Prepared for The Australian Egg Corporation Ltd.

Food-borne pathogens and animal botulism issues surrounding the on-farm composting of layer chicken waste and mortalities – a review

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Prepared by:
Nalini Chinivasagam
(Department of Primary Industries and Fisheries)

and

Geoff Runge

PREFACE

MAJOR OBJECTIVES OF THE PROJECT

Objective 1: To review literature of pathogen risk (*Salmonella*, *Campylobacter*, *E. coli* - as an indicator, *Clostridium perfringens*, *and Clostridium botulinum*) in composting manure (cage, barn and free range from indoor shed.

Objective 2: To assess the pathogen risk to humans and stock from the land application of composted poultry manures. Mortalities and egg waste (cracked and broken eggs – with emphasis on layers – from cage, barn and free range production systems.

Objective 3: To provide guidelines to producers and regulators on the safe application of composted poultry solids to land with emphasis on pathogens that is of most risk to humans and livestock.

Objective 4: To include and discuss data from testing of compost samples.

Table of Contents

Executive Summary 1

Chapter 1 Introduction 7

1.1 Background of literature searched 8

1.2 Chicken waste composts and mortality composts 9

1.3 The composting process 9

1.4 Summary 11

Chapter 2 Food-Borne Pathogens 12

2.1 Food-borne pathogens, poultry and the environment 12

2.2 *Salmonella,* general background 15

2.3 *Salmonella*, the layer industry and public health 15

2.4 *Salmonella,* On Farm biosecurity 20

2.5 *Campylobacter* general background 22

2.6 *Campylobacter* and chickens 24

2.7 Biosecurity measures for *Campylobacter* 24

2.8 *Clostridium botulinum* – general background 25

2.8.1 Serological types and cattle botulism 26

2.9 *Clostridium perfringens* 28

2.10 Biosecurity measures for *Cl. perfringens* 29

2.11 *Listeria monocytogenes* 29

2.12 Biosecurity measures for *L. monocytogenes* 30

2.1.3 Summary 31

Chapter 3 Composting, poultry carcasses and manure 32

3.1 Chicken carcass composting 32

3.2 Chicken carcass composting processes 33

3.3 Emergency composting of carcasses 39

3.4 Composting chicken waste other than carcasses 40

3.5 Case studies of on-farm composting of chicken manure 41

3.6 Temperatures and pathogen reduction 42

3.7 Summary 44

Chapter 4 Pathogens, Pathways and an "Index Organism" of Compost Safety 45

4.1 The pathways 45

4.2 Prevalence of pathogens in source material 46

4.3 Co-composting raw material 50

4.4 Nematodes as vectors for the transfer of pathogens 51

4.5 An "index" organism for compost safety 53

4.6 Summary 55

Chapter 5 Survival of pathogens 56

5.1 Animal wastes and pathogen survival 56

5.2 Composts and pathogen survival 60

5.3 Carcass composting and pathogens 61

5.4 Composting of wastes and pathogens 62

5.5 Summary 67

Chapter 6 Survival and re-growth of *Salmonella* 68

6.1 *Salmonella* and composts 68

6.2 Re-growth of *Salmonella* 73

6.3 Summary 76

Chapter 7 Australian Data 77

7.1 Introduction 77

7.2 Methods 77

7.3 Results and discussion 78

7.4 Summary 82

Chapter 8 *Clostridium botulinum*, cattle and composting 88

8.1 Botulism, cattle and source of infection 88

8.2 Chickens as a possible source of *Cl. botulinum* 90

8.3 Distribution and site specificity of *Cl. botulinum* types 91

8.4 Persistence of spores in the environment 92

8.5 Ecological aspects on the persistence of spores 96

8.6 Composting and *Cl. botulinum* 97

8.7 Case studies of bovine botulism outbreaks - poultry waste and other sources 101

8.7.1 Persistence of the organism after an outbreak 103

8.7.2 Outbreaks in Queensland 104

8.7.3 Decomposing carcass and ensilaged poultry litter 105

8.7.4 Carcass in stockpiled litter, a source 105

8.7.5 Single source responsible for a cluster of outbreaks 106

8.7.6 Preventative measures 108

8.7.7 Other outbreaks 108

8.8 Production and stability of botulinum toxin in the environment 108

8.9 Inactivation studies 111

8.10 Diagnostic techniques for Toxin types C and D 113

8.11 Summary 114

Chapter 9 Guidelines 116

9.1 Introduction 116

9.2 General overview of guidelines 119

9.2.1 US EPA Guidelines 119

9.2.2 Queensland Stock Act 125

9.2.3 England, Scotland. Wales and Northern Ireland 126

9.2.4 Other Australian Guidelines 126

9.2.5 US FDA Guideline for Fruits and Vegetables 128

9.3 Specific details within relevant existing Guidelines 128

9.4 Possible criteria relevant for chicken waste composts 131

9.5 Summary 134

Chapter 10 References 135

Acknowledgements 150

Appendix 1 Details of selected composting methodologies 151

Appendix 2 Summary of botulism cattle outbreaks (Advisory committee on the microbiological safety of food ad hoc group 2006) 156

List of Tables

Table 2.1 Notifications of communicable diseases, Australia in , 2005, by state or territory (Owen *et al.* 2007) 13

Table 2.2 Most common *Salmonella* serovars in egg layers and eggs (2006) Source – Australian Salmonella Reference Centre – 2006 Annual Report 17

Table 2.3 Top 10 human isolates of Salmonella, Australia, 2005, by state or territory (Owen *et al.* 2007) 19

Table 3.1. Level of temperature and length of time necessary to destroy some pathogens present in primary products of composts – adapted from Déportes *et al.* (1995) Note that typical range of composting temperature is 55 -65^{0}C. 43

Table 4.1 Levels of coliforms in clean bedding material – adapted from Rendos *et al.* (1975) 51

Table 6.1 Survival times, moisture content and time temperature relationships of different *Salmonella* strains during different composting processes Adapted from Ceustermans *et al.* (2007) 69

Table 7.1 Field data and physical parameters of composts examined in this study 84

Table 7.2 Result of microbiological tests and moisture determinations 86

Table 8.1 The slow destruction of type C toxin under semi natural conditions May 1975 (minimal temp in March /April 3-11°C), after Graham *et al.* (1978) 109

Table 8.2 Time temperature relationship for inactivation of *Cl. botulinum* type C toxin adapted from Hubalek and Halouzka (1988) 110

List of Figures

Figure 1.1 Summary of the composting process for animal wastes 10

Figure 3.1 Cross section of static compost pile showing distribution of various feed stocks within the pile (González and Sánchez 2005) 34

Figure 3.2 Recorded temperature in the poultry carcass “mini composter” (Lawson and Keeling 1999) 36

Figure 3.3 Typical Temperature Profile in a Two Stage Composter (Hog Producers Sustainable Farming Group 1996) 38

Figure 4.1 Possible pathogen pathways – adapted from Déportes *et al.* (1995) 46

Figure 5.1 Adapted from Hutchison *et al.* (2005a) 59
A - Temperature profiles of various manure heaps over time.
B - Pathogen decline in cattle manure

Figure 8.1 The reported distribution of botulinum neurotoxin types in the Australian environment (Szabo and Gibson 2003) 94

Figure 8.2. Internal temperatures of bird carcasses and ambient temperatures. (Wobeser and Galmut 1984) 96

Figure 8.3 Survival pattern of *Cl. sporogenes* in soil and on parsley (after spiking with spores) -adapted from Girardin *et al.* (2005) 100

Figure 8.4 Schematic presentation of the cycle of *Cl. botulinum* type B on a cattle farm involved in an outbreak by brewers’ grain - after Notermans *et al.* (1981) 103

Figure 9.1 Decision tree for the land application of biosolids as in United States Environmental Protection Agency guidelines (2003) 123

Executive Summary

Chicken carcass composting and the composting of chicken wastes are both feasible processes used overseas and in Australia. The literature on composting, especially on pathogens and chicken carcass and waste composting (from cage, barn and free range) and pathogen survival, is not extensive with limited peer reviewed literature. However, when this literature is supported by studies based on animal manures and other composted material, there is a solid basis for the necessary background knowledge on pathogen survival. The literature on composting of bio-solids also provides supporting information.

Composting of layer wastes is widely practiced in the USA. A range of carcass composting techniques have been discussed. Chicken carcass composting studies, including limited data on pathogen survival, have been published especially in the USA. Carcass composting is not permitted in some countries, for example the UK, because of the risk of animal botulism.

On the basis of a survey of the literature, the bacterial pathogens of importance in both general composting and carcass composting in Australian layer operations are *Salmonella* spp. in terms of food safety and *Cl. botulinum* (Types C and D) in terms of animal health (specifically cattle botulism).

Criteria for layer waste composting need to be developed encompassing the three major risk areas:

- Human health
- Cattle health
- Environmental aspects that can have both a direct or indirect impact on the above two criteria

The issues surrounding *Cl. botulinum* are more complex than the food borne pathogens. However, adhering to good composting practices can aid in the production of compost acceptable from a food safety perspective and cattle safety.

Composting needs to be carried out as per best practice, with turning and aeration that are conducive for carcass breakdown and the avoidance of anaerobic conditions suitable for proliferation of *Cl botulinum*. The toxins are suggested to be heat labile. However, the spores are more resistant to a composting process than normal bacteria.

A range of pathways for the movement of pathogens to the food chain as well as transfer in the environment are potentially present. The possibility of pathogens being in the source material and thus re-entering the production system via the on-farm waste management system needs to be managed by implementing suitable biosecurity measures such as control of vectors and the use of segregation. or barriers.

Salmonella has been associated with both layers and eggs and also one of the major organisms of interest in national outbreaks. *Cl. botulinum* specifically Types C and D have been isolated in several Australian environments as well as linked to large cattle outbreaks of botulism attributed a single source (chicken litter) in Queensland. Thus the possible risks associated with spreading compost to areas where cattle have access needs to be considered.

Cattle botulism outbreaks with links to rotting chicken carcasses via litter have occurred in various countries as well as Australia. Maggots within such carcasses are known to be the key sites of toxin production. *Cl. botulinum* spores can remain dormant, transfer in the environment, grow and produce toxin when conditions are right. Bio-composts have been shown to harbour *Cl. botulinum* (54% positive for vegetative cells and 25% having the potential to produce toxin). Inoculation studies have shown that spores can persist in the soil and transfer to crops.

Salmonella could act as an "index organism"in composts and be included in guidelines. Further *Salmonella* has the potential to persist in the environment and re-grow under certain conditions. The aging periods of compost can support either re-growth or re-colonisation of compost by organisms from external sources. The value of *Escherichia coli* as an "indicator organism" and its relationship to *Salmonella* is not well supported.

An Australian study testing thirteen partially composted samples and one fully composted sample produced a range of outcomes. Botulinum toxin was detected in one sample. This sample was a poorly composted sample which contained a rotting chicken carcass. *Salmonella* was present in six of the 14 compost samples. In three of these samples the *Salmonella* level was <3 MPN/g with another sample being 4 MPN/g. Of the remaining two samples one had a level of 15 and the other 4300 MPN/g. Overall, 12 of the 14 compost samples had a *Salmonella* level of 4 or less MPN/g (with 11 being <3 MPN/g). Hence, despite the fact that most of the samples were partially completed composts, we found little evidence of high levels of *Salmonella.* A total of six different *Salmonella* serovars were detected – Amsterdam, Infantis, Mbandaka, Montevideo, Senftenberg and Singapore. Importantly *S.* Typhimurium was not detected in this limited survey.

The sole completed compost examined in this study was sourced from a full range of potential layer waste input – daily mortalities, cracked and broken eggs and cage manure. This completed compost, produced by a piling process resulted in a product of good physical parameters, an absence of food-borne pathogens, an absence of the spore-forming *Cl. perfringens* and no botulinum toxin.

This is evidence that a well managed composting process can deliver a good quality, safe compost under practical on-farm conditions. There is also a need to explore if smaller composting units such as heaps or small units may be

more manageable to operate within a farm location due to resource limitations.

A range of guidelines on the methods of processing and the microbiological quality of bio-solids (and other) exist which can form the basis for a set of criteria to ensure microbiological safety of compost. Based on the USEPA guidelines (United States Environmental Protection Agency 2003), the safety of the product could be achieved via process control i.e. Processes to Further Reduce Pathogens (PFRPs) which are linked to “Class A” biosolids.

Composting should be performed using standard recommended procedures for best practice composting in terms of pathogen reduction and pathogen transfer to the environment (includes aeration, management of run-off, and control of wild animals)

Microbiological criteria should be focussed on *Salmonella* levels and reflect the end-use of the product. This approach can be dealt with by using a decision tree approach. For end-uses such as vegetables that undergo minimal processing, the *Salmonella* level should set at absent in 50 g (dry weight). For use on pasture, the existing generally used criterion of < 10 *Salmonella* per 50 g (dry weight) could be used and supported by a with-holding period based on pasture re-establishment, soil incorporation and so on (30 to 60 days has been nominated).

From a cattle perspective a decision tree approach needs to take into account the possible risks from *Cl. botulinum.* Another measure which can be adopted is to strictly limit/prevent cattle access to areas where composted carcass waste has been spread (the rational behind the British guidelines).

A HACCP plan can be developed to have documented process controls during each compost operation. This would allow the history of the process to be monitored and the possibility of taking corrective action.

Above all, the education and training of all personnel and farmers dealing with the composting such operation is vital

Chapter 1

Introduction

The Australian egg industry consisted of a flock size of 13,175 million hens that produced 203 million dozens of eggs in 2005 with this production being dominated by cage (70%) followed by free range (20%) and barn (5%) respectively (http://www.aecl.org/). This overall production system generates waste in the forms of faeces and other material such as egg waste and carcasses (spent hens and daily mortalities). This waste (as well as litter generated from meat chicken production) contains valuable nutrients that can be recycled. Most recycling practices such as composting are not only a cost effective means of managing waste but also return a valuable resource back to the environment for agricultural purposes.

However, there are concerns in terms of pathogen transfer and the need to develop safe, sound and workable guidelines. Such hygiene regulations need to serve the animal production facilities satisfy environmental hygiene concerns and be cost effective to the farmer (Heinonen-Tanski *et al.* 2006). Both the meat chicken industry (Runge *et al.* 2007) and the egg industry currently are proactive in terms of working towards solutions of using such waste with minimal impact to the environment.

Kelleher *et al.* (2002), in a comprehensive review on poultry waste disposal strategies, discuss various co-composting options impacting on a successful end product. Whilst the composting process has been around for a long time, the increase in wastes generated at a producer level has highlighted the need

for environmentally sound and safe composting systems. The issues of both zoonotic and food borne pathogens are becoming a current major focus. Increasingly these waste management strategies are focussed on processes that allow litter and other poultry waste to be fully utilised as valuable resources for soil amendment in agricultural applications.

In addition, composting mortalities on the farm can be a part of the overall waste management strategy. While it is possible to remove and/or bury solid waste, innovative solutions such as composting can provide environmentally friendly solutions that still meet the need for economic realities of poultry production. Poultry carcasses resulting from death by natural occurrences represent a large amount of organic matter that requires environmentally and biologically safe disposal (Blake 2004).

There is also growing pressure from legislators in understanding the issues surrounding the presence and transfer of zoonotic pathogens as a consequence of either the direct use of animal manures or due to the use of composted or treated manures for agricultural purposes. The concerns surround both direct as well as indirect use of manures

1.1 Background of literature searched

Scientific literature from both agricultural and medical databases were searched and reviewed. General internet based searches were also carried out to look for reports, fact sheets and composting protocols for the poultry industry and other animal industries. Pathogen survival was reviewed in a

generic manner – covering both composts as well as bio-solids. The basis for this generic approach was that both of these organic materials (composts and bio-solids) have undergone a biological process of conversion. In addition, food-borne pathogens are common to these environments. Wherever available, literature on the survival of the zoonotic pathogens from poultry and their survival in manures and the environment was sought. Thus a combined approach was undertaken - allowing an understanding of pathogen survival within a composting scenario. This approach was adopted due a lack of extensive peer reviewed literature specific to some aspects of the review.

1.2 Chicken waste composts and mortality composts

This review deals with the following composting activities within the poultry industry:

a) Carcass (spent hens and daily mortality composting)

b) General composting (to include chicken waste, broken eggs)

1.3 The composting process

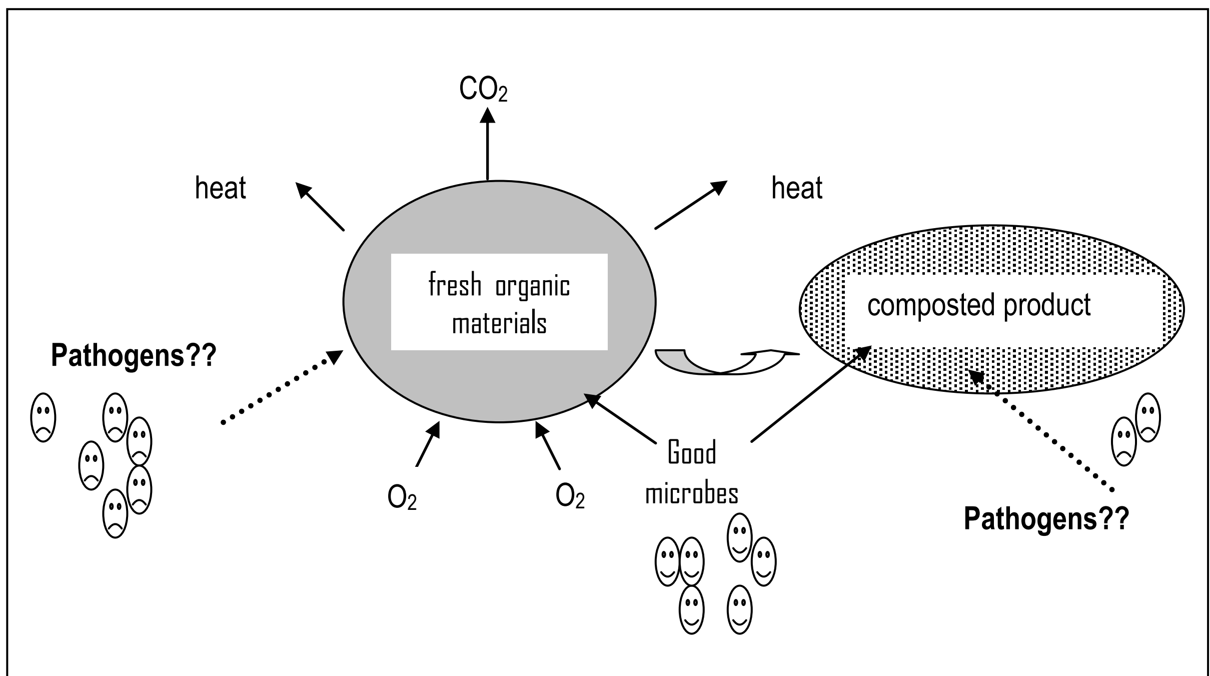
Composting is an aerobic process using intrinsic micro-organisms to convert organic material to an utilisable form and, in the process, destroying pathogens and converting nitrogen from volatile ammonia to stable organic forms (Imbeah 1998). The activity of intrinsic micro organisms involves both the mesophilic and thermophilic phases under aerobic conditions. The breakdown organic material occurs in two stages:

- stage 1- involves a high rate of biological activity and rapid composting and high temperatures

- stage 2 – involves a lower biological activity and lower temperatures (Keener *et al.* 2000).

Figure 1.1 provides a summary of the composting process.

Figure 1.1 Summary of the composting process for animal wastes



Importantly in the context of this review, it is imperative that it is recognised that composting occurs within an environment where pathogens are known to exist.

Governo *et al.* (2003) in their white paper on composting describe the two basic forms of the composting processes as follows:

Windrow composting

- materials are placed in long rows
- height and shape of rows depend on the aeration equipment used for turning
- aeration is by warm air rising from the pile
- mechanical turning is used
- air flow rate is dependent on porosity and availability of oxygen.

In-vessel composting

- performed within a closed structure
- a more controlled and costly process
- combines most composting techniques within a single environment
- a process that can be used when available land is limited.

1.4 Summary

- Pathogens are potentially present in waste
- Concerns about pathogens – often in the absence of scientific data – are held in many quarters – including regulatory authorities
- Composting is recognised as a valuable management tool that can be used to address pathogen issues

Chapter 2

Food-Borne Pathogens

2.1 Food-borne pathogens, poultry and the environment

This section provides a general background of the pathogens of significance to this review. These pathogens can be present in poultry waste and have the possibility of re-entering the poultry operation due to the presence of the composting operation on site or transfer via improperly treated manures or wastes to the food chain impacting on humans.

Clostridium botulinum, though a food-borne pathogen is considered a limited risk to public health however this aspect will be briefly discussed in this section. The factors associated with cattle botulism and composting will be discussed in detail under Chapter 8.

Food-borne pathogens such as *Campylobacter, Clostridium perfringens* and *Salmonella* are all associated with poultry and poultry waste (Shane 1992, Limawongpranee *et al.* 1999; Chalmers *et al.* 2008). *Listeria monocytogenes*, commonly linked with the environment, can also be associated with the free range poultry production systems (Esteban *et al.* 2008). *Arcobacter,* an emerging pathogen, is linked with poultry (Wesley and Baetz 1999) and may be of future significance. This section discusses the importance of these organisms from a public health point of view.

Based on the national statistics on gastrointestinal outbreaks (Owen *et al.* 2007) the dominant gastrointestinal disease in Australia in 2005 was campylobacteriosis (16,468 cases) followed by salmonellosis (8,441 cases) (Table 2.1). The incidences of listeriosis (54 cases) and human botulism (3) are at much lower levels.

Table 2.1 Notifications of communicable diseases, Australia in , 2005, by state or territory (Owen *et al.* 2007)

Gastrointestinal disease	State or territory								
	ACT	**NSW**	**NT**	**Qld**	**SA**	**Tas**	**Vic**	**WA**	**Aust**
Botulism	0	0	0	2	0	0	1	0	3
Campylobacteriosis	402	NN	248	4416	2089	760	6109	2444	16,468
HUS*	0	11	0	2	1	2	3	1	20
Listeriosis	3	25	0	7	6	0	9	4	54
Salmonellosis (NEC)	95	2179	393	2613	577	302	1481	801	8441
Shigellosis	7	135	196	80	48	5	105	156	732
SLTEC VTEC	0	16	0	9	40	2	8	12	87
Typhoid	0	28	0	3	2	0	11	8	52

NN – non-notifiable

* HUS Haemolytic uraemic syndrome

Thus, based on the above data on human outbreaks and the nature of these pathogens in the environment (see section below), the key pathogens that need to be considered from a composting point of view for the purpose of this review are listed below. These organisms will be discussed in this Chapter.

Organisms of primary importance

Organism	Reason for consideration
Salmonella	• Ability to survive in the environment • Dominant association with poultry • One of the major food-borne pathogens • Key serovars linked with egg related outbreaks in Australia
Cl. botulinum (Cattle)	• Good survivor in the environment • Spore former • Cattle mortality – chicken waste, associated with previous outbreaks in Australia • Chicken – chicken waste link
E. coli	• A common indicator organism

Organisms of secondary importance

Organism	Reason for consideration
Campylobacter	• Poor survivor in the environment • Poor survival during composting • Dominant association with poultry • One of the major food-borne pathogens • Prevalent in barn, cage, free range birds
Cl. perfringens	• Good survivor in the environment • Spore former • Chicken – chicken waste link • A food-borne pathogen
Listeria monocytogenes	• Present in the environment • Possible link with free range poultry • A food-borne pathogen
Arcobacter	• An emerging food borne pathogen

2.2 *Salmonella,* general background

Salmonella are closely linked with poultry and poultry production. The organism is shed in faeces and can contaminate soil, pasture, streams and lakes, which serve as the source of organisms to colonise other animals (Jay *et al.* 2003). The organism also can survive in soil for months (Jay *et al.* 2003). S*almonella* Newport was seen to survive for 184, 332, and 405 days in manure, manure-amended non sterilized soil and manure-amended sterilized soil, respectively under experimental conditions (You *et al.* 2006). Thus, the organism has the potential to impact on the safety issues surrounding the production of animal waste products such as composts. Whilst thermophilic temperatures linked to composting can contribute to *Salmonella* reduction, the organism has shown a potential to grow in litter and manure and a capacity to adjust to dry environments (Eriksson De Rezende *et al.* 2001). Due to its ubiquitous nature, *Salmonella* is not likely to be eradicated from the food chain, Thus simple measures, such as improved on-farm biosecurity and the proper storage and use of animal excreta as fertiliser could do much to improve animal health and food safety (Humphrey 2004).

2.3 *Salmonella*, the layer industry and public health

Salmonella has been associated with numerous food-borne outbreaks, and thus is a key food-borne pathogen. A total of 8,441 salmonellosis cases were reported in Australia in 2005, a rate of 41.5 cases per 100,000 population and a 6.6% increase from the rate reported in 2004 (Owen *et al.* 2007). The national notification rate for 2005 showed an increase of 14.1% over the mean

rate for the previous 5 years (Owen *et al.* 2007). Thus, the incidence of salmonellosis seems to be on the rise.

Salmonella Enteritidis (SE) outbreaks due to the consumption of contaminated Grade A eggs have been a threat to public health and to the economic viability of the egg industry in other countries (Gast and Beard 1993). However, SE has been not an issue for the egg industry in Australia (Table 2.2). In Australia, *Salmonella* Typhimurium has been reported in both layers as well as eggs, Table 2.2. One of the largest egg-associated outbreaks of food borne illness in Australia for many years occurred between June and December 2005. Five outbreaks of *Salmonella* Typhimurium phage type 135 were identified in Tasmania, leading to 125 laboratory-confirmed cases. Foods containing raw egg or foods contaminated through inadequate food handling and/or storage procedures were the possible vehicles for infection. A particular poultry farm was reported as the common source of eggs (Stephens *et al.* 2007)

Table 2.2 lists the dominant serovars isolated from eggs and layers in 2006, with *S.* Mbandaka replacing *S.* Typhimurium as the previous dominant serovar since 2005.

Table 2.2 Most common *Salmonella* serovars in egg layers and eggs (2006) Source – Australian Salmonella Reference Centre – 2006 Annual Report,

Chicken – layers (791)#		Eggs (84)#	
Mbandaka	20.9%	Anatum	36.5%
Typhimurium	15.8%	Montevideo	19.0%
Agona	10.7%	Ohio	19.0%
Montevideo	9.4%	Singapore	9.5%
Kiambu	3.5%	Typhimurium	7.9%
Ohio	3.5%	Infantis	3.2%
Tennessee	3.4%	*Muenster	1.6%
Livingstone	2.9%	Oranienburg	1.6%
Cerro	2.5%	16:1,v:-	1.6%
Bredeney	2.3%		

* Reported as a appearing in poultry for the fist time in 2006

\# Total number of isolates

Salmonella Infantis was observed in the late 1990s to be dominating in layer flocks and in raw egg products in south-east Queensland. The organism was thus considered to be of potential significance to the Australian egg industry, with regard to egg-borne transmission and related public health issues (Cox *et al.* 2002).

A study (Cox *et al.* 2002) carried out to assess these possible risks showed that:

- the incidence of. *Salmonella* Infantis was lower than the incidence of other *Salmonella* serovars during a survey of raw egg products
- when commercial point–of-lay hens were orally challenged with 10^9 cfu/ml *S.* Infantis, the organism was persistently shed via the faeces for up to 6 weeks and was also able to colonise the gastrointestinal tract and other internal organs of the birds

The Australian egg industry is presently basically free of *Salmonella enterica* serovar Enteritidis (SE) and a current national surveillance program has been developed in the event of an outbreak within the egg industry (Sergeant *et al.* 2003). This program has a 95% confidence of detecting infection if it were present in 1% of layer flocks (equivalent to three flocks) at a prevalence of 5 in 1,000 birds being infected.

S. Enteritidis is one of a small group of serovars, which includes *S.* Typhimurium, that is highly invasive in hens and can contaminate egg contents *in viva* under commercial conditions (Humphrey 2004 3168). Flock recycling (induced moulting), within layer operations can increase *Salmonella* (Enteritidis) problems in the flock {Seo *et al.* 2000)

From an Australian perspective the dominant serovar isolated from human outbreaks in 2005 was *Salmonella* Typhimurium (Owen *et al.* 2007. Table 2.3 lists the top ten serovars isolated from outbreaks.

Table 2.3 Top 10 human isolates of Salmonella, Australia, 2005, by state or territory (Owen *et al.* 2007)

Organism	State or territory									Total %
	ACT	NSW	NT	Qld	SA	Tas	Vic	WA	Aust	
S. Typhimurium 135	14	188	1	135	23	175	198	68	802	16.60
S. Typhimurium 197	1	113	0	140	5	2	280	4	545	11.30
S. Typhimurium 170*	14	328	0	48	3	6	64	9	472	9.80
S. Saintpaul	3	42	48	271	13	2	24	33	436	9.00
S. Typhimurium 9	11	155	5	33	57	10	124	11	406	8.40
S. Virchow 8	2	28	10	182	6	1	7	12	248	5.10
S. Typhimurium 44	6	67	0	59	28	6	53	9	228	4.70
S. Birkenhead	0	85	0	128	0	0	6	1	220	4.50
S. Chester	1	30	14	87	14	1	10	29	186	3.80
S. Hvittingfoss	5	23	5	129	1	0	19	3	185	3.80
Sub-total	57	1059	83	1212	150	203	785	179	3728	77.00
Other isolates	6	217	35	370	90	63	134	197	1112	23.00

Source: National Enteric Pathogenic Surveillance System.
* Reported as *Salmonella* Typhimurium phage type 108 in some states and territories.

Salmonella Typhimurium is responsible for 40–70% of cases of human salmonellosis in Canada (Leon-Velarde *et al.* 2004). Testing commercial layer flocks and pullet environments in Canada during a 20-month period revealed a prevalence of 5.5% of *Salmonella* Typhimurium DT104 (Leon-Velarde *et al.* 2004).

The prevalence and levels of *Salmonella* associated with the production environment (i.e. farm) will influence the extent of contamination of the waste. Contamination of *Salmonella* can occur from layer flocks to eggs and ultimately to waste (cracked eggs, manure and mortalities) finally destined for on-site composting. Surviving environmental stress may be *Salmonella* strain dependent (in a slaughter house situation) and could also be a case in the external environment (Someya *et al.* 2005). This suggests that some serovars may be selected over the others in the waste.

2.4 *Salmonella,* On Farm biosecurity

Rodents (Barbour and Nabbut 1982; Davies and Breslin 2001; Kinde *et al.* 2005; Lapuz *et al.* 2007), litter beetles (Skov *et al.* 2004), wild birds (Davies and Breslin 2001), ground beetles and centipedes (Davies and Breslin 2003b) have been associated with *Salmonella* in operations such as cage layer flocks, barn egg production, free range flocks and egg processing facilities. A naturally infected mouse which died after intermittently excreting small numbers of *S.* Enteritidis in its droppings for 19 weeks had 10^4 organisms/g of liver and 10^3/g of macerated intestine and contents (Davies and Wray 1995) suggesting that rodent can harbour *Salmonella* for long periods of time. Similarly litter beetles have also been shown to be a reservoir for *Salmonella* Indiana (Skov *et al.* 2004).

Various *Salmonella* serovars were also isolated from different fly species from cage layer facilities, while *S*. Mbandaka was isolated from a beetle species from manure pit beneath the house (Olsen and Hammack 2000) Free-range chickens have access to outside soil and water, which could provide exposure to additional vectors of infection (Wales *et al.* 2007). Following an outbreak of *S*. Enteritidis, the depopulated production environment continued to demonstrate the presence of the organism. *Salmonella* was found to persist for 8 months in soil in free range paddocks and free range house (Davies and Breslin 2003a). *Salmonella* was isolated from vectors such as wild mice, foxes and cats (Davies and Breslin 2003b).

S. Enteritidis strains were isolated from wildlife and from the environment of a layer farm (Liebana *et al.* 2003). The same *S*. Enteritidis clones were present in both wildlife vectors (mice, rats, flies, litter beetles and foxes) and farm environment (such as cages, beams, floor and egg belt). Some of the clones present in mice were also present in the egg contents and spent hens (Liebana *et al.* 2003). .This example shows the need to maintain biosecurity on farm, which if not maintained can compromise the production system

In order to determine the epidemiological link between the *S.* Enteritidis contamination in a rat-infested chicken layer farm, an attached egg processing facility and liquid egg samples, several *S.* Enteritidis isolates were analysed by pulsed-field gel electrophoresis (PFGE) and bacteriophage typing. Similar pulsed-field patterns were present in *S.* Enteritidis isolates from liquid eggs, rats and effluent water suggesting a genetic link between these isolates. This

demonstrates the role played by rats in the transfer of *Salmonella* and that rats pose a public health risk (Lapuz *et al.* 2007) and need to be monitored.

Thus, there is a need for biosecurity measures to be adopted with on-farm composting. Failure to manage wildlife vectors is likely to negate even the most effective cleaning and disinfection regimes in layer (and broiler houses) (Wales *et al.* 2006).

The existing code of practice for Biosecurity in the Australian egg industry deals with a HACCP based approach for on-farm hygiene aspects including rodent (Grimes and Jackson 2001).

2.5 *Campylobacter* general background

Campylobacter is found in the intestinal tracts of a wide range of both domestic and wild animals that show no sign of disease (Wallace 2003). *Campylobacter* species are responsible for intestinal colonization in poultry and food-borne enteritis in humans (Shane 1992). *Campylobacter jejuni* is the most significant of the three thermophilic campylobacters which do not grow under aerobic conditions (Wallace 2003). Birds and, in particular, poultry are known to be the primary reservoir for *C. jejuni* with the organism generally being considered to be a commensal that has evolved to grow at 42°C to mirror the avian gut (not 37°C as in the mammalian gut) (Park 2002). Campylobacters are ubiquitous in nature and large environmental reservoirs

of *Campylobacter* are present in avian populations as a part of the commensal microflora (Carrillo *et al.* 2007).

In 2005 there were 16,468 notifications of campylobacteriosis in Australia (where campylobacteriosis is notifiable in all jurisdictions except New South Wales) (Owen *et al.* 2007). A common vehicle for infection is contaminated poultry meat as carcasses can carry more than 10^8 *Campylobacter* cells (Humphrey *et al.* 2001).

Park (2002) provides a succinct summary of the nature of this major food and water borne pathogen and has stated that *Campylobacter*:

- has uniquely fastidious growth requirements
- has an unusual sensitivity to environmental stress compared to other food borne pathogens,
- lacks many of the well characterised adaptive responses associated with resistance to stress in other bacteria
- has a minimal capacity for recognising and responding to stress
- lacks the ability to grow below 30°C
- is sensitive to heat (though thermophilic in nature)
- is very sensitive to desiccation
- is readily inactivated by pasteurization treatments and domestic cooking processes

In spite of all these factors, *Campylobacter* is still able to persist in the food chain and in doing so remains the most common cause of bacterial food-borne illness (Park 2002). However Humphrey *et al.* (2007) suggest that the

belief that *Campylobacter* is a sensitive organism is based on laboratory studies. Current work in this area is showing that campylobacters may be more robust than previously thought and thus may represent a greater challenge to food safety (Humphrey *et al.* 2007).

2.6 *Campylobacter* and chickens

The incidence of *Campylobacter* can vary from 0 -100%, depending on such factors as the age of the bird, natural resistance and hygiene. (Wallace 2003). Two species of *Campylobacter* can be found in chickens. An epidemiological investigation in Austrian broiler flocks over a 3 years period resulted in a dominance of *Campylobacter jejuni* (88%) with the rest being *Campylobacter coli* (12%) (Neubauer *et al.* 2005). Generally broilers show infection at 2-3 weeks of age and within a week all birds within a flock are infected and remain thus until slaughter. Layers show the similar pattern of infection but the isolation rate from the birds begins to fall from around 12 weeks of age to about 30% after a year (Wallace 2003).

2.7 Biosecurity measures for *Campylobacter*

The biosecurity measures in terms of vectors adopted for *Salmonella* can also be included for *Campylobacter* as well. There is a potential for manure / compost heaps to possibly contribute to pathogen transfer via vectors, depending on the role played by the various vectors in pathogen transmission. More specifically the following have been observed as potentially playing a role in the management of the prevalence of *Campylobacter* in a production system.

- Litter beetles to harbour *Campylobacter;* however, was considered not to have the ability to transfer from one flock to another (Skov *et al.* 2004)
- flies have been shown to be a source of *Campylobacter* (Gregory *et al.* 1997)
- cattle in surroundings are a source of *Campylobacter* (Gregory *et al.* 1997)
- manure heap location. The odds of *Campylobacter* colonization were 5.2 times higher for flocks with manure heap >200 m from the poultry house. The presence of a manure heap at <200 m from the broiler house was significantly associated with a decreased risk of colonization in chicken flocks (Arsenault *et al.* 2007). The authors could not explain these unusual findings.

Overall, measures adopted for vector control in terms of creating barriers or minimising odours (will be discussed under guidelines) could aid in management of both *Salmonella* and *Campylobacter* movement from the composting environment to the production facility.

2.8 *Clostridium botulinum* – general background

Clostridium botulinum is responsible for botulism, a fatal illness in both humans and animals (Jay 1978). *Cl. botulinum* is widely distributed in environmental sources such as soil (Smith 1975a; Wobeser *et al.* 1987) and aquatic environments (Segner *et al.* 1971a; Huss 1980). The organism was

first isolated in 1895 by Van Ermengen who named the organism named *Bacillus botulinus* (Jay 1978).

Cl. botulinum is an anaerobic spore forming organism with spores that are able to survive in the environment for decades (Mitscherlich and Marth 1984, cited in Böhnel *et al.* (2002) and under most environmental circumstances e.g. dry heat (Critchley 1991). The ingestion of a highly toxic, soluble exotoxin produced by the organism is responsible for the symptoms of the food-borne botulism (Jay 1978). This exotoxin, also known as botulinum neurotoxin, varies depending on the different serotypes. Toxins are formed within the organisms and are released during autolysis. These toxins are among the most toxic substances known to man and contain 30,000,000 mouse LD_{50}/mg. (Jay 1978).

2.8.1 Serological types and cattle botulism

Based on the serological specificity of the toxins of *Cl. botulinum,* seven types are recognised i.e. A, B, C, D, E, F and G (Jay 1978). Group C is also recognised as C alpha and C beta, with type C being commonly associated with chickens (and birds) (Dohms 2003). Types C and D seem to have a relationship between bacteriophages for toxin formation, details of which are discussed in Szabo and Gibson (2003). Since poultry are commonly affected by type C toxin and cattle are susceptible to types B, C and D, the consumption of poultry litter can result in cattle being affected with type C toxin (Jean *et al.* 1995). It is suggested that if one gram of pure toxin is

adequately distributed in feed it can kill 400,000 adult cows, cited in Martin (2003).

It appears that not all strains are capable of producing toxins. Types C and D strains isolated from fodder and soil in South Australia resulted in the type D strain yielding spontaneous mutants not capable of toxin production in culture media possibly due to high mutation rates (Eales and Turner 1952). Types A, B, E and F are responsible disease in humans (Jay 1978). There are four naturally occurring types of botulism, i.e. food borne botulism (ingestion of preformed toxin), infant botulism (toxin produced in intestinal tract), wound botulism (toxin production in infected wound) and botulism due to intestinal colonisation in children and adults (due to either surgery or antibiotics) (Johnson *et al.* 2007).

A draft report by the Advisory Committee on the Microbiological Safety of Food Ad Hoc group (2006) was prepared against a backdrop of increased incidences of cattle botulism and thus the possible impact on the human food chain. The outcomes of this report were as follows:

- the consumption of meat and milk from affected cattle will not pose a risk due to the fact that the toxin delivered from a food source is rapidly and irreversibly bound and unlikely to cause intoxication
- acknowledgement that a risk assessment carried out in France did not identify a significant risk to the public from food associated with botulism in cattle.

- no evidence was identified to suggest any clinical human cases were linked to consumption of meat or milk from affected herds

2.9 *Clostridium perfringens*

Food poisoning strains of *Cl. perfringens* are present in soil, water, foods, dust and the intestinal tract of humans and other animals (Jay 1978). *Cl. perfringens* is also a spore forming organism and is able to withstand conditions such as drying and heating. The organism plays a role in various disease conditions, such as necrotic enteritis of chickens (McCrea and Macklin 2006). However the organism has also been isolated in healthy broiler chickens (Chalmers *et al.* 2008).

The organism may also cause wound infections and human food poisoning outbreaks (Niilo 1980). They organisms have optimum growth temperatures between 37 – 45°C with the ability to grow at pHs between 5.5-8.0 (Jay 1978).

Cl. perfringens has been isolated in 96% of the composts in Greece (Lasaridi *et al.* 2006), indicating the organism has the ability to survive the composting process.

Interestingly *Cl. perfringens* strains can produce an inhibitor effective on strains of *Cl. botulinum* type A (Smith 1975b). In mixed culture, an inhibitor strain of *Cl. perfringens* repressed growth and toxin production by a *Cl. botulinum* type A strain even though it was outnumbered by the latter by about about 40 times. The inhibitor strain also repressed growth and toxin

production of *Cl. botulinum* in mixed culture of soils, the natural environment of the latter organism. This indicates that there is a possible beneficial attribute of *Cl. perfringens* when present along with *Cl. botulinum* (Smith 1975b). Smith (1975b) notes that botulinum inhibitory strains of *Cl. perfringens* are rare and that the inhibition is dependent on the medium used. These studies were all performed in laboratory cultures. It is not clear if this inhibition would occur in natural situation such as compost piles.

2.10 Biosecurity measures for *Cl. perfringens*

Cl. perfringens has been linked to dirty transport containers and thus can potentially contaminate subsequent flocks (McCrea and Macklin 2006). This observation can be related to any surface areas or equipment that is used in relation to a composting operation. Following the use of pressure washing, with sodium hypochlorite spray and quaternary ammonium spray with 48-h drying showed that the greatest bacterial reduction of 2 to 3 log10 cfu/mL, was observed after 48 h of drying (McCrea and Macklin 2006).

2.11 *Listeria monocytogenes*

Listeria monocytogenes is widely found in nature and is carried by most species of both wild and domestic animals and is commonly linked with the soil environment (cultivated, uncultivated soils mud and moist soils). (Sutherland *et al.* 2003). *Listeria monocytogenes* was able to grow for a period of 2 days in fresh chicken manure at 20°C with a resulting 1-2 log units increase in CFU (Himathongkham and Riemann 1999). Prolongation of the storage time to 6 days resulted in *L. monocytogenes* not decreasing below the

initial level. These changes were accompanied by an increase in pH and an accumulation of ammonia in the manure (Himathongkham and Riemann 1999).

Listeria survived in stored animal slurries and dirty water for up to three months and less than one month in solid manure heaps where temperatures greater than 55°C were obtained. Following manure spreading to land, *Listeria* survived in sandy arable and clay loam grassland soils for more than one month (Nicholson *et al.* 2005a). In composted rural sewage sludge *Salmonella* decayed at a rate greater than *Listeria* (Pourcher *et al.* 2005). Experimental in-vessel biowaste composts used to study the survival of seeded *Listeria monocytogenes* resulted in the organism being present only in 4-week-old composts and never in older composts suggesting that proper composting may prevent long-term survival of *L. monocytogenes* (Lemunier *et al.* 2005). Faecal wastes (poultry manure and dirty water) inoculated with *L. monocytogenes* levels indicative of normal levels and then spread on to grass pasture resulted in the organism surviving for 42 and 128 days respectively (Hutchison *et al.* 2005c).

2.12 Biosecurity measures for *L. monocytogenes*

L. monocytogenes is a general environmental organism commonly found in moist and cool environments. The organism is commonly found in food processing environments such as floors, drains and wet areas in factories. (Sutherland *et al.* 2003). General measures adopted in maintaining the overall

hygiene for production environments and drains can be adopted for internal areas would not be easy for external environments.

2.13 Summary

- the major pathogens of concern based layer waste composting *Salmonella* and *Campylobacter*,
- *Campylobacter* has a poor survival potential
- the layer industry has been linked to *Salmonella* Typhimurium *S.* via the presence of this serovar in both eggs and layers and is one of the top ten serovar isolated in humans 2005.
- vectors and their role in transmitting pathogens impacts on the presence of a compost operation linked to a production facility and needs to be considered
- meat and milk from cattle linked with cattle botulism outbreaks has shown to be of minimal risk to human

Chapter 3

Composting, poultry carcasses and manure

3.1 Chicken carcass composting

Carcass composting is said to have begun in the poultry industry during the late 1980's with dead birds being found to be biodegradable in only 30 days (Murphy and Handwerker 1988, cited in Kalbasi *et al.* (2005)). Composting continues to have an impact as an environmentally sound and cost effective way to stabilize and reduce the volume of agricultural wastes with added benefits to most producers. Overall, composting has major benefits from an environmental perspective but not without continued concerns with respect to pathogen issues. Carcass composting is relatively less capital intensive than incineration and rendering, a better alternative to burial in areas with shallow water tables and also provides for quick removal and isolation of farm mortalities from the production system (Kalbasi *et al.* 2005). The pathogen issue has prompted regulatory agencies to establish rules and standards to prevent undesirable environmental impacts from improper carcass disposal in a manner that may contaminate air or water resources (Kalbasi *et al.* 2005).

The size and layout of the of the carcass composting facility are one of the key issues in terms of facilitating the complete destruction of pathogens (Kalbasi *et al.* 2006) and may vary from producer to producer. Improper animal mortality disposal can lead to a number of environmental hazards such

as odour, as well as the key issue of pathogen spread by air, water and soil - both to humans and animals (Gerba and Smith 2005).

Kalbasi *et al.* (2005) describe carcass composting as a temporary process burying dead animals above the ground in a mound, incorporating a carbon source and supporting heating by thermophilic organisms. This process kills most pathogens and digests carcass tissue under aerobic conditions. The carcass compost pile contains:

a) relatively large amounts of water, high nitrogen, low carbon content, and low porosity

b) is surrounded by a co composting material of good porosity, high carbon, low nitrogen, and low to moderate moisture.

This work also suggested that pathogen reduction could be enhanced by using a 50:50 mixture of composted carcasses as a carbon source for the new composting process.

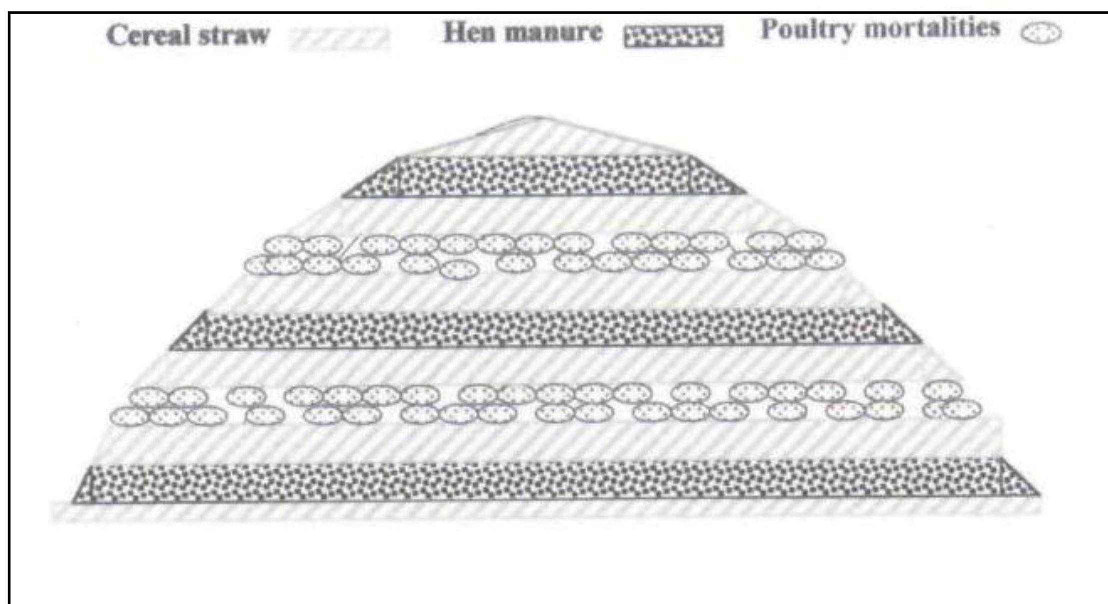
3.2 Chicken carcass composting processes

Chicken carcass composting as a stand alone process for carcasses has been dealt with by several studies (listed in this section). Experimental studies (Lawson and Keeling 1999; González and Sánchez 2005) have dealt with some factors associated with composting poultry carcasses. Composting poultry carcasses themselves pose certain risks simply in terms of the size of the carcass material within a composting environment. Large clumps of solids (e.g. a whole carcass or parts of one) reduce the efficiency of heat inactivation

in a composting pile because they take longer to heat than smaller particles (Wilkinson 2007).

Some basic issues on carcass composting were dealt with in a set of trials carried out by González *et al.* (2005). Static piles (3-4 m wide at base by 1.5 - 1.8 m high) using hen manure and poultry mortalities were created with the first, third and fifth layers consisting of three sub-layers alternative straw, hen manure and straw. The poultry mortalities were placed in the second and fourth layers. Finally, the pile was covered with a 10 cm thick straw layer. The pile was moistened using liquid effluent from liquid hen manure lagoon (Figure 3.1).

Figure 3.1 Cross section of static compost pile showing distribution of various feed stocks within the pile (González and Sánchez 2005)

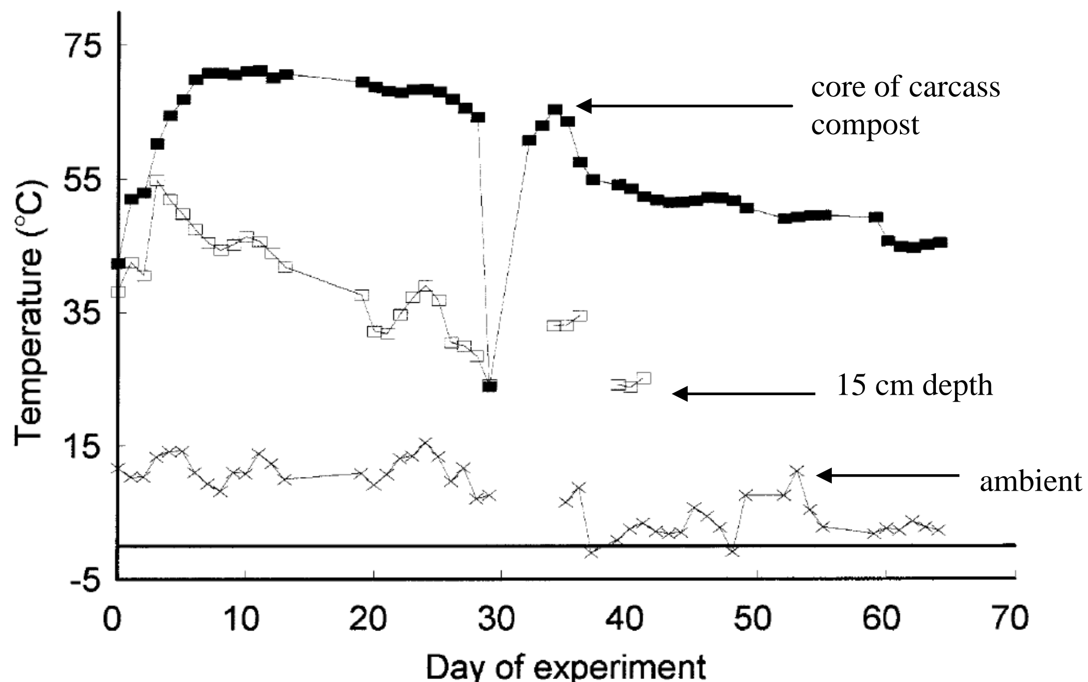


Farm waste such as cardboard and non-marketable eggs was included in the later trials. This system enabled the inclusion of daily poultry mortalities.

In the primary stage, high temperatures (53-66°C) within the curing pile were reached and the poultry mortalities were completely degraded while straw was only partially degraded. No turnings were made in this stage. The secondary stage used a standard dynamic pile system with turning and watering. The temperature reached was over 60°C for summer, but not winter trials. A marketable product with excellent appearance was obtained. However no microbiological studies in terms of the safety of this product were assessed.

Another study assessed the suitability of an experimental “mini composter” (1.5 m X 0.75 m X 0.75 m) constructed from wood and capable of composting daily poultry mortalities (Lawson and Keeling 1999). The bin was loaded with layer carcasses, turkey litter, wheat straw and water (ratio 1: 2 : 0.1 : 0.25 respectively) using a repeated layering system. After 16 days the composter was full. Figure 3.2 illustrates the temperature profiles with core temperatures of 60 - 70°C being reached. .

Figure 3.2 Recorded temperature in the poultry carcass "mini composter" (Lawson and Keeling 1999)



The hygiene measures adopted by Lawson and Keeling (1999) to manage pathogen transfer on site were as follows:

- preventing rodent access by wire mesh
- prevent contamination of the hard core beneath the bin by placing the bin on stock-board.
- fencing to keep off habitual raiders along with trapping of pests where appropriate and legal.
- controlling run-off from composting systems to prevent contamination of any water sources (eg dams on farm) or surrounding waterways or even adjacent on-farm compost piles.

- clear separation of composting facilities from the production system to prevent cross contamination to the production system.

Other carcass composting processes are briefly described as follows:

Georgia method (Ritz and Worley 2005)

Details are described in Appendix 1. Some key measures to manage contamination are:

- The composter set on a concrete slab with overhead roof to prevent leaching into the soil, burrowing vermin and pests from under the compost
- Proper management of the system by the operator
- Temperatures of 130 – 150°F for a period of 7-21 days – suggested as a means of killing pathogens (exceeding the human waste treatment requirements of the Environmental Protection Agency (i.e. 130°F for 15 days).

North Carolina method (Carter *et al.* 1996)

Details of this process are listed in Appendix 1. Factors to prevent run-off, such as the need for a roof and solid base to prevent run-off are listed.

Alabama method (Anonymous 2001)

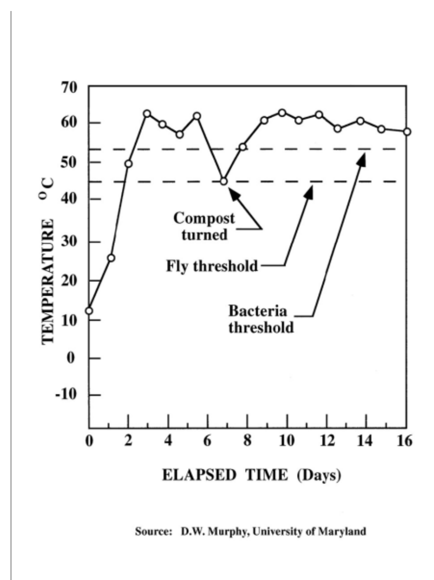
This is a three stage process where physical movement of the stages on completion of each stage is described. The owner will typically spend only 20

minutes per day in loading the bins (dead birds) for a 60,000-bird production facility. Careful attention is needed to adhere to the guidelines.

British Columbia Method (Hog Producers Sustainable Farming Group 1996)

Chicken litter is used along with the carcasses for composting. The carcasses should not touch each other to prevent localised wet spots and poor composting. In a properly operating compost operation, new material added to the bins reaches temperatures of 50 to 65 ºC within 24 to 48 hours. This is a two stage process where partially composted product is moved from one bin to another. In the second bin the temperature should reach the 55-65 ºC range in one or two days, and should peak in the 60-70 ºC range in 7 to 10 days Figure 3.3. Temperature is an important parameter in the control of fly larvae and pathogens.

Figure 3.3 Typical Temperature Profile in a Two Stage Composter (Hog Producers Sustainable Farming Group 1996)



Virginia Method (Collins 1996)

Research at the University of Maryland, and field application in other poultry states, has shown that normal mortalities can be handled efficiently and safely by composting the dead poultry (Collins 1996). From an environmental point of view Collins (1996) suggests an impervious floor to deal with contamination of the groundwater and other surrounding areas.

All the above processes lack details on specific pathogen information related to composting.

3.3 Emergency composting of carcasses

These processes are designed to deal with culling large numbers of birds following a disease outbreak. Catastrophic carcass composting has been previously discussed (DeRouchey *et al.* 2005; Wilkinson 2007). Wilkinson (2007) suggests that traditional composting methods can be used either inside or outside the poultry house for composting in windrows of large mortalities along with litter /saw dust. Additionally Shane (2006) recommends in house composting by either layering, shredding and piling or mixing and piling, with all these operations be carried out by personnel adhering to biosecurity driven protective measures due to the nature of such disease outbreaks (e.g. avian influenza virus).

3.4 Composting chicken waste other than carcasses

Composting is a common method adopted for the breakdown of agricultural/food waste (Ranalli *et al.* 2001; Cekmecelioglu *et al.* 2005a) human biosolids (Pourcher *et al.* 2005) and general wastes (Herrmann and Shann 1997; Déportes *et al.* 1998).

Such systems can either operate on a large or small scale basis and are cheap and effective to manage depending on the scale of production. Conventional composting systems contain raw materials such as green waste, biosolids and manure, mixed together with the aim of constructing a windrow of uniform physical-chemical properties (Wilkinson 2007). Composting of chicken waste and egg waste would be akin to composting manures along with food waste and the studies listed below aim to address some of the pathogen issues within such composting environments.

While normally composting of chicken manure is carried out with amendments, composting of un-amended chicken manure is possible, as long as the manure is not too moist (Elwell *et al.* 1998). Alternatively chicken manure can be a component along with agricultural (yard trimmings) and food waste (Elwell *et al.* 1996).

Composting poultry litter has shown to be a feasible process (Logsdon 1993; Brodie *et al.* 2000; Tiquia and Tam 2002) either using simple stacking or using an amendment. During deep stacking of broiler poultry litter *Salmonella* and *E. coli* (which were inoculated into the litter) were eliminated between the

2nd and 4th day (Kwak *et al.* 2005). The pathogen destruction was attributed to not just the heat generated but also ammonia as well as microbial competition (Kwak *et al.* 2005). When cage manure (1-3 day old layer manure) was composted with broiler or turkey litter (1 year old) (at a ratio of 4:1) in a trial that lasted 57 days, *E. coli* was eliminated and *Salmonella* was never detected even though the compost temperatures were generally low (< 33°C) (Haque and Vandepopuliere 1994). The use of poultry litter as an amendment to compost hatchery waste (eggs, shells and membranes) resulted in a better elimination *Salmonella* (presence absence test) when litter was used as when compared to when litter was not used. However both treatments resulted in 99.99% reduction of *E. coli* (Das *et al.* 2002).

3.5 Case studies of on-farm composting of chicken manure

Some large operations in the USA (listed below) compost chicken manure where state regulations require all concentrated animal feeding operations (CAFOs) to have an Agricultural Waste Management Plan (Kreher). The case studies listed below are supported by the New York State Energy Research and Development Authority (NYSERDA) and Cornell University.

Brey farm, is in Sullivan County, New York and has 200,000 laying hens that produce 60,000 pounds of manure daily. Due to the high cost of hauling, nuisance complaints, and Concentrated Animal Feeding Operations (CAFO) regulations, a composting operation was set up to manage chicken manure. Mortalities are also composted in a large, three-bin covered system for the last eight years. The advantages are claimed to be the production of an

energy saving, marketable commodity, chemical-free fly control, odor reduction and runoff containment (Bonhotal 2006).

ACE Farm, in Orange County California, has 130,000 laying hens producing 39 million eggs and 3000 yards of manure annually. Composting is carried out with 50% chicken manure and 50% leaves for a 10 – 12 week period and 3 month curing period (Etzel).

Kreher's Poultry Farms is a large egg farm in Erie County in New York which has over 500,000 hens and is designated as a CAFO operation. The farm uses a dry collection process and composts in windrows, to achieve a dry granular product which is a fertilizer substitute (Kreher).

3.6 Temperatures and pathogen reduction

In all composting processes, temperatures are deemed to play a key role in the management of pathogens (Kalbasi *et al.* 2006). During active carcass composting (the first phase), pathogenic bacteria are inactivated by high thermophilic temperatures. These bacteria may survive in the compost environment if high temperatures do not persist for a sufficient period of time (Kalbasi *et al.* 2006). The use of absorptive and reactive carbon sources supports proper moisture, pH, nutrient and temperature distribution that enhances microbial activity (Kalbasi *et al.* 2006). Conditions such as clumping of solids (which can isolate material from the temperature effects), non-uniform temperature distribution (which can allow pathogens to survive in colder regions), short-circuiting of the feed substrate and reinoculation after

the high temperature phase can reduce pathogen inactivation during the composting process (Haug 1993).

The focus on temperature and pathogen reduction is based on the type of data cited by Déportes *et al.* (1995)(see Table 3.1).

Table 3.1. Level of temperature and length of time necessary to destroy some pathogens present in primary products of composts – adapted from Déportes *et al.* (1995) Note that typical range of composting temperature is 55 -65^{0}C.

Organism	Lethal temperature and necessary time
Salmonella spp.	15-20 min at 60^{0}C, 1 h at 55^{0}C
Escherichia coli	15-20 min at 60^{0}C, 1 h at 55^{0}C
Entamoeba histolytica	68^{0}C, time not given
Taenia saginata	5 min at 71^{0}C
Necator americanus	50 min at 45^{0}C
Shigella spp.	1 h at 55^{0}C

The aeration rate seems to have a relationship with composting temperature. Lau *et al.* (1992) looked at the use of aeration during swine waste composting to help fulfil the regulatory requirements (imposed by USDA) for composting to be classified as a PFRP (Process to Further Reduce Pathogens). Essentially, the regulations require that the minimum operating temperatures must be maintained at 55°C or above for 3 days. Lau *et al.* (1992) found that maintaining suitable aeration rates (0.04-0-08 l/min kg VM) resulted in both surface and core temperatures being in excess of 55°C for the required three days.

However the composting processes discussed in this section deal more specifically of the composting process with limited outcomes on pathogen studies. There also appears to be a lack of peer reviewed scientific literature in this area.

3.7 Summary:

- chicken carcass and manure composting procedures have been documented particularly in the USA.
- carcass composting is often performed as a two stage process
- In Stage 1, a rapid process, temperature plays a key role in both supporting the thermophilic microbial activities (45-55°C) characteristic of composting as well as in aiding pathogen destruction

Chapter 4

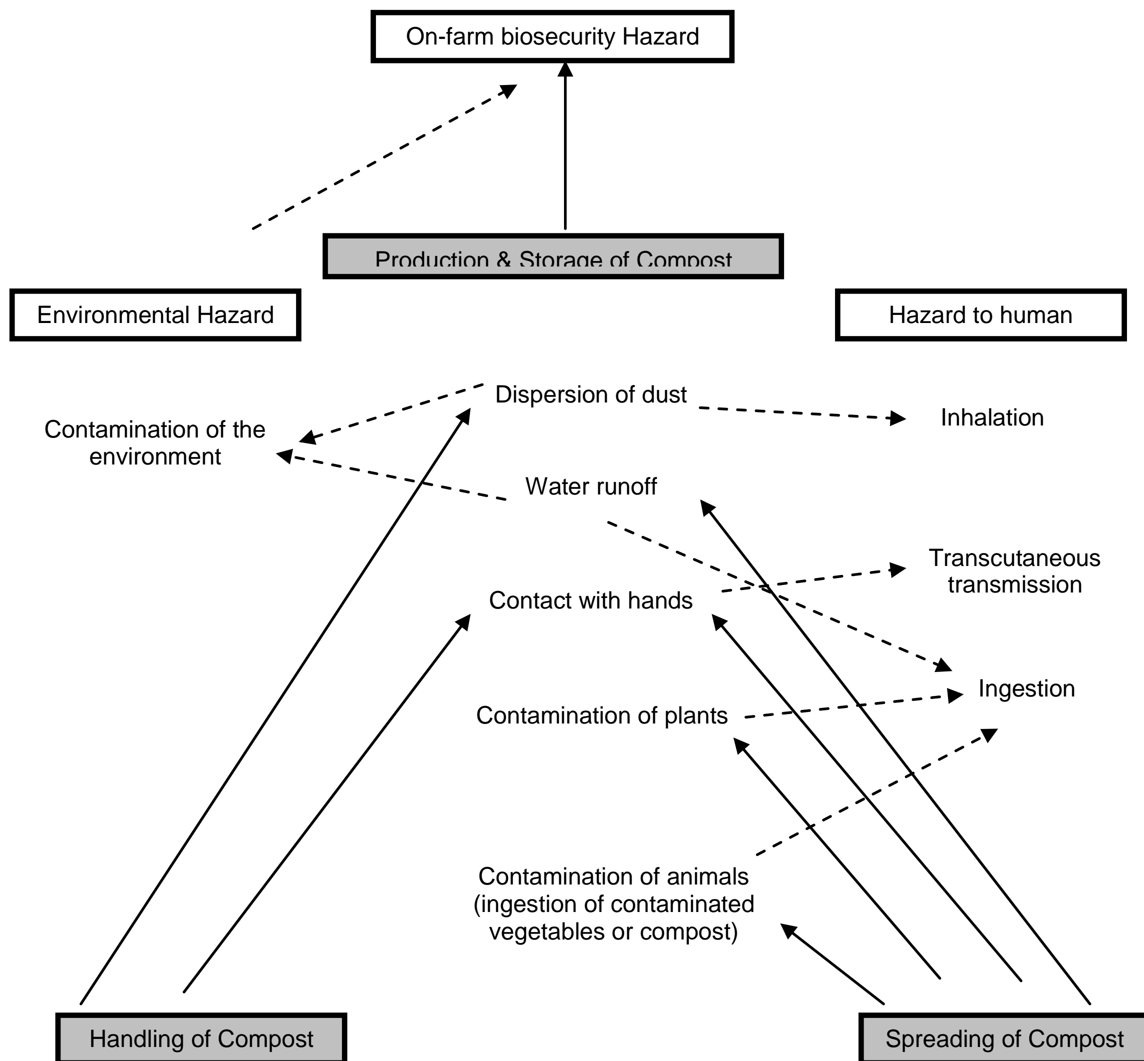
Pathogens, Pathways and an "Index Organism" of Compost Safety

4.1 The pathways

There are a number of possible pathways in which pathogens present in a compost can move into the environment (and ultimately into humans) and directly to humans. Figure 4.1, adopted from Déportes *et al.* .(1995) illustrates these possible pathways for pathogen transmission. Clearly, these pathways are applicable for layer waste composting.

However an additional pathway that needs to be considered is the pathway between the composting facility and the farm. In the context of manure management and contaminating water ways Goss and Richards (2007) state that manure handling and on-site waste (water) treatment systems form the most important hazards; both can comprise confined and unconfined source elements. Thus, there is a need to understand the modification of pathogen numbers through these pathways on their impact in the environment. There is a need to be aware of these risks where waste management, use of waste in the surrounding environment and the production all occur within the same environment.

Figure 4.1 Possible pathogen pathways – adapted from Déportes *et al.* (1995)



4.2 Prevalence of pathogens in source material

As identified previously, chicken wastes, both carcass and other farm waste (the key components of composts) are a potential source of zoonotic pathogens. While the composting processes described previously should result in a decline of these pathogens, there is a need to set this potential

decline in the context of the prevalence of pathogens entering the compost process. An understanding of the prevalence (and levels) of these pathogens would aid in understanding the levels of risk through the different composting stages. A further benefit would be an ability to implement safe operating procedures that would lead to minimal pathogen transfer to the environment and the food chain.

Barn (spent hens and breeders, 10 – 26 months)

The prevalence of both *Salmonella* and *Campylobacter* seems to be high in both layer and breeder flocks. In Germany, *Campylobacter* colonized all tested laying hen and breeder flocks (100%), whereas 65% of these flocks were positive for *Salmonella* (Rasschaert *et al.* 2007). These colonisation levels were much higher than that of the broiler flocks also tested in this survey (Rasschaert *et al.* 2007).

Free range production

As thee are similarities between free range broiler production and free range egg production in terms of exposure to environmental sources of food-borne pathogens, it is worth reviewing the data on these free range chickens. A survey of *Campylobacter*, *Salmonella*, *Listeria* and Shiga toxin-producing *E. coli* involving 60 flocks from 34 free-range farms in the Basque Country (Northern Spain) resulted in *Campylobacter* being the most prevalent (70.6%) of the four pathogens, isolated followed by *L. monocytogenes* (26.5%), and *Salmonella* (2.9%) (Esteban *et al.* 2008). No *E. coli* 0157 or other STEC's were isolated. The authors suggest under the conditions of free-range rearing

might reduce *Salmonella* levels but not those of *Campylobacter* or *L. monocytogenes* (Esteban *et al.* 2008). A study (Rivoal *et al.* 2005) assessing sanitary barriers for free range broilers showed that the chickens were *Campylobacter* free until they had access to the open area when they were rapidly colonised from *Campylobacter* strains isolated from the soil. Free-range and/or organic meat chickens in the USA had carcass positive rates for *Salmonella* that varied from 25% to 60% (Bailey and Cosby 2005). It is worth noting that studies on free-range layers have shown that, occasionally, birds will have *Campylobacter* in the liver (Crawshaw and Young 2003).

Cage production

A review by Shane (1992) discuss the relationship between cage hens and the prevalence of *Campylobacter.* Cage-housed hens maintained for table-egg production are frequently infected with *C. jejuni* with flock prevalence rates ranging from 13 to 62%, based on cloacal swab assays However, the eggs from cage-housed table-egg flocks known to carry *C. jejuni* were uninfected. As far as excretion of the organism is concerned infected chicks have the ability to excrete *C. jejuni* for up to 63 days when housed under conditions which inhibit coprophagy (Shane 1992).

Eggs

Campylobacter jejuni was not isolated from any of 276 eggs sampled from 23 egg farms in New York State (Baker *et al.* 1987). Even eggs contaminated with a high faecal load were free of *Campylobacter* suggesting a die-off of the organism on egg surfaces (Baker *et al.* 1987) Thus whole eggs are not a

dominant source. It is possible that damaged/cracked eggs may contain *Campylobacter*. *Campylobacter* has been shown to have limited ability to penetrate the egg shell as *Campylobacter* was not recovered from any of 500 fresh eggs obtained from commercial broiler-breeder flocks that were actively shedding *Campylobacter* in faeces (Sahin *et al.* 2003).

<u>A mixture of systems</u>

A study (Methner *et al.* 2006) assessing the influence of the housing system on *Salmonella* prevalence in layers found an overall prevalence of 32.2% irrespective of housing system with the breakdown for *Salmonella* prevalence being as follows:

- 46.3% in conventional cage systems
- 33% in organic farming with free range management systems
- 21.9% in floor management systems with free range
- 23.4% in floor management systems without free range

A baseline study (Namata *et al.* 2007) assessing the prevalence of *Salmonella* in the Belgian layer flock found that the main risk factor was The main risk factor

t was rearing flocks in cages compared to barns and free-range systems with *Salmonella* being isolated from dust and faeces from all systems.

Studies were carried out on the composting of chicken dung in southeast France where the reductions in levels of *Clostridium perfringens* during composting was 80% (Omeira *et al.* 2006). On comparing the microbiological properties of litter generated from layer and broiler chickens reared under

intensive and free-range production systems *Cl. perfringens* counts were lower in chicken litter from intensively raised broilers and layers whereas the highest level was recorded in free range layers (4.40 log cfu/g). The higher *Cl. perfringens* counts obtained in free-range systems was suggested to be due to the birds picking habit in backyard soil where the organism is common. During picking the chickens can ingest the spores that can survive and multiply in the enteric system, before being shed in the manure (Omeira *et al.* 2006).

4.3 Co-composting raw material

In addition to the key composting components, spent hens, culled birds, broken eggs and manure, pathogens can enter the process via the type and quality of raw co-composting materials used in the composting process as a carbon source. As the C:N ratio for composting can range from 10:1 to 50:1, with 25:1 being the most common ratio (Glanville and Trampel 1997), there is a need for considerable co-composting material to be added. The common carbon sources used for composting are sawdust, straw, rice hulls, green waste and cotton waste and other such resources that are economically viable as a carbon source within Australia.

It is possible that the carbon source also could be a source of pathogens if the material has been contaminated at any stage from external sources such as birds or other mammals. Table 4.1 shows the levels of coliforms present in clean bedding material used for cattle – material that are also associated with

a composting process. The presence of coliforms suggests the possibility of the presence of pathogens.

Table 4.1 Levels of coliforms in clean bedding material – adapted from Rendos *et al.* (1975)

Type of bedding	Total coliform count
Sawdust	5.2×10^7
Shavings	6.6×10^6
Straw	3.1×10^6

A litter survey involving 3 Australian states (Chinivasagam *et al.* 2006) assessing the levels and prevalence of *Salmonella* and *Campylobacter* in broiler litter just after final pick-up resulted in 60% of the litter being positive for *Salmonella* and 36% of the litters being positive for *Campylobacter*. Thus though *Campylobacter* can be dominant at the stage of pick-up the organisms poor survival potential may have resulted in the lower prevalence after final bird pick-up.

4.4 Nematodes as vectors for the transfer of pathogens

As previously discussed pathways within a production environment can be responsible for the continued presence and transfer of pathogens. Where improperly treated manures or composts, harbouring pathogens such as *Salmonella* and *E. coli,* are used for soil enhancement, nematodes have been suggested as possible vectors to transport these organisms within the pockets of soil to contaminate food crops (Caldwell *et al.* 2003; Kenney *et al.*

2005; Anderson *et al.* 2006a; Kenney *et al.* 2006). Several typical studies are described below.

Kenny *et al.* (2005) found that populations of *C. elegans* persisted in compost amended soil for at least 7 days but declined in unamended soil. Under laboratory conditions, *C. elegans* shed around 100 organisms of *E. coli* O157:H7, *Salmonella* serovars Poona and *L. monocytogenes* per worm. When *Salmonella* serovar Newport was fed to *C. elegans*, viable *Salmonella* organisms were isolated in nematodes two generations removed from exposure to the pathogen (Kenney *et al.* 2005).

C. elegans has been shown, in laboratory studies to be attracted to colonies of a range of pathogens (seven strains of *Escherichia coli* O157:H7, eight serotypes of *Salmonella*, six strains of *L. monocytogenes*), surviving up to 7 days (Caldwell *et al.* 2003).

A study on *Diploscapter* sp. strain LKC25 indicated that the nematode can shed pathogenic bacteria after exposure to pathogens (Gibbs *et al.* 2005). This study confirmed the potential of this nematode to serve as a vector of food-borne pathogenic bacteria in soil, with or without amendment with compost, to the surface of pre harvest fruits and vegetables in contact with the soil.

4.5 An "index" organism for compost safety

E. coli is termed an indicator organism – to indicate the possible presence pathogens such as *Salmonella*. However this traditional approach has been questioned due to conflicting data with respect to the traditional indicator organism and its relationship to various pathogens. The fact is enumeration of faecal coliforms or *E. coli* is relatively easer to perform than for example, *Salmonella*. (Gerba *et al.* 1979).

A recent study (Anderson *et al.* 2006b) on *E. coli* from humans, cattle and horses found diverse complex unstable populations, with the diversity varying according to the host. The unstable and diverse nature of *E. coli* populations observed has practical implications with the use of the organism as a "water quality indicator". They also state that this issue is further complicated by the fact that the survival rates of the different strains of *E. coli* in environmental waters vary, with some subtypes persisting longer than others (Anderson *et al.* 2006b). It is unknown if varying *E. coli* subtypes are present within composts and, if so, the impacts of such phenomena on:

a) the levels of destruction of these organisms at thermophilic temperatures

b) their persistence at the final stages of composting and their ability to indicate compost safety.

In evaluating strategies for sanitary qualities of compost Christensen *et al.* (2002) observed that the species composition of the faecal coliforms varied depending on the stage of the material. The raw material contained *E. coli* of

faecal origin, while the finished compost contained a different faecal coliform that were of non-faecal origin such as *Klebsiella*. Thus, Christensen *et al.* (2002) questioned the use of faecal coliforms as an “indicator organism” because the species composition varied based on the composting stage.

An overview of the literature confirms that *Salmonella* is commonly isolated from composts and biosolids, is well characterised and could thus be used as “an index organism” as an alternative to *E. coli*. Indeed, *Salmonella* has been included in various standards of guidelines commonly dealing with sanitation areas, such as wastes and waters.

As an example, an USEPA guidelines offer two microbiological for determining Class A biosolids (United States Environmental Protection Agency 2003). Specifically these guidelines are <1000 faecal coliforms MPN (Most Probable Number) of dry solids or a *Salmonella* level of <3 MPN/4 g of dry solids. Thus operators now have the option of meeting either guideline before releasing composted sludge for soil conditioners or additives (United States Environmental Protection Agency 2003).

An Australian study on pig effluents (Chinivasagam *et al.* 2004) did not observe a relationship between the presence of *Salmonella* and the presence the common indicator organism’s thermotolerant coliforms (faecal coliforms) / *E. coli*. Similarly an Australian study (Chinivasagam *et al.* 2006) on broiler litter did not observe a relationship between the presence of *E. coli* and the presence of *Salmonella*.

Clostridium perfringens, an organism that does not show re-growth potential and is a spore former and thus more resistant than other organisms, has be suggested as a better indicator of the composting process (Desmarais *et al.* 2002). Despite this recommendation *Cl. perfringens* has not been widely accepted unlike *Salmonella* see above.

The selection of an “optimum indicator organism” in terms of composted or co -composts materials of animal origin needs further research.

4.6 Summary

- A range of pathways for the movement of pathogens to the food chain and to humans are possible
- All layer production systems have been associated with the major food borne pathogens and thus the possibility of these pathogens being in the source material must be considered
- The major food-borne pathogen in Australian layers that poses a risk in terms of layer waste products is *Salmonella* – chiefly due to the ability of this organism to persist in the environment
- Since composting occurs within the layer operation, management procedures must address the risk of pathogen transfer to the production system
- *Salmonella* could act as an “index organism“ in composts and be included in guidelines

Chapter 5

Survival of Pathogens

In this Chapter, the survival of pathogens in both animal wastes (a source material for composts and an area dominant in pathogen survival data) and composts is reviewed.

5.1 Animal wastes and pathogen survival

The major influence in composting of animal wastes is the pathogen levels and their survival in waste itself. Pathogens can be normal inhabitants of animals and are thus a key component of their waste, waste treatment and related activities surrounding animal production. A range of studies in UK on "pathogens and wastes" have focused on the development of management strategies for waste (Hutchison *et al.* 2004; Hutchison *et al.* 2005a; Hutchison *et al.* 2005b; Hutchison *et al.* 2005c; Nicholson *et al.* 2005b). These studies deal with the survival of *Campylobacter*, *Salmonella*, *E. coli* O157 and *Listeria* in animal wastes. In Chapter 2, we have already identified *Salmonella* as the only major food-borne pathogen of risk in layer composting. Hence, for the purpose of this review only the *Salmonella* aspects of these papers will be discussed with respect to management.

Some issues from these studies are as follows:

- “incorporation to soil” – increased aging of manure before soil incorporation reduces pathogen levels. This effect is moderated by seasonality
- “persistence” in relation to the rate of decline of zoonotic agents - E.g. waste spread onto a grass pasture yields pathogens detectable up to 64 days later
- “pathogen survival and nature of waste” - e.g. the rates of pathogen decline in liquid (slurry) and solid (farmyard manure) wastes show little difference
- “best practice options” - e.g. a number of the correlations identified to be used as the basis of a best-practice disposal document for farmers, thereby lowering the microbiological risks associated with applying manures of contaminated livestock to land
- “cost effective treatment options” - e.g. Studies on bedding heaps demonstrate a simple and cheap treatment to be used to help prevent the spread of zoonotic agents through agricultural environments.

Thus the collective outcomes of the above studies indicate that pathogen survival in the environment is dependent on various factors. An understanding of the key issues in the management of waste treatment processes, re-use of animal manures and implications surrounding environmental re-use is vital in developing sound workable management options. In fact there is considerable concern surrounding the transmission of

pathogens via waste through to the human food chain. However, such information is not widely available for composted or co composted bird wastes or carcasses, especially in peer reviewed literature.

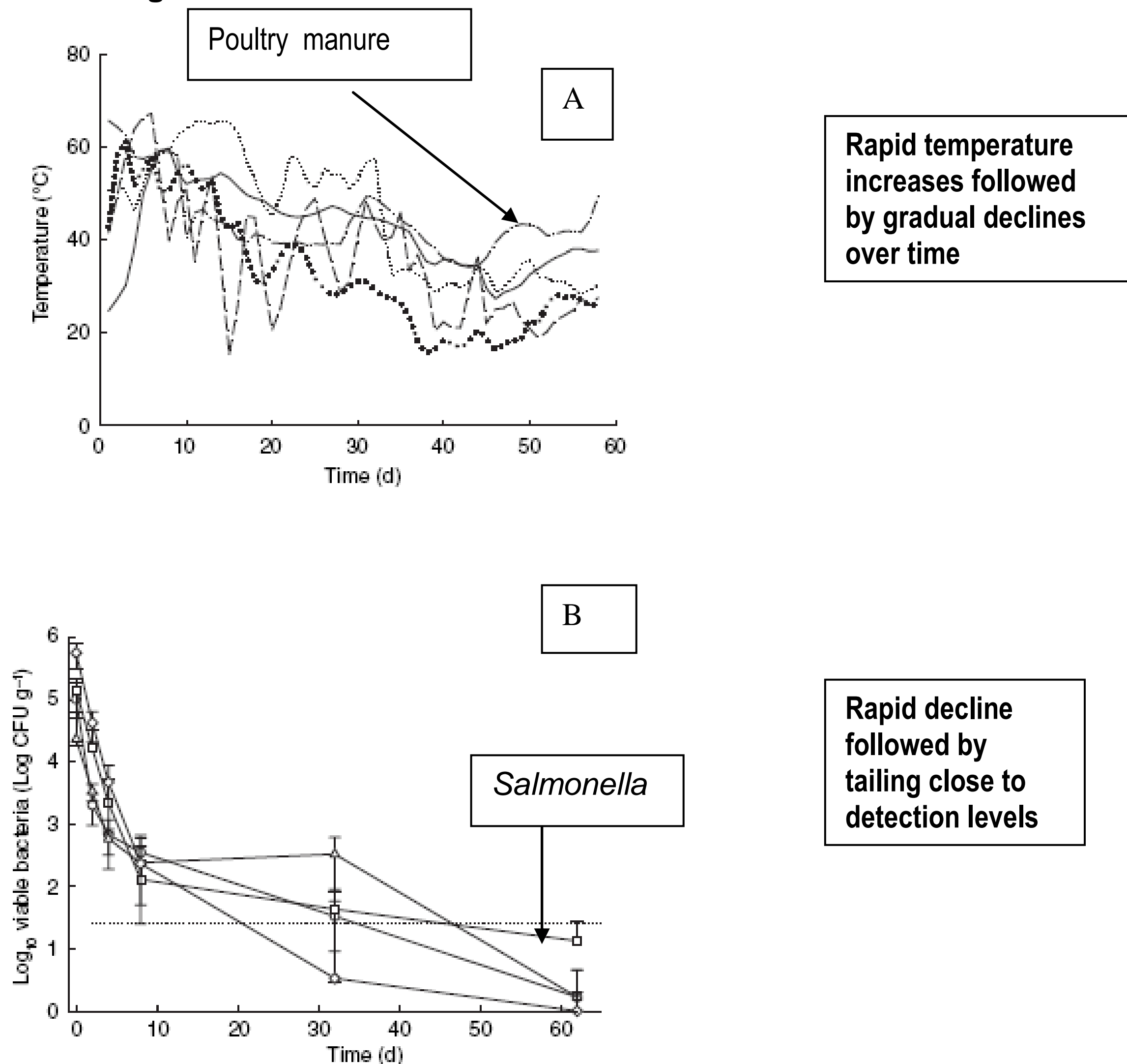
In a two year study, Hutchison *et al.* (2005a) monitored the survival of key pathogens in manures over time. They used freshly collected solid farm yard manures (<72 h after deposition) from dairy cattle, beef cattle, laying chickens and grower pigs and inoculated them with strains of *Salmonella*, *E. coli* (O157), *Listeria* and *Campylobacter.* Temperature profiles of the manures were also monitored.

The survival pattern of the organisms is illustrated in Figure 5.1. In general, there was an initial decline that was "linear and rapid". This linear decline extended to 8 days for most pathogens. However, *Salmonella* showed effects of "tailing" after 4 days. More specifically *Salmonella* levels in chicken manure show a decrease up to 50 days after which it seems to increase. The following could be inferred from this data:

- an initial rapid decline occurs in levels of these organisms
- "tailing" was significant with low levels of bacteria surviving for extended periods of time
- such phenomena could mean that low levels of organisms could persist among other normal manure organisms
- complex microbial interactions within a specific environment could support the potential re-growth of pathogens
- "tailing" an issue that needs to be considered in management.

This type of information can form a basis for understanding pathogen survival, not just in animal wastes but also in composted chicken materials – an area not as well studied as with animal manures. This is important as composted carcass/waste material is destined to have an impact on the food chain via soil, water (run-off) or air (aerosols). Such information can also support the development of guidelines backed by scientific outcomes.

Figure 5.1 Adapted from Hutchison *et al.* (2005a)
A - Temperature profiles of various manure heaps over time.
B - Pathogen decline in cattle manure



5.2 Composts and pathogen survival

As previously described composting of biological material is an efficient biological process driven by beneficial micro organisms in a nutrient rich system. This nutrient rich environment in many ways is conducive to support the survival of pathogens, organisms originating from a completely different environment – the animal.

A study involving the composting of rural sewage sludge in a mixture with straw over 7 months was carried out by Pourcher *et al.* (2005). The following outcomes were noted:

- the temperature at the bottom of the pile did not exceed 50°C while 66°C was achieved elsewhere within the pile
- regular turning was carried out (0, 27, 48, 76, and 111 and 202 days)
- the levels of *E. coli* ranged from 10^6 cfu/g at the beginning to 10^2 cfu/g at the end (7th month)
- *Clostridium perfringens* levels ranged from 10^4 cfu/g at the beginning to 10^2 at the end
- *Salmonella* levels ranged from a maximum of 9.6 MPN/g at the start to non detectable levels after 1 month and remaining thus to the end
- *E. coli* levels ranged from 10^4 to 10^2 during the period *Salmonella* was absent in the compost. This questions the role of *E. coli* as an indicator for the presence of *Salmonella.*
- *L. monocytogenes* was present after 4 months of composting suggesting that *L. monocytogenes* has a greater survival potential during composting than *Salmonella*

5.3 Carcass composting and pathogens

A poultry facility would yield about 0.1% daily mortalities and disposing these mortalities has been identified as an environmental issue (Conner *et al.* 1991). There have been few published papers on pathogen reduction studies however the available information is discussed.

Laying hen carcases (125) were composted in a wooden compost bin over autumn and winter months. The process took 8 weeks and effectively decomposed the carcases to leave only leg and breast bones. The compost was turned once, which ensured that all the material reached the high temperatures (60° to 70°C) required to control pathogens. *Salmonella* was fully heat-inactivated, indicating that many poultry-associated bacterial pathogens would also have been inactivated (Lawson and Keeling 1999).

A two stage mortality composter was used to estimate pathogen survival with and without the use of bulking material (Conner *et al.* 1991). The study involved either directly spiking the carcass or by adding vials of pathogens to the different sections of the pile to determine pathogen die-off in the vials. Inactivation of coliforms did not occur until the product was transferred to the secondary bin. The primary bin did reach and stabilise at temperatures in excess of 50°C. However the coliforms were inactivated in the secondary bin that reached temperatures in excess of 60°C.

In the second segment of the study *Salmonella* Typhimurium was inoculated into the carcasses at 10^6 cfu per carcass and placed in the compost bin (Conner *et al.* 1991). *Salmonella* was inactivated at temperatures of 65°C, 52°C, and 50°C, with these temperatures being influenced by the levels of bulking material (wheat straw). However *Salmonella* survived in only 2 of the 63 vials over a period of 33 days. This study concluded that aeration via transfer to the second bin was required to achieve sufficient temperature to initiate pathogen inactivation in the carcasses (Conner *et al.* 1991).

Several studies (Blake 2004; Bendfeldt *et al.* 2006; Flory *et al.* 2006) have examined the composting of poultry carcasses derived from avian influenza outbreaks. A pile temperature of above 54°C which is 14 – 21 days through two composting cycles is regarded as sufficient to kill the virus (Bendfeldt *et al.* 2006).

5.4 Composting of wastes and pathogens

Pathogen survival during composting can depend on a number of factors and thus their survival within a composting ecosystem is a complex interaction. Compost parameters such as moisture and temperature play a complex role not only in pathogen destruction but the composting process itself. The composting process is not generally supportive of the survival of pathogens associated with intensive animal operations such as poultry farms.

Seasonality seems to have an impact on the high thermophilic temperatures (≥55 ° C) and their duration of the windrows. Composting of food waste, manure and bulking agents across seasons was evaluated in terms of maximum *Salmonella* inactivation across 90-150 days of composting (Cekmecelioglu *et al.* 2004). Temperatures ≥55°C lasted 68 to 87 days in summer and 25 to 55 days in winter. Pathogen inactivation was not consistent in winter. During composting the thermophilic temperature ranges (>_55°C) lasted 68 to 87 days in summer and 25 to 55 days in winter. Re-growth of *Salmonella* was observed, often to high levels, over several time intervals during winter composting (453.3 and 5.5 MPN/g dry compost) (Cekmecelioglu *et al.* 2004). The optimum formulation for composting was 43.3% food waste, 28.3% manure and 28.3% bulking agents with an optimum C/N ratio and moisture content of 25 4/1 and 61 8%, respectively.

Moisture content seems to have an impact on pathogens destruction during windrow composting. Moisture content also seems to interact with temperature. Gong *et al.* (2005) detected *E. coli* and *Salmonella* during composting at 54°C to 67°C, with results indicating that *E. coli* in compost of high moisture content was more heat sensitive than *E. coli* in compost of low moisture content. Turner (2002) using a laboratory strain of *E. coli* (used as marker for pathogens) observed that *E. coli* inactivation at 50°C in compost depended on the moisture content and the nature of material with *E. coli* sometimes viable after 72 h. However, when the temperature was increased to 55°C, rapid inactivation of *E. coli* occurred with levels below detection limit in 2 h. These results indicate that the inactivation is not merely temperature

dependent, but is also affected by the moisture content and the nature of the material. An 80% reduction of *Cl. perfringens* was observed during the composting of chicken dung (waste) where a moisture level of 40% was suggested as being a key to the composting process (Guillouais and Couronne 2003).

The survival of pathogens during composting may be dependent on the strains of organisms present. Co composting of municipal soild waste compost with poultry litter resulted in the detection of *Salmonella* and *E. coli* after 47 days of composting suggests that both pathogens appear to be capable of surviving in temperatures in excess of 60° C. The surviving *Salmonella* and *E. coli* may be strains selected to adapt to thermophilic growth (Cooperband and Middleton 1996).

Compost amendments may have an impact on pathogen survival. Hatchery wastes (containing non fertile eggs, dead chicks, and broken eggshells**)** composted in a bin system with poultry litter was more efficient in reducing *Salmonella* compared to waste simply amended with saw dust and yard trimmings. However both type of amendments were capable of 99.99% reduction of *E. coli* (Das *et al.* 2002).

Temperature alone may not be the key to pathogen destruction during aerobic thermophilic composting. *E. coli* and *Salmonella* have been detected during composting at 54 – 67° C (Gong *et al.* 2005). Studies on pathogen survival in industrial compost (i.e. biosolids from waste water treatment plant and

biowastes, food scraps and yard waste) resulted in *Salmonella* and *E. coli* still being present at 60° C, but non detectable during the curing process where temperatures decreased from 62^oC-40° C (Droffner and Brinton 1995). This information suggests that the temperature or the time of high temperature is not an easy parameter to correlate to the destruction of *Salmonella* or *E. coli* which is not simply temperature dependent but a more complex process (Droffner and Brinton 1995) .

Water activity, a measure closely related to microbial growth, can have an impact on pathogen reduction. A model generated from data gathered from composting stacked poultry litter which was not turned indicated that the parameter with the most influence on temperature was water activity. Piles with a higher water activity were 20^oC hotter than piles with lower water activity allowing the producer to adjust water activity to ensure better heating. Inoculation studies resulted in a maximum survival of 2 h, 32 h and 28 h respectively for *Campylobacter, E. coli* and *Salmonella* (Jeffrey 2001)

Pathogens may survive in the surrounding production environment which may have an impact on composting in terms of possible cross contamination. The persistence of *Salmonella* (in this case *S.* Enteritidis PT4) was studied on a free-range breeding chicken farm which had been depopulated. The organism persisted not only in dry faeces, litter and feed but also in soil (for 8 months) (Davies and Breslin 2003b).

Re-growth of pathogens (this issue will be dealt with in detail later) may be linked with temperature. In a study on the composting of municipal solid waste *Salmonella* was absent after composting for 27 days (temperature maintained between 54 and 66°C but re-appeared after 57 days in an uncovered heap when temperatures declined to 28.3°C. This could be result of re-growth in the absence of other competing organisms (Déportes *et al.* 1998).

Mathematical models have played a role in planning of windrow composting processes. In developing a mathematical model to understand composting of spent broiler litter Sobratee *et al.* (2008) recommended faecal enterococci as the better indicator to document the efficacy of the sanitization phase during process control while *E. coli* was more appropriate in the documentation of sanitary quality of finished composts. These workers make a point on understanding process control. They inicate that the attainment of inhibitive temperatures (>55°C) may lead to a debilitation of the microbial community structure that can eventually result in decomposition suppression and inhibited pathogen removal (Sobratee *et al.* 2008). Simulation models have indicated that moisture contents of 40% to 60% (summer) and 40% to <60% (winter) were appropriate to inactivate *Salmonella* and *E. coli* 0157.H7 (Cekmecelioglu *et al.* 2005c). Further outcomes of this study were that higher moisture contents did not inactivate the pathogens during winter and took a month longer to eliminate them in summer (Cekmecelioglu *et al.* 2005c).

These studies give an insight in to understanding parameters surrounding pathogen die-off

Finally the failure to detect pathogens does not mean that the absence of pathogens but could mean levels being below the detection limit or some pathogens surviving in an unculturable form due to the stresses undergone within the composting environment. Such factors need be taken into account when testing the end product for pathogen presence (Cooperband and Middleton 1996).

5.5 Summary

- comprehensive studies on animal wastes can provide a basis for understanding pathogen survival and management in composts. To date, such studies are not common
- a range of factors play an integrated role in compost. However temperature, including a uniform distribution of temperature, has been suggested to play a role in the reduction of pathogen levels in compost
- Chicken carcass composting studies, including limited data on pathogen survival, have been published

Chapter 6

Survival and Re-growth of *Salmonella*

Salmonella spp. are a major food-borne pathogen and the focus of many studies into the efficacy of various composting processes. In this Chapter, studies on the survival and the potential for the re-growth of this key pathogen are covered.

The importance of *Salmonella* is shown by the fact that guidelines for biosolids often specifically mention the organism. As an example, the, USEPA guidelines the application of for Class A sewage sludge composted biosolids to land, requires the levels for *Salmonella* should be <3 MPN/4 g total solids – dry weight (USEPA 1993).

6.1 *Salmonella* and composts

There have been extensive studies on the survival of *Salmonella* in various composts. Table 6.1 shows some studies that have examined the relationship between *Salmonella* survival and various compost parameters. A key issue is that complex interactions dictate the survival of this organism.

Table 6.1 Survival times, moisture content and time temperature relationships of different *Salmonella* strains during different composting processes Adapted from Ceustermans *et al.* (2007)

Salmonella species	Concentration at the start	Type of composting	Type of organic waste	Moisture Content (%)	Temperature (^{0}C)	Time – temperature	Survival time	Reference#
Salm. Enteritidis	10^7 CFU g^{-1} raw compost feed	Bench-scale composting system	Cow manure and sawdust	59.6 ± 5.7	45	n.a.	>24 h, <48 h	1
Salm. Typhimurium	10^5-10^6 CFU g^{-1} waste	Reactor composting	Municipal waste and sludge	60	±55 ±65	n.a.	>4 days, <5 days <2 days	2
Salm. Newport	2.4 X 10^6 CFU g^{-1} compost	Aerobic composter	Sewage sludge	n.m.	60-70	n.a.	>19 h, <25 h	3
Salm. Typhimurium	2.5 X 10^4 CFU g^{-1} raw material	Pilot plant (field test)	Refuse and sewage sludge	45	20-55	±39 days >40^{0}C ±31 days >50^{0}C	<50 days	4
Salm. Cairo	1.3 X 10^5 CFU g^{-1} raw material				25-75	±45 days >40^{0}C ±41 days >50^{0}C		
Salm. Infantis	1.4 X 10^5 CFU g^{-1} raw material					±32 days >60^{0}C ±22 days >70^{0}C		
Salm. Typhi	5 X 10^4 CFU g^{-1} raw material				40-65	±50 days >40^{0}C ±47 days >50^{0}C ±40 days >60^{0}C		
Salm. Cairo	Same as in field test	Ventilated incubator	Sewage sludge	50	50	n.a.	>6 days, <7 days	4
Salm. Paratyphi B	2.3 X 10^6 CFU ml^{-1}	Ventilated incubator	Sewage sludge	50	50	n.a.	<2 days	4
Salm. choleraesuis var Kunzendorf	2 X 10^{10} CFU ml^{-1}	Compost piles in an open front	Pig carcasses, spelt hulls and straw	60	27-62	n.m.	>3 days, <7 days	5)
Salm. enterica subsp. *enterica* W775	10^8 CFU g^{-1}	Industrial composting in enclosed facility	Biowaste	60-65	60 20-55 20-45	n.a. ±6 days >50^{0}C ±13 days >40^{0}C	<10 h >1 days, <7 days >7 days, <14 days	6
Salm. Enteritidis *Salm.* Hadar *Salm.* Braenedrup *Salm.* Corvallis *Salm.* Menchen *Salm.* Newport *Salm.* Virchow	n.m.	Semi-industrial pilot plant	Municipal solid waste	55-65	37-55	±20 days >40^{0}C ±12 days >50^{0}C	<25 days	7
Salm. Typhimurium Q	10^7 cells g^{-1}	Water bath	Waste water sludge and sawdust	Low, medium, high	40-55	2 days at 40^{0}C 5 days at 55^{0}C 13 days at 40^{0}C	>5 days, <9 days	8
Salm. Senftenberg W775	10^8 – 10^9 CFU ml^{-1}	Compost heap with turning	Sewage sludge, spelt hulls and straw	65-72	43-49.7	n.a.	>18 days, <28 days	9
Salm. Senftenberg W775	10^8 – 10^9 CFU ml^{-1}	Compost heap with perforated pipe system	Sewage sludge, spelt hulls and straw	70-75	19.5-33.9	n.a.	>78.4 days, <158.9 days	9

Salmonella species	Concentration at the start	Type of composting	Type of organic waste	Moisture Content (%)	Temperature (^{0}C)	Time – temperature	Survival time	Reference#
Salm. Typhimurium Q	n.m.	Industrial composting facility	Municipal waste	n.m.	59-62	n.a.	>44 days, <56 days	8
Salm. Typhimurium Q	10^7 cells g^{-1}	In-vessel	Waste water sludge and sawdust	50	22-70	$\pm$18 days >40^{0}C $\pm$14 days >50^{0}C $\pm$9 days >60^{0}C	>11 days, <20 days	8
Salm. Typhimurium Q	10^7 cells ml^{-1}	Composting container	Food wastes and leaves	55	22-70	$\pm$8 days >50^{0}C $\pm$7 days >60^{0}C	>7 days, <11 days	8
Salmonella spp.	n.m.	Windrow composting	Spent pig litter	60	38-67	$\pm$20 days >40^{0}C $\pm$19 days >50^{0}C $\pm$18 days >60^{0}C	>14 days, <21 days	10
Salmonella spp.	$\geq$561.4 MPN g$^-$1 dry compost	Forced aerated in-vessel system	Food waste, mulch hay, manure and wood shavings	71.5	14-56.6	$\pm$4 days >40^{0}C $\pm$2.5 days >50^{0}C	>12 days	11
Salmonella spp.	n.m.	Windrow composting	Municipal solid watse	39.2-59.6 36.3-47	31.5-62.6 16.7-66	$\pm$8 days >50^{0}C $\pm$16 days >50^{0}C	>8 days, <12 days >13 days, <21 days	12

references are as follows

1 Lung *et al.* (2001)
2 Krogstad and Gudding (1975)
3 Wiley and Westerberg (1969)
4 Knoll (1961)
5 Garcia-Siera *et al.* (2001)
6 Ceustermans *et al.* (2007)
7 Hassen *et al.* (2001)
8 Droffner and Brinton (1995)
9 Paluszak *et al.* (2003)
10 Tiquia *et al.* (1998)
11 Cekmecelioglu *et al.* (1998; 2005b)
12 Déportes *et al.* (1998)

Tiquia *et al.* (1998) studied the effect of windrow composting on the survival of *Salmonella* from spent pig litter (mixture of partially decomposed pig manure and sawdust). Temperature was the main factor in terms of survival, with 64 – 67°C for 2-3 weeks killing the *Salmonella*. This elimination corresponded with the progressive decrease in faecal coliforms, by the end of the composting process (day 91) faecal coliforms were reduced to around 100 organisms / g of compost.

Lemunier *et al.* (2005) experimented with in-vessel laboratory reactors with forced aeration for 12 weeks at 25°C. They used biowaste (organic waste mixtures) for composting. The mixtures were seeded with *Salmonella enterica* subsp. *enterica* serovar Enteritidis and *E. coli*. The seeded *Salmonella* survived in all composts including in mature composts (90 days), suggesting mature composts may support the survival of *Salmonella* at room temperature. In contrast, *E. coli* was only observed in 4 week old compost and not in mature composts. This emphasises that *E. coli* levels can decline prior to those of *Salmonella* spp.

The importance of antagonistic bacteria capable of killing *Salmonella* has been shown by Millner *et al.* (1987). Bacteria sourced from the 70°C zone of a compost pile showed no ability to inhibit *Salmonella*. In contrast, bacteria sourced within the 55°C compost zone suppressed *Salmonella* growth by 100 – 10,000 fold (Millner *et al.* 1987). These laboratory studies were confirmed when Millner *et al.* (1987) found that *Salmonella* did not survive when inoculated into unheated zones (25 – 40°C) of a compost pile. In a similar

study, Pietronave *et al.* (2004) found that the survival of an inoculated strain of *Salmonella arizonae* in organic compost was related to the microbial stability and diversity in the compost.

Salmonella enterica sp. *enterica* serotype Senftenberg strain W 775, selected due to its higher resistance to heat compared to other salmonellas, was used as an indicator of compost safety (Ceustermans *et al.* 2007). Composting was carried out within enclosed facilities in open air. The outcomes were:

- Elimination of *Salmonella* was temperature dependent (In open compost heaps elimination occurred at >60°C)
- Moisture, along with temperature, was a key parameter associated with *Salmonella* reduction (survival rate increases 0.5 $\log_{10}$ unit when there is a reduction of 5% in moisture content)
- When competition with intrinsic flora was present, eradication was faster.

The issue of intrinsic flora and their ability to kill *Salmonella* leads to the importance of studies such as those by Ryckeboer *et al.* (2003) who profiled the populations of microbes in the composting of bio-wastes. The microbial groupings were based on the organisms being either Mesophilic (24°C) or Thermophilic (55°C). The mesophiles decreased during the thermophilic phase of the composting but increased again when the temperatures declined. The fact that the microbial populations differed within the thermophilic and mesophilic phases suggests that “the dominant population”

dictate temperature variations within a composting process. This suggests that overheating can eliminate beneficial populations.

Overall, it is clear that the normal microbial populations have a role in determining the temperatures achieved during compost, contributing to the temperature related killing of pathogens and also have a key role in the antagonistic suppression/elimination of key pathogens such as *Salmonella*. These interactions are complex but certainly are a key parameter in understanding pathogen destruction. Understanding these interactions will result in well designed composting processes.

6.2 Re-growth of *Salmonella*

Some of the issues of *Salmonella* persistence suggest that this organism either persists through unfavourable conditions or the organism actually increases in numbers under a given set of conditions in a fluctuating environment with time, such as the compost micro environment.

An Australian study by Sidhu *el al.* (1999) stated that testing for an indicator organism alone cannot ensure the bio-safety of composted biosolids. They also comment that the behaviour of *Salmonella* in composted biosolids can only be confidently predicted by monitoring its regrowth potential. In another study, evaluating the role of indigenous micro organisms in the re-growth of *Salmonella*, Sidhu *et al.* (2001) found a strong negative correlation between the *Salmonella* inactivation rate and the maturity of the compost. The *Salmonella* inactivation rate was seven times higher in biosolids that had been

composting for two weeks as compared to compost that had been stored for two years. This suggests that antagonistic effects due to indigenous micro organisms and compost age are both key factors in the bio-safety of biosolids during long term storage.

A study by Russ and Yanko (1981) used compost that had been thoroughly dried for one year in the laboratory. The compost had no detectable *Salmonella.* However, when re-hydrated (to levels of 9%, 16% and 21%) and incubated (4^0C, 28^0C, 36^0C and 44^0C) the samples adjusted to 21% moisture and incubated at 28^0C and 36^0C yielded *Salmonella*. The authors suggested that the indigenous *Salmonella* that initiated this growth survived in a desiccated state for a year and the provision of the proper moisture and temperature conditions resulted in repopulation of the organism. This study also linked this re-population to the C:N ratio which was 15:1 or below - suggesting that this parameter could act as a long term “nutritional indicator” for monitoring re-population of *Salmonella* where C was the rate limiting factor.

However Zaleski *et al.* (2005) make a key point about the difference between “re-growth” and “re-colonisation”, where re-growth is defined as an increase in viable numbers of an indigenous microbial population following a decline that has occurred previously, whereas re-colonisation is defined as re-introduction of bacteria to a substrate such as biosolids. Thus, Zaleski *et al.* (2005) in studies carried out on biosolids attribute the increase in *Salmonella* following a rainfall event to re-colonisation rather than re-growth. This conclusion was

based on the very low original levels of *Salmonella* in the biosolids after composting. Laboratory studies also supported this fact, as *Salmonella* were not able to survive and regrow in the composted biosolids or in a soil environment, even when conditions of moisture and temperature were favourable. Contaminating bird faeces were suggested as being the source of the reappearance of *Salmonella* rather than re-growth.

Burge *et al.* (1987) suggest that if *Salmonella* was present at undetectable levels and was re-introduced into compost from infected animal or other sources, the nature of the compost or its components would dictate such re-growth. They extracted a dilute mineral salt component from composts and showed that the availability of this "substrate" dictated *Salmonella* re-growth in composts. This study emphasises the nature of the compost and not just the number or the state of the *Salmonella* dictates the re-growth of this organism under varied circumstances.

The re-growth / re-colonisation issue plays a key role in the safety of composted carcasses or waste, especially during the longer term curing stages. These issues will also a play a role once composts are moved out for commercial use and need to be dealt with in standards and guidelines.

6.3 Summary:

- *Salmonella* has the potential to survive under a range of conditions during composting
- Intrinsic micro-flora have a role in composting as well as compost safety. They have been shown to play a role in reducing the level of pathogens such as *Salmonella*
- Re-growth (from initial low levels following completion of compost) by *Salmonella* is possible
- Re-colonisation of aged composts from external sources of *Salmonella* is also possible

Chapter 7

Australian Data

7.1 Introduction

The Australian study performed in this project focused on assessing the status of compost from producers with compost piles set up for the purpose of this study. Samples from 14 different composts and collection of chicken faeces were examined for the levels of key pathogens – *Campylobacter* and *Salmonella*. The levels of the standard indicator organism – *Escherichia coli* – were also determined. The presence or absence of *Cl. botulinum* toxins C and D was determined by an ELISA. This ELISA does not distinguish between toxins C and D. However both toxins are of significance to cattle. As well, levels of *Clostridium perfringens* – an organism known to be commonly capable of surviving the compost process (Pourcher *et al.* 2005) – were determined. The percentage moisture content and percentage dry matter were determined.

7.2 Methods

The fifteen operations tested were selected on a State basis and covered composts sourced from cage, barn or free range operations. Composting was carried out onsite using available carbon sources. Typically the original source material or primary source was as follows:

- daily bird mortalities
- cracked and broken eggs
- spent hens

The carbon sources used were feed mill dust, cage manure, used litter from free range operation, green waste, barn litter, straw, lawn clippings, timber chips and recycled compost (Table 7.1). The composts were processed either in piles or windrows and turning was carried out for some of the piles.

Data on the physical characteristics such as texture and odour were recorded along with the temperature of the composts of the piles or windrows. These compost characteristics are listed in Table 7.1. Comments were also recorded which described events such as rain or other improvements such as the “need for more C material” were also recorded.

7.3 Results and discussion

The levels of *E. coli* ranged from a minimum of <3 MPN /g to 9400 cfu /g. The highest levels around 10^4 organisms per gram, were associated with three windrow samples and one pile. However the composts that had low *E. coli* counts (4 or <3 MPN/g) were also derived from a mixture of piles and windrows. This suggests that the process used (windrow or pile) may not have an impact on the levels of *E. coli*. It may be that the more important factor is how the composts (be it windrow or pile) are managed that helps pathogen die-off.

A finding of interest was that compost number 15 had the highest levels of *Salmonella* but had an *E. coli* level of <3 MPN/g indicating that *E. coli* was below detection limit. Thus *E. coli,* the common indicator organism, failed to indicate the presence of the pathogen *Salmonella*. It is worth noting that this

compost consisted of cracked and broken eggs which may have contributed to this observation.

The fact that *E. coli* levels for some composts ranged from levels below the detection limit (i.e. <3 MPN/g to around 10^4 cfu/ g may be as a result of some piles either not reaching sufficient temperatures or showing an unequal heat distribution pattern. The higher temperatures of the windrows (and piles) should have been achieved at the early stage of the composting process resulting in reduction of *E. coli* levels in a comparable manner in all samples of compost regardless of compost age. Hence compost age should not be a factor in the varying levels of *E. coli* detected in this study.

The levels of *Cl. perfringens* from composts ranged from levels below detection (<100 cfu/g - one sample) to a highest level of 5.1 X 10^5 cfu/g. In general the *Cl. perfringens* levels were high. This result suggests that *Cl. perfringens,* which is a spore former, probably had a better survival potential in the composted product than non spore forming organisms.

Cl. botulinum is also a spore forming organism like *Cl. perfringens.* Hence the *Cl. perfringens* results may be some indication of the levels of *Cl. botulinum,* if present in these composts. Control of *Cl. botulinum* by composting is a challenge. If the compost temperatures reached were high enough to kill the vegetative cells, the spores could have survived the composting process. Subsequently sporulation under suitable conditions within the compost may result in an increase in number of the organism.

One of the samples was positive *Cl. botulinum* toxins C or D based on an ELISA. This sample was partially composted, mushy and contained a decomposing chicken carcass with maggots and a strong putrefying odour.

No sample, other than the manure sample, yielded *Campylobacter*. This is an expected result given the well recognised poor ability of this organism to survive in the environment. The manure sample was a composite sample – containing both fresh and aged (14 day) layer manure. Hence, it should not be assumed that the level of *Campylobacter* we established (24,000 MPN / g) is typical of fresh layer manure. More extensive studies would be needed to address this issue.

Salmonella was present (in 25 grams) in six of the 14 compost samples, with one of these positives originating from a source material of cracked and broken eggs. In these six presence / absence positive samples the level was shown to be <3 MPN/g for three samples with another sample being 4 MPN/g. Of the remaining two samples one had a level of 15 and the other 4300 MPN/g the latter being a pure cracked and broken egg compost. Overall, 12 of the 14 compost samples had a *Salmonella* level of 4 or less MPN/g (with 11 being <3 MPN/g). Hence, despite the fact that most of the samples were partially completed composts, we found little evidence of high levels of *Salmonella.* This may be due to a good killing of *Salmonella* in the early compost stages or simply the absence of *Salmonella* in the source material. Issues on the possible re-growth potential of the organism as previously described or re-colonisation due to external factors (vectors) also needs to be

considered during the extended periods of exposure of composts heaps to the outer environment.

A total of six different *Salmonella* serovars were detected – Amsterdam, Infantis, Mbandaka, Montevideo, Senftenberg and Singapore. The six serovars identified in this study are not in the top ten isolates of *Salmonella* associated with human outbreaks in the year 2005 (the latest available data) (Owen *et al.* 2007) (see Table 2.3 in Chapter 2). When our results are compared with the common serovars isolated from layers and eggs in 2006 (Australian Salmonella Reference Centre 2006) (see Table 2.2 in Chpater 2), four of the six serovars we found (Infantis, Mbandaka, Montevideo and Singapore) were commonly found in layers and/or eggs. While serovar Typhimurium was present in 15.8% of layer isolates and 7.9% of egg isolates in the national figures (Australian Salmonella Reference Centre 2006) (see Table 2.2 in Chapter 2) and accounted for five of the top ten isolates (five different phage types) in human outbreaks in 2005 (Owen *et al.* 2007) (see Table 2.3 in Chapter 2) , this serovar was not detected in our study.

The single manure sample examined was a composite of fresh and older (14 day) manure. This composite manure had a level of *Salmonella* of 4600 MPN per g. More extensive studies of manure samples are needed before concluding if this result is typical of *Salmonella* levels in layers in general.

Cleary, two major factors play a role in the presence of pathogens in compost - the presence of these pathogens in the source material and the ability of the composting process to reach conditions suitable to eliminate the pathogens (if present).

Only a single compost in this study had completed the composting process. This sample (sample 7) contained daily bird mortalities, along with cracked and broken eggs, and the carbon source was cage manure and straw. Thus, though having the all the components (i.e. chickens, broken eggs and layer manure, common to most layer operation) the product was composted to a stage where the *E. coli* levels were low at 93 MPN/g. Importantly, however the *Cl. perfringens* level was below detection (<100 cfu/g), a result not observed in any other samples. Furthermore, the *Salmonella* level was <3 MPN/g (below detection) and absent in 25 g. No botulinum toxin was detected and the compost had good physical attributes. These findings mean that a good compost can be produced, with minimum pathogen risk and based on good composting practices.

7.4 Summary

- Of the possible food-borne pathogens potentially present in layer waste based composts, our results indicate that only *Salmonella* needs to be considered.
- As one sample was positive for botulinum toxin (type C/D), the results indicate the need to consider the risks of botulism if cattle are exposed to layer waste compost.

- The sole completed compost examined in this study was sourced from a full range of potential layer waste input – daily mortalities, cracked and broken eggs and cage manure. This compost, produced as a heap with active turning, had good physical parameters, an absence of food-borne pathogens, an absence of the spore-forming *Cl. perfringens* and no botulinum toxin. This is evidence that a well managed composting process can deliver a good quality, safe compost under practical on-farm conditions.
- It also needs to be explored if smaller composting units such as heaps or small units may be more manageable to operate within a farm location compared to large windrows, which require more resources to operate.

Table 7.1 Field data and physical parameters of composts examined in this study.

Sample	Farm waste that was composted	Carbon source used	Compost method	Container/Pile/ Windrow dimensions	Time since compost was last turned	Condition/maturity of compost at sampling					Composting process completed	Comments
						Moisture	*Texture*	*Uniformity*	*Odour*	*Temperature*		
1	a) Everyday bird mortalities b) Cracked & broken eggs	Feed mill dust & cage manure 40:60	Windrow	W 4.3m, L 5.1m, H 1.7m	4 days	Just right	*Intermediate, no bones	*Medium	*Modorate	*Warm	No	Done outside on pad, heavy rain during first week. Moisture appears Ok using squeese test. Not completed because not friable - lack of aeration?
2	a) Everyday bird mortalities b) Cracked & broken eggs	Straw and cage manure (saw dust)	Pile Windrow	W 6.3m, L 7.9m, H 2.2m	5 days	Just right	*Intermediate, no bones	*Medium	*Moderate	*Hot	No	Has responded to turning. Odd whole eggs have dried out leaving dried yolk inside coated with dired albumen. Shell appears to be undamaged. Possibly internal eggs
3	Everyday bird mortalities	Used litter from free range/barn sheds	Tumbler/barrel for 28 days then windrow	W 5m, L 22m, H 4m	No turning	Wet and dry layers	*Intermediate	*Low	*Varied-strong & moderate	*Varied in windrow	No	Needs turning & perhaps moisture.
4	Everyday bird mortalities	Cage manure & green waste	Windrow	W 5m, L 11.5m, H 1.7m	N/A	Varied-just right & too dry	*Rough, hard bones & feathers	*Low	*Moderate	55-60C, where dry 40-45C	No	More turning, moisture & carbon.
5	Spent hens	Barn litter & green waste	Windrow	W 4m, L 80m, H1.55m	N/A	Varied-just right	*Intermediate	*Low	*Moderate	50-60C	No	Needs more carbon material & turning
6	Spents hens	Barn litter	Windrow	W 0.95m, L 23m, H 4m	No turning	Varied	*Intermediate, soft & hard bones	*Low	*Strong	45-50C	No	Needs turning, supposed to be sitting for 6 months?
7	Everyday bird mortalities with cracked & broken eggs	Cage manure & straw	Pile	D 7.5m, H 2.2m	7 days	Just right- tending to dry	N/A	*High	*Weak	*Hot	Almost	Started in Jun, too dry (drought). Rain in Aug. Turning since late Sep, large lumps are now small.
8	Everyday bird mortalities with cracked & broken eggs	Feed mill waste & cage manure	Pile	D 7m, H 2m	N/A	Just right- tending to dry, odd to wet patches	*Intermediate	*Medium	*Moderate	*Hot	No	This is a much better batch than pile, couple more turns & it will be as good as Jim's.
9	Cage manure	Raw cage manure	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10	a) Everyday bird mortalities with cr b) Spent hens	Lawn clippings & straw	Windrow	W 2.3m, L 7.9m, H 1.5m	N/A	Too dry	Rough	*Low	*Moderate	a) *Cool b) Hot to warm	No	No moisture added other than wetting the birds. Process stopped or slowed due to lack of moisture.
11	Spent hens	Free range shed litter & timber chips/waste	Started as windrow, finished as pile	D 4m, H 1m	Had 2 turns	Varied-just right to dry, wet (rain)	Rough	*Medium	*Strong	Varied-*warm (dry) to hot (moist)	No	Bones and poutlry carcasses/feather.

Table 7.1 Continued

Sample	Farm waste that was composted	Carbon source used	Compost method	Container/Pile/ Windrow dimensions	Time since compost was last turned	Condition/maturity of compost at sampling					Composting process completed	Comments
12	Spent hens	Free range shed litter & timber chips/waste	Started as windrow, finished as pile	D 6m, H 2.1m	Had 3 turns	Varied-just right to dry, wet (rain)	Rough	*Medium	Strong	Varied-*warm (dry) to hot (moist)	No (more turning)	Added 1000L of water at each turn. Some bones, feather, poultry broken down carcases. Clay base has reduced the amount of moisture being sucked up from ground during rain.
13	Spent hens	Recycled partial compost & green waste	Pile	N/A	35 days	Just right patches, too dry patches (centre	Rough, hard & soft bones	*Medium	Weak	*Hot (except in dry spots)	No	Only turned once, needs another 2 turns & water.
14	Spent hens	N/A	Windrow	N/A	N/A	Just right, too dry-some patches	Intermediate	*Medium	Weak	*Hot-wet patches Warm-dry patches	No	Another 1-2 turns & moisture will finish this off. Better product that sample 1.
15	Cracked & broken eggs	Recyled compost	Pile	N/A	No turning	Mostly just right, some too wet	Intermediate	*Low	Strong	*Warm	No	The compost has not worked as well using the partially composted material. Works better with green

*Rough	70% of particles are <20mm diameter
*Intermedi	90% of particles are <20mm diameter
*Low	Dominant colour or texture represents less than 70% of material
*Medium	Dominant colour or texture represents up to 95% of material
*High	Dominant colour or texture represents more htan 95% of material
*Strong	Manure/dead animal smell
*Moderate	Dry manure/ammonia smell
*Weak	Soil/musty smell
*Hot	Difficult to touch
*Warm	Similar to body temperature
*Cool	Cool to touch

Table 7.2 Results of microbiological tests and moisture determinations

Samples	*E. coli* (cfu/g)	*E. coli* (MPN/g)	*Clostridium* (cfu/g)	*Salmonella* (MPN/g)	*Salmonella present in 25 g*	*Salmonella* serotyping	*Campylobacter* (MPN/g)	*Cl. botulinum* toxin (Type C & D)	% Moisture
1	9400	n/a	24600	<3	-ve	NA	<3	-ve	35.2
2	1600	n/a	10100	<3	-ve	NA	<3	-ve	28.7
3	n/a	4	600	<3	-ve	NA	<3	-ve	42.0
4	n/a	<3	17100	<3	+ve	1 culture *S. Montevideo*	<3	-ve	24.4
5	2200	n/a	510000	4	+ve	2 cultures Both *S. Mbandaka*	<3	-ve	38.7
6	200	1100	130000	<3	-ve	NA	<3	+ve for C or D	n/a
7	n/a	93	<100	<3	-ve	NA	<3	-ve	20.9
8	4600	n/a	7200	<3	+ve	1 culture *S. Singapore*	<3	-ve	32.5

Samples	***E. coli*** **(cfu/g)**	***E. coli*** **(MPN/g)**	***Clostridium*** **(cfu/g)**	***Salmonella*** **(MPN/g)**	***Salmonella present in 25 g***	***Salmonella*** **serotyping**	***Campylobacter*** **(MPN/g)**	***Cl. botulinum*** **toxin (Type C & D)**	**% Moisture**
9	186000000	n/a	1290000	4600	+ve	9 cultures 8 being *S. Singapore* 1 being *S. Montevideo*	24000	-ve	54.4
10	2700	n/a	5000	15	+ve	4 cultures All *S. Infantis*	<3	-ve	11.6
11	n/a	240	2900	<3	-ve	NA	<3	-ve	37.1
12	n/a	<3	3100	<3	-ve	NA	<3	-ve	37.6
13	n/a	<3	100	<3	+ve	1 culture *S. Amsterdam var 15+*	<3	-ve	21.1
14	n/a	43	400	<3	-ve	NA	<3	-ve	25.4
15	n/a	<3	500	4300	+ve	12 cultures 3 being *S. Mbandaka* 9 being *S Senftenberg*	<3	-ve	44.3

Chapter 8

Clostridium botulinum, cattle and composting

8.1 Botulism, cattle and source of infection

The interest in terms of *Cl. botulinum,* layer waste and cattle is a result of outbreaks of bovine botulism across the world (examples discussed later in this Chapter). More specifically, this Chapter will look at the following:

- the conditions supporting spore /organism /toxins persistence and modes of transfer in environments of risk
- knowledge from previous cattle botulism outbreaks as a means of developing background knowledge in a context of composting

Bovine botulism outbreaks can occur as a result of the either the ingestion of the preformed toxin (Kozaki and Notermans 1980; Gregory *et al.* 1996) or spores of the organism (Notermans *et al.* 1978; Souza *et al.* 2006).

The first and perhaps the most common pathway involves the ingestion of toxin and has been described by Jean *et al.* (1995) as follows:

- ingestion of preformed toxin
- the toxin penetrates the gastrointestinal tract, reaches the neuro muscular junction via the circulatory system,
- the toxin irreversibly binds to the nerve plates, resulting in symptoms of disease

The second pathway is toxin formed within the gut of the animal as a result of the ingestion of bacteria or spores that can continue to grow within the rumen and gut. This occurs with the consumption sufficient number of organism via contaminated feed or rotting vegetable matter, causing what is known as toxico-infectious botulism in cattle (Taylor 2004).

Holdeman **(1970)** provides a summary of the factors necessary for an outbreak of botulism, particularly due to types C and D:

- presence of viable organisms
- environment that will support growth and toxin production
- ingestion and absorption of toxin
- susceptibility of host

For toxin formation to occur, the genetically determined toxigenic bacteria strains, along with substrate for growth and multiplication needs to be present (Böhnel *et al.* 2002)

Particularly in Australian environments phosphorous deficient range cattle have been known to eat or chew bones from carcasses of wild and domestic animals to satisfy cravings for phosphorus or protein (Szabo and Gibson 2003, Taylor 2004, Freeman and Bevan 2007). This practice leadis to the ingestion of neurotoxin originally sourced from the gut of the decaying animals. Though cattle outbreaks in Australia have been due to types C or D, an outbreak in South Australia was implicated as type B in cattle that were vaccinated against type C and D (Freeman and Bevan 2007).

Fitzpatrick (2006) provides a summary of conducive factors for outbreaks (and losses) related to botulism in Alice Springs, Barkly and Katherine regions of the Northern Territory as follows:

- the deficiency of protein and phosphorous, through much of the dry season, a factor of these soil types including pasture
- a habit of carcass and bone chewing, even when dietary protein and phosphorus are adequate
- the distribution of the bacteria, where types C and D are present in all pastoral regions of the NT
- the conditions for bacterial growth i.e. an anaerobic environment with optimum temperatures around 23°C (15-35°C) – conditions found in rotting carcasses.
- the toxin having the potential to last for a year at 30°C but rapidly is inactivated at 37°C. This leading to the potential of some carcasses in tropical environments to continue to be toxic
- potential for carcasses of birds other decaying animals to remain infective
- susceptibility of unvaccinated or improperly vaccinated cattle. This would depend on the amount of toxin produced. Cattle that recover from an outbreak can develop natural immunity.

8.2 Chickens as a possible source of *Cl. botulinum*

Chickens are susceptible to *Cl. botulinum* type C (Roberts *et al.* 1973; Dohms *et al.* 1982; Dohms and Cloud 1982; Pecelunas *et al.* 1999). Following an outbreak within a commercial operation (~ 15,000 birds per shed) in the Delmarva region due to type C, preformed toxin (Dohms *et al.* 1982) could not

be demonstrated from litter feed and water. However *Cl. botulinum* type C was present in litter and feed as well as alimentary tract etc. of the affected birds. Thus, the litter seemed to be a source of the organism, rather than pre-formed toxin ingested by the chickens.

In an outbreak involving 368 chickens within a commercial operation preformed toxin of *Cl. botulinum* could not be detected within the litter (or decomposing carcasses within the shed as a source) (Roberts and Collings 1973). However spores and the organism were demonstrated in the litter and in the soil outside the shed respectively. This study provides some indication that the presence of the organism or its spore can be linked to toxin production. The absence of the toxin does not indicate the total absence of the organism or its spore (Böhnel and Lube 2000).

8.3 Distribution and site specificity of *Cl. botulinum* types

These organisms are detected worldwide and are commonly present in soil but with limited prevalence in different localities (soil) (Holdeman 1970; Gessler and Böhnel 2006b,). Böhnel *et al.* (2002) are very specific with the fact that different types have a distinct geographic distribution. They indicate that the types are site specific (topotypes), depend on the location i.e. present in soil, water (sea), agricultural settings, the presence of prevailing animals and other unknown factors. As an example toxin producing clostridia were detected in 23% of Argentine soil samples, with a higher percentage of positive samples (27%) being from non-virgin soils (cultivated) compared to virgin soils (16%) (Luquez *et al.* 2005). Further more types C and D were not

isolated in these (Argentinean) soils suggesting a link between distribution and geographic locations as previously suggested. In contrast soil from a national park in Zambia inhabited by multitude of wild life contained botulinum toxins B and D (Karasawa *et al.* 2000) perhaps indicating a geographic distribution.

As an example of differential prevalence, cattle can be affected by *Cl. botulinum* types B, C and D, with the latter two types being commonly associated with outbreaks in Europe, Australia, and South Africa and Type B being of importance in North America (Jean *et al.* 1995).

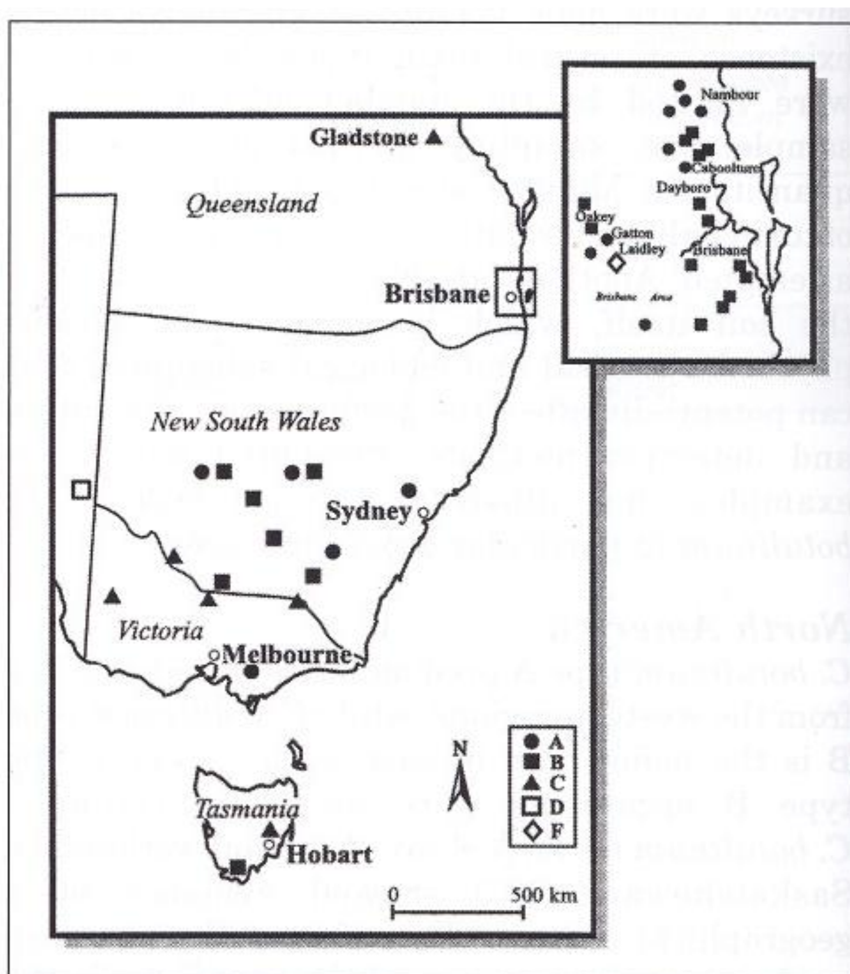
8.4 Persistence of spores in the environment

Botulinum spores can persist in the environment for a long period after an outbreak (Notermans *et al.* 1981). There was a strong association between the previous occurrence of avian botulism in a marsh and the ability of the soil samples from that marsh to produce toxin (59%) (Wobeser *et al.* 1987). Similarly soil from another marsh with no history of disease for 11 years was able to yield toxin indicating a long residual effect after an outbreak *et al.* (Wobeser *et al.* 1987). *Cl. botulinum* Type D has been distributed in South Australian soils (Eales and Turner 1952). Subsequently Szabo and Gibson (2003) have reported the presence in Australian environments the presence of different neurotoxin types at various locations (Figure 8.1).

Other conditions such as the complex soil structure (i.e. neutral or alkaline soils, pH, organic matter content), can provide suitable micro-environments which are supportive of the growth of *Cl. botulinum* (Smith 1975a). Thus, suggesting a combination of factors can determine spore or toxin production within different pockets of micro environments in products such as composts (Böhnel and Lube 2000).

The resistant nature of the spore can result in dormant spores being present in the environment (Böhnel *et al.* 2002). *Cl. botulinum* type C has been isolated at pH of 8-9 in lake environments (Wobeser *et al.* 1987). Given the right conditions for growth the spores can either germinate resulting in toxin or the spores themselves being further transfered within the environment. Thus, along with soil the aquatic environments have a role not only in supporting the growth of the organism but also in the distribution of the spores/organism (Huss 1980).

Figure 8.1 The reported distribution of botulinum neurotoxin types in the Australian environment (Szabo and Gibson 2003).



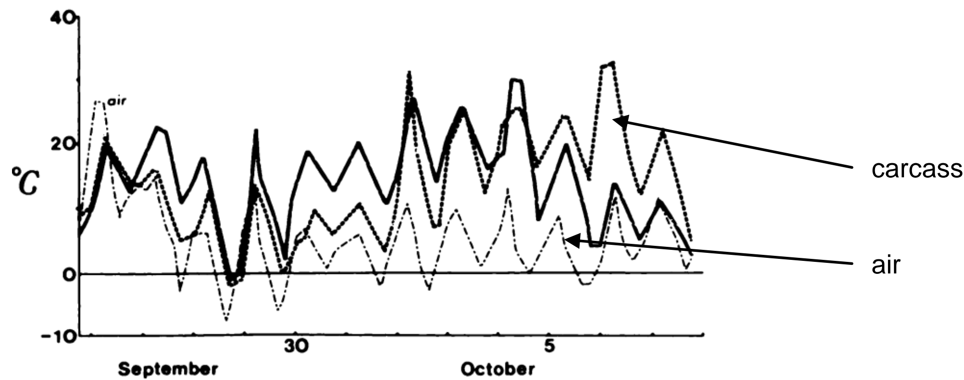
Spore persistence has shown to be more common than the toxin in soil and sediments around pond environments (Souza *et al.* 2006). *Cl. botulinum* spores and toxins of type C and D were present in cattle drinking ponds (300 ponds), used on 130 farms. *Cl. botulinum* spores were also present in superficial soil samples (19%) and the sediments (10%). However, botulinum toxins (type C and D or classified as belonging to the CD compound) were detected in only 6 samples (2%) of the 300 ponds (Souza *et al.* 2006). The

older and shallower ponds had a higher frequency of detection of botulinum spores or toxins.

The toxins are known to be heat labile (Losikoff 1978). However if the toxins are present in the environment they could move with surface run-off waters following heavy rain. An understanding of the factors that trigger spore germination, vegetative cell growth and toxin production in soil reservoirs from which they can be mobilised to surface waters needs to be developed from a watershed management point of view (Long and Tauscher 2006).

Decomposing animal carcasses and rotting vegetation provide anaerobic conditions that prevail seem to support the growth of the organism in the environment (Critchley 1991). Rotting animal material is a key source providing suitable growth conditions for the organism. The optimum temperature for the growth of *Cl. botulinum* is above 30°C (Wobeser and Galmut 1984). The internal temperatures of rotting carcasses can be substantially different to the ambient temperatures and can provide a microclimate for botulinum growth, even under non-optimum weather conditions (Wobeser and Galmut 1984). Figure 8.2 illustrates both the rotting carcass and cooler ambient temperatures.

Figure 8.2. Internal temperatures of bird carcasses and ambient temperatures. (Wobeser and Galmut 1984)



8.5 Ecological aspects on the persistence of spores

The persistence of spores is closely linked to the conditions of the environment. Most ecological studies in terms of the continued presence of botulinum toxin/spores/genes in an environment after an outbreak have been in relation to avian botulism outbreaks involving migratory birds. These outbreaks also involve type C (Graham *et al.* 1978; Takeda *et al.* 2006), the type also involved in cattle outbreaks. *Cl. botulinum* is a strict anaerobe but has been shown to grow on plants (Girardin *et al.* 2005) and grass (Böhnel *et al.* 2003). This suggests the possibility of micro-environments created by extracelluar components providing an anaerobic growth niche where the organisms are protected within biofilms on the plant.

The greatest concentration of botulinum spores are found in carrion, larvae, grubs or invertebrates feeding on the carcass and in soil under the carcass (Critchley 1991). Maggots feeding on the carcasses were suspected as the

dominant location (rather than sediments, water or macro zoobenthos) where massive growth of toxigenic clostridia with subsequent toxin production occurred; possibly contaminating the surrounding environment via the carcass (Zechmeister *et al.* 2005). Other studies have also detected *Cl. botulinum* type C in maggots (collected from rotting carcasses) (Haagsma *et al.* 1972, and cited in (Hubalek and Halouzka 1988). Maggots (mature larvae) originating from household bin waste also contained both the toxin and the organism, and thus are a possible vector of *Cl. botulinum*. (Böhnel 2002). Rotting carcasses (in a compost situation) may have the potential to support *Cl. botulinum* along with other invading organisms such as maggots and the concentration these scavenging organisms may also be of great concern. Thus the composting process should be performed with an awareness of the need to manage maggot proliferation during the process.

8.6 Composting and *Cl. botulinum*

Böhnel *et al.* (2002) are of the view that composting conditions will not destroy botulinum spores in biocomposts (sewage, garden waste). In fact they are of the view that the organism will multiply during the subsequent curing and storage of the final product. Composting is considered to have a high "hygienisation" potential due to the heat generated during the process which however may not have an impact on the destruction of *Clostridium* spores (Böhnel and Lube 2000). *Clostridium* spores are known to survive heating at 100°C at pH 6.0-7.0 for more than 110 minutes (Mitscherlich and Marth 1984 cited in Böhnel *et al.* (2000)). Thus Böhnel *et al.* (2000) make the key critical point that the common belief that all pathogen input via plant and animal (plus

human) material are destroyed during composting resulting in “hygienic product” without a health risk is incorrect. To address this concern a study assessing the presence of *Cl. botulinum* was carried out to understand the safety of bio composts produced in Germany (Böhnel and Lube 2000). The outcomes of this study were as follows

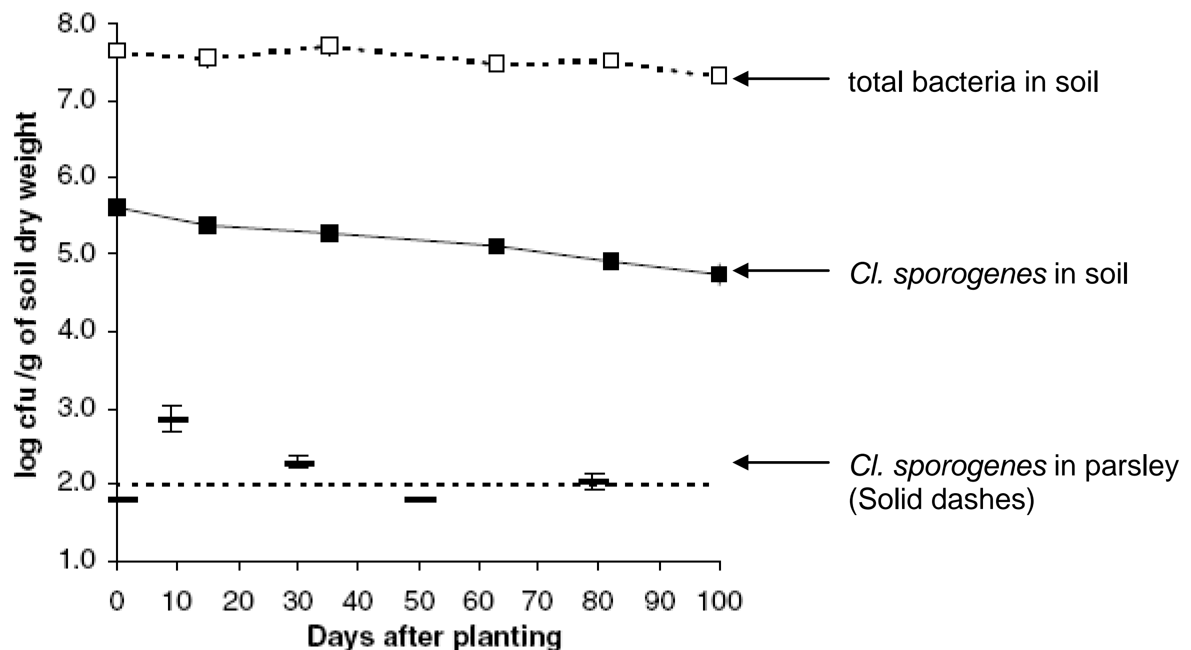
- *Cl. botulinum* (spore or vegetative) form was present in 54% of the bio composts tested
- *Cl. botulinum* toxin production was demonstrated in 25% of the incubated samples leading to the conclusion that the required nutrients were available in the bio composts to potentially instigate toxin production
- typing showed equal distribution of the two groups - A, B and E and C and D
- management of processes may play a role in this prevalence in organism and toxin
- the possibility of botulinum spores further transferring in the environment via run off following rain is possible
- an understanding of the accumulation of the spores and the environmental factors that trigger toxigenesis needs to be gained to assess future implications of these findings

A study following on from Böhnel *et al.* (2000) set out to test the persistence and mobility of *Cl. botulinum* spores by spiking compost (Gessler and Böhnel 2006a). Compost was spiked with *Cl. botulinum* type D spore (10^3 – 10^5 spores /g) after which the composts were spread on to experimental sites. The samples were collected from an upper layer (0-5 cm) and deeper layer

(10-30cm). The plots that received 10^3 spores/g were positive for 757 days. The organism was never detected from the depths of 10-30cm. In plots that received 10^5 spores/g, the spores were still present after 939 days. The spores were also detected at depths of 10-30cm indicating that *Cl. botulinum* also had the ability to transfer to the lower levels of the compost site.

The outcomes of the Gessler *et al.* (2006a) study suggest that *Cl. botulinum* has the ability to persist and transfer in the environment ultimately contaminating food crops via composts and manures. Another study (Girardin *et al.* 2005) addressed this issue by spiking studies using *Cl. sporogenes* – a non-pathogenic surrogate *for Cl. botulinum.* This study was carried out under field conditions to simulate the actual environmental conditions that impact on the survival of the organism. *Clostridium sporogenes* was introduced to soil through organic fertilizers and its persistence in soil and transfer to plants (via splashing, air transmission and plant internalisation) were assessed through a cultivation period of over two successive years. In soil, the spores of *C. sporogenes* declined by less than 0.7 log cycles (a log cycle is a 90% reduction of the original population) within 16 months and were detected on parsley leaves throughout the experiment. The levels of spores of *C. sporogenes* remained relatively constant during the course of the experiment, suggesting that they survived rather than proliferated in soil and on parsley (Figure 8.3). Nonetheless this survival was regarded as a risk in terms of crop contamination (Girardin *et al.* 2005).

Figure 8.3 Survival pattern of *Cl. sporogenes* in soil and on parsley (after spiking with spores) -adapted from Girardin *et al.* (2005)



However Girardin *et al.* (2005) make the point that "In European countries, the use of amendments based on animal manure and sewage sludge for crop fertilization is increasingly monitored by Regulations and/or Codes of Practice that are increasingly restrictive with regard to microbiological agents". These regulations were cited as being:

- Safe Sludge Matrix. Guidelines for the application of sewage sludge to industrial crops. In: www.water.org.uk and www.adas.co.uk/matrix Water UK and ADAS (2001) (as cited in (Girardin *et al.* 2005)).
- Arrêté du 8 janvier 1998 fixant les prescriptions techniques applicables aux épandages de boues sur les sols agricoles. In: French Official Journal of the 31 January 1998 France.

Girardin *et. al* (2005) further state that "the delay of 10–18 months currently required by the French Regulations may not have an impact on preventing the presence of spores of *Cl. botulinum* in the crop environment as with the present study. These authors make the key point that "The increasing use of composted wastes as crop fertilizers and the absence of regulation limits concerning pathogenic spore-forming bacteria in these fertilizers may enhance the risk of produce contamination by these pathogens".

Curci *et al.* (2007) reported on a study where five carcasses of cattle that died from botulism (type D toxin, one by the CD complex), were composted in individual isolated bed. The temperature of the beds was monitored during the period and oscillated from 40.5 to 52.4°C. C. All samples were negative for botulinum toxin using the mouse bioassay and only 2 out of 200 samples were positive for spores. Thus the authors concluded that composting carcasses of cattle that died from botulism eliminated botulinum spores and toxin from the remains. However this outcome would also depend on the level of toxin and/or spores that prevailed prior to composting.

8.7 Case studies of bovine botulism outbreaks - poultry waste and other sources

Cattle botulism has been associated with the consumption of poultry related waste by grazing cattle which will be discussed in studies listed in this section.

In 2004 an "ad hoc" group was set up to investigate a marked increase in cattle botulism increase in England, Wales and Ireland (Advisory committee on the microbiological safety of food ad hoc group 2006). From 1997-2002 an average of only four botulism incidents were reported in England and Wales. However the reporting increased to 29 incidents in 2003 and 34 in 2004. Among the various suspected cases the dominant source was poultry litter (see Appendix 2 of this this review which lists details of the associations of 55 of these outbreaks from 2003-2005) (Advisory committee on the microbiological safety of food ad hoc group 2006).

A summary of key exposure pathways is set out below (These can have implications on an on-going composting process or a poorly composted final product)

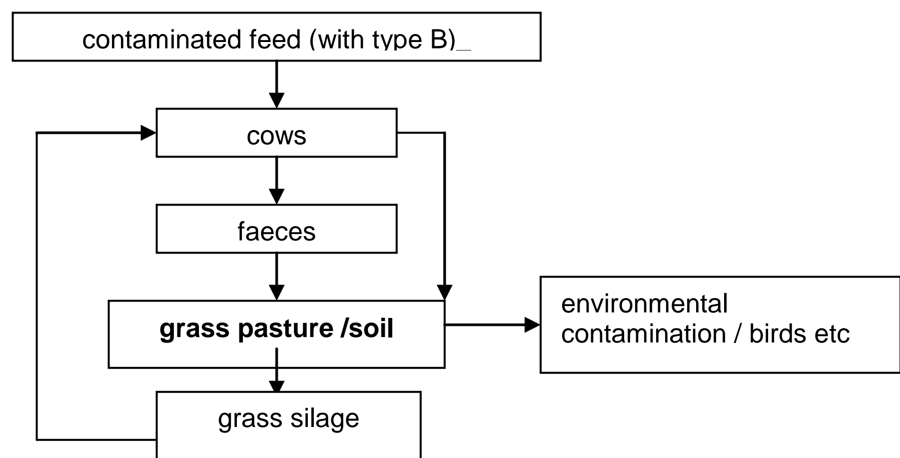
- direct and indirect contact from stacked litter
- direct and indirect contact from spreading of litter /stacked litter on fields
- spread litter on neighbouring /adjacent farm
- presence of adjacent poultry farm

The following sections overview some outbreaks that describe pathways and environmental links. Most of these involve poultry litter – partially composted, piled or directly used and in instances with the presence of decaying carcasses. These studies provide valuable background knowledge within the context of on-farm composting of poultry waste/carcasses

8.7.1 Persistence of the organism after an outbreak

This outbreak describes how a single event of contamination can result in the persistence of the organism in the environment. Several outbreaks in dairy cattle occurred in 30 farms in Netherlands in 1976 and 1977 as a result of brewers' grain contaminated with type B (Notermans *et al.* 1981). This study described the link between the presence of *Cl. botulinum* in the grass silage and the subsequent return of the organism to the environment via shedding of contaminated faeces (Figure 8.4).

Figure 8.4 Schematic presentation of the cycle of *Cl. botulinum* type B on a cattle farm involved in an outbreak by brewers' grain - after Notermans *et al.* (1981)



The continuous presence of the organism due to spreading of manure increased the levels of organism over a two year period from 100/g to 600/g in the faeces. However, no clinical symptoms were evident in the animals. The grass used to prepare silage was not contaminated immediately on the onset of disease. However the soil 2.5 years later was found contaminated with 100

cells/g. A cyclic build up seems to have occurred via a single incident of contaminated feed (grass silage) resulting in the contamination of the environment. Faeces of foraging water fowl, a vector, were also contaminated with type B along with types C and D. These water fowl are capable of spreading of the organism to previously non-contaminated pasture.

8.7.2 Outbreaks in Queensland

An outbreak in Queensland in 1988 (Trueman *et al.* 1992) resulted in the deaths of 237, 109 and 30 heads of cattle across three farms at the same time, where the cause of deaths appeared to be feed, where among other ingredients litter was also present.

A second major outbreak in Queensland involved the loss of over 5000 head of feedlot cattle when one batch of chicken litter being used as a feed supplement for cattle as a source of protein proved to be contaminated (Douglas 2001). The outbreak occurred on two properties at around the same time with the same source of litter from a feed supplier (DPI & F Queensland case files). The first outbreak resulted in the death of 3017 head of cattle leading to a final mortality rate of 87% in one of the feedlots and 2455 head of cattle in a second feedlot. Chicken litter was component (2-9%) in the feed fed to the cattle. On examining the suspected batch of chicken litter a significant amount of chicken carcass fragments were found in the litter. Extensive testing resulted in *Cl. botulinum* Type D toxin being detected in the following - a chicken carcass found in the chicken litter at one of the feedlots, chicken litter from both of the feedlots and serum from an animal from the

cattle (DPI & F Queensland case files). Overall this outbreak resulted in the practice of feeding chicken litter to cattle being outlawed in Queensland (Taylor 2004).

Cattle outbreaks have also occurred in Queensland where cattle had eaten piled-up litter ready to be spread on pasture, thus leading to legislation denying cattle access to such material, (which included chicken faeces and litter) (Taylor 2004).

8.7.3 Decomposing carcass and ensilaged poultry litter

Decomposing carcasses can pose a risk during breakdown. An outbreak involving deaths of 68 cattle from 150 animals in Ireland resulted from ensiled poultry litter used as feed (McLoughlin *et al.* 1988; Neill *et al.* 1989). In this outbreak decomposed poultry carcasses were detected in the ensilaged poultry litter. Botulinum toxin detected in the samples where decomposed poultry carcases were present (McLoughlin *et al.* 1988). The concentrations of the toxin in these samples were 28.4, 15.8 and 1673 LD /50g of sample.

8.7.4 Carcasses in stockpiled litter, a source

Incompletely composted material spread to pasture can pose a risk to cattle as well as contribute to the persistence of spores in the environment. An outbreak involving cattle and piled poultry litter in the UK was described by Smart *et al.* (1987). Poultry litter along with poultry faeces, wood shavings and the occasional broiler carcass were stacked for 2.5 months during which time heating of the pile occurred. The product was then spread over 10

hectares of pasture as manure, where 24 cattle were grazing. The cattle consumed silage spread over this area. Seven animals were affected with botulism probably by feeding on contaminated pasture.

Type C toxin was detected in the serum, contents of the alimentary track of the affected animal, decomposing chicken muscle which contained 25,000 MLD of botulinum toxin /g. However *Cl. botulinum* type C (organism) was detected in chicken muscle, poultry litter and the gut of the cattle indicating a possible link with the carcass via the litter. The use of poultry waste as a grassland fertilizer can increase the possibility of *Cl. botulinum* type C (present in poultry) being more widely distributed in the farming environment, particularly adjacent to poultry production units (Smart *et al.* 1987).

Incomplete carcass breakdown in material spread to land can cause problems. A suspected outbreak in Scotland involving poultry litter occurred where the toxin source was poultry carcasses contained in litter waste spread on to stubble grazing – many decomposing chicken carcasses were present throughout the field (Appleyard and Mollison 1985).

8.7.5 Single source responsible for a cluster of outbreaks

Hogg *et al.* (1990) reported an outbreak of botulism on three closely sited dairy farms. Farm 1 was top dressed with poultry litter. Farm 2 had no contact with poultry litter but had a brook linking it to the poultry farm. On Farm 3 litter was stockpiled over winter and was then thickly applied to pasture. On Farm 3 cattle also had access to the pile.

On Farm 1 the silage was found contaminated with clostridia species. On Farm 2 testing of the water failed to provide a conclusive link (there was no toxin or spores of *Cl. botulinum* detected). On Farm 3, type D *Cl. botulinum* toxin and spores were present in the desiccated chicken carcass taken from the pasture. Types C and D spores were identified in the poultry litter in the field and the poultry litter within the manure heap.

On the poultry farm *Cl. botulinum* type C and D toxin was present in decomposing carcasses present within the piled litter. *Cl. botulinum* was absent in the mud samples (a possible link to the brook) collected in the vicinity of the poultry farm. A mixture of the spores of types C and D or type D alone was detected within the intestinal content of the dairy cattle, suggesting a link with the chicken litter. No toxin was detected in the serum, liver and the intestine of the chickens.

A summary of the issues surrounding this cluster of outbreaks from an environmental contamination point of view is as follows:

- guarantee of a complete removal of carcasses from litter may not be possible
- birds that have died due to botulism may be a potent source of toxin, especially if such birds are allowed to decompose in litter
- the ecology of *Cl. botulinum* in poultry units and in crops to which poultry litter has been applied needs to be evaluated

8.7.6 Preventative measures

A summary of the role of the presence of carcass material and its relationship to botulism in cattle either due to direct consumption or via the environment has been provided (Livesey *et al.* 2004; Otter *et al.* 2006). Some of the key points listed are as follows:

- the presence of carcasses is a risk and hence under “The Animal By-products Regulations 2003 (SI 1484)” carcasses need to be disposed off by rendering or incineration.
- access to cattle needs to be prevented in areas of potential risk in terms of the presence of carcass material

8.7.7 Other outbreaks

An outbreak in Brazil resulted in the death of 155 from 201 animals across three different farms with the source being week old deep-stacked poultry litter from a single source (Ortolani *et al.* 1997). A Canadian outbreak involving the deaths of 34 cows due to type D was attributed a localised high concentration toxin within a haylage from a silo (Martin 2003).

8.8 Production and stability of botulinum toxin in the environment

Whilst heat may have an impact on toxins, the destruction of toxins within the environment will depend on the prevailing conditions. Stability of the toxin in the environment can depend on several complex factors. Stability of toxin of *Cl. botulinum* type C (contained in vials) under natural conditions in lake water at temperatures 3-11°C, was investigated by Graham *et al.* (1978). The toxin

destruction was slow and an appreciable amount of toxin remained when the experiment was terminated after a year (see Table 8.1).

Table 8.1 The slow destruction of type C toxin under semi natural conditions May 1975 (minimal temp in March /April 3-11°C), after Graham *et al.* (1978)

Days	Mouse Lethal Doses (Thousands per ml)		
	1A	1B	1C
6	5-10	5-10	5-10
7	5-10	5-10	5-10
42	5-10	1-5	1-5
97	0.5-1	0.5-1	0.5-1
133	0.5-1	0.5-1	Not done
168	0.5-1	0.5-1	Not done
344	0.5-0.8	Not done	Not done

Seasonality can have an impact on toxin stability. The study of Hubalek and Halouzka (1988) assessing the thermal stability of *Cl. botulinum* type C toxin is summarised in Table 8.2. This study was carried out at a pH of 6.5, in the absence of air and viable bacteria and in a constant temperature environment. This study reported the constant persistence of toxin below temperatures of 30°C with longer persistence at temperatures below 10°C, suggesting the possibility of the persistence of toxin produced in summer over winter periods.

Table 8.2 Time temperature relationship for inactivation of *Cl. botulinum* type C toxin adapted from Hubalek and Halouzka (1988)

Temperature	Time required for inactivation by	
^{o}C	90%	99%
-70	> 5 years	> 5 years
-20	4 years	> 5 years
+5	30 days	6 months
+20	3 days	21 days
+28	2 days	14 days
+37	2 days	1 days
+42	5 h	9 h
+56	< 30 min	< 30 min
+60	< 20 min	< 20 min
+80	< 5 min	< 5 min

(The shaded areas represent temperatures that can possibly be achieved due to composting)

In studies on poultry litter, the maximum toxin production occurred at pHs from 6.5 to 7.0, where the toxin is stable. However at pH lower than 6.5 inactivation of the toxin is possible due to fermentation within the deep stacked waste (Ortolani *et al.* 1997). Type C strains have shown the ability to produce toxin on grass substrates at a pH of 5.3 and a water activity of 0.985, suggesting the ability for such strains to be able to produce toxin in wilted grass silages (Notermans *et al.* 1979).

8.9 Inactivation studies

Most studies on the factors surrounding the growth and germination of spores have involved toxin types relevant to food environment rather then the natural environment (Long and Tauscher 2006). However data derived from such studies may be useful in determining the optimum growth parameters of the organism. *Cl. botulinum* under natural conditions has to face competing organisms which may be inhibitory in terms of growth and toxin production (Jay 1978). Knowledge of such complex interactions will aid in understanding the dynamics of the organism in the environment.

Vegetative cells of *Cl. botulinum* are killed in a few minutes at 60°C and the toxins being proteins are more sensitive to heat. Maintaining a temperature of 78°C for 1 minute are used as a guideline for inactivation in foods (Szabo and Gibson 2003)

Cl. botulinum growth associated with decomposing carcasses and to a lesser extent vegetable matter occurs where conditions are alkaline and anaerobic (Critchley 1991). However the released toxins are thermolabile and are rapidly inactivated as the pH rises (Critchley 1991).

The nature of the substrate, the pH and temperatures, redox potential all play a role in the growth as well as the ability for toxin production of the organism. Studies carried out under laboratory conditions have indicated that *Cl. botulinum* type C was able to grow at a minimum temperature of 12.8°C and the minimum pH for growth was 5.62 (Segner *et al.* 1971b). *Cl. botulinum*

was also able to grow and produce toxin under laboratory conditions at a pH as low as 4.6 (Wong *et al.* 1988). Specifically types C and D have the ability to produce toxins at 40 – 42°C, where a drop in temperature over the winter months may allow the toxin to remain stable (Critchley 1991).

Thermal death studies have been carried out to plot the survival of *Cl. botulinum* under different temperatures (Campanella and Peleg 2001) and such data is extensive in the literature on sterilising (canning) foods using much higher temperatures than is typical of compost processes. Thermal inactivation of pathogens has been studied for *Cl. botulinum* at of 72°C for 15 seconds and thermal inactivation curves for this process has been produced (van Asselt and Zwietering 2006) The proteolytic types (C and D) have shown to have a lower heat resistance than the rest of the types (van Asselt and Zwietering 2006).

Type C and B toxins have been shown to be stable for an hour in the presence of cattle rumen fluid experimental conditions (Kozaki and Notermans 1980). The authors used this finding to conclude that cattle exposed to toxin containing feed were at risk. In contrast Allison *et al.* (1976) have shown type C toxin inactivation was rapid in the presence of the bacterial fraction of the rumen, suggesting a role of microbial proteases for such inactivation.

8.10 Diagnostic techniques for Toxin types C and D

The mouse bioassay is the only internationally accepted test and the most important and common test (Thomas 1991; Böhnel *et al.* 2002; Chaffer *et al.* 2006) and has the advantage of being able to prove the presence of toxins (and active toxigenic bacteria) (Böhnel *et al.* 2002). However a negative mouse bioassay test can mean that: the spores are dormant, the toxigenic bacteria are present but genetically not activated, toxigenic bacteria are present but conditions (i.e. temperature, water activity) prevent toxin production, the toxin was simply destroyed by other metabolites or the levels can be low (Böhnel *et al.* 2002). .

An ELISA for the identification of *Cl. botulinum* toxins C and D was developed by Thomas (1991) as an additional diagnostic tool along with mouse inoculation. The mouse inoculation test is a highly specific test but has a low sensitivity due to insufficient toxin present to kill the mice. Hence this ELISA was suggested as a valuable tool for the diagnosis of botulism in animals (Thomas 1991). Toxicity could either be detected by direct analysis of the samples or by cultivation of the sample to support the growth of the spores/organism which are then subjected to toxin detection Various immunoassays based on monoclonal antibodies as well DNA based PCR methods for detection of different toxin types have been described in literature (Szabo and Gibson 2003).

An PCR with the potential to detect *Botulinum neurotoxin Cl (BoNt Cl)* directly from environmental samples such as sediments has been recently developed to assess the risk of avian botulism from aquatic habitats (Zechmeister *et al.* 2005). Zechmeister *et al.* (2005) have compared direct PCR detection of the toxin genes in the environment and the culture of clostridia and the subsequent confirmation of the toxicity of these cultures via ELISA or mouse bio assay. They found that in areas with a high botulism risk the ratio of positives in the PCR compared to the culture was around 2. In low risk areas the ratio was much higher suggesting the toxin genes were located outside clostridia cells. This suggests that a combination of both techniques, PCR and culture / toxin assays, can be used to predict the risk level of an environmental botulism outbreak (Zechmeister *et al.* 2005).

8.11 Summary

- *Cl. botulinum* specifically Types C and D have been isolated in several Australian environments
- large outbreaks of botulism attributed a single source (chicken litter) have occurred in Queensland
- other outbreaks with links to rotting chicken carcasses via litter have occurred in various countries
- chickens are also susceptible to botulism, being Types C & D
- *Cl. botulinum* spores types can remain dormant, transfer in the environment, grow and produce toxin when conditions are right
- heat has shown to destroy the toxin (e.g 28 – 37°C in 2 days 42 °C in 5h and 60°C in < 30 minutes, (however toxin production can occur at

40 - 42 ^{o}C). The toxins are stable in Northern Territory soil environments at 30 ^{o}C.

- most spore inactivation studies are linked to food where spores require higher temperatures for inactivation e.g. known to survive heating at 100°C (pH 6.0-7.0) for >110 minutes
- bio compost following a composting process have been shown to harbour *Cl. botulinum* spores (54% vegetative cells and 25% were able to demonstrate toxin production)
- inoculation studies have shown that spores can persist in the soil, and transfer to crop.
- rotting carcasses and maggots within such carcasses are known to be the key sites of toxin production
- the pathways shown in most cattle outbreaks are manageable with the development of strict guidelines

Chapter 9

Guidelines

9.1 Introduction

Compost is a valuable resource in terms of soil conditioning for agricultural purposes. However, limitations do occur with respect to composting chicken waste and carcass from the perspectives of food safety, cattle botulism and the implications for the general environment. A series of guidelines have been summarised in this Chapter, the full details being covered by the original documents.

In the absence of any chicken specific composting guidelines and especially due to the nature of the waste, the location of the composting processes and the related risks associated with pathogens, a comprehensive and best possible approach to manage this situation has been produced based on the cited guidelines. One of the key issues is the need to develop process control documents to ensure microbiological safety and to educate the farmers dealing with these composting operations. This also would provide the basis for end-user confidence when using these products in the environments.

However many critical issues, especially that of *Cl. botulinum* need to be taken in to account in the management of composting. Further, the aspects of human food safety and cattle botulism are driven by different key factors and should be viewed separately for the purpose of management.

Importantly, all positions of a compost pile may not be heated uniformly and the age of the manure may be different in different pile locations leading to the conclusion that such composts cannot be regarded as free of enteric micro organisms (Heinonen-Tanski *et al.* 2006). Maturing compost heaps normally attain temperatures ranging from 55 to 65°C and are generally regarded as conforming to the recommended biological risks and sanitation standards for composts stipulated by either the EU or US-EPA (Moore *et al.* 2007) However composted products derived from animal sources are further required by the EU biohazard safety regulatory legislation to either attain 70° C for over 3 h during maturation or via separate treatment at 70°C for 1 h before being considered for disposal on land (Moore *et al.* 2007). During the "hygienisation" process of composting (i.e the early stages of composting) not all spores (e.g. from *Cl. botulinum*) will be destroyed (Böhnel *et al.* 2002). Indeed, national regulations such as those in Germany do not provide for the elimination of spore formers (Böhnel *et al.* 2002). It is worth noting that Hazard Analysis Critical Control Point processes could be developed to help to deal with this situation (Böhnel *et al.* 2002).

Specific guidelines for chicken waste composts are limited and thus a comprehensive approach needs to be adopted based on knowledge derived from available guidelines, which are generally most developed for human waste.

This Chapter deals with the USEPA human biosolid guidelines (United States Environmental Protection Agency 2003) as well as Australian State guidelines for human waste.

The USEPA human biosoild guidelines (United States Environmental Protection Agency 2003), amongst other treatment options, also deals with composting, i.e. in-vessel, static pile and windrow and thus can provide some guidance to chicken waste/carcass composting. However when reviewing guidelines based on human bio-solids, it is important to consider that bio-solids from human sources are far less likely to carry *Salmonella* as the organism is only prevalent under situations of outbreaks. In contrast, in chickens *Salmonella* can be a normal gut inhabitant. Another key point is that the environmental impact of pathogens is generally considered in the human biosolids guidelines. The unique nature of carcass composting (size of bird carcass) requires special consideration in terms of considering composting parameters recommended for human bio-solid or even animal manures.

Based on the present review the organisms of most risk in terms of composting chicken waste and carcasses are the following:

- *Cl. botulinum* - due to the ability of the organism to survive a composting process and the risk to cattle
- *Salmonella* - a food borne pathogen which has the ability to re-grow following a composting process.

9.2 General overview of guidelines

The guidelines that are reviewed in this section are as follows:-

- Environmental Regulations and Technology, Control of Pathogens and Vector Attraction in Sewage Sludge (Including Domestic Septage) Under 40 CFR Part 503 EPA/625/R-92/013 Revised July 2003 (United States Environmental Protection Agency 2003)
- Queensland Stock Act
- Draft report by a UK group (Advisory committee on the microbiological safety of food ad hoc group 2006)
- A range of state guidelines
- US FDA guidelines (US Food and Drug Administration 1998)

9.2.1 US EPA Guidelines

The EPA guidelines (United States Environmental Protection Agency 2003) are based on control of pathogens in sewage sludge. While this document deals with viruses and parasites relevant to human waste, for the purpose of this review, only bacterial pathogens will be discussed here.

This document has a focus on pathogen control to protect both public health and the environment. The pathogen reduction requirements for sewage sludge are based on two major criteria:-

1 Requirements designed to control and reduce pathogens

2 Requirements designed to reduce the attraction of vectors that transmit these pathogens

The guidelines use a combination of microbiological and technological requirements (e.g. composting) to ensure pathogen reduction. These requirements are both performance based (microbiology) and technology based with composting is recognised as one such technology.

Because of concern over the effect of pathogens from biosolids on animal health (in the present context *Cl. botulinum*) the 503 regulations require that sewage sludge undergo pathogen treatment prior to land application. There are two treatment categories, which are designated as Class A and Class B.

For Class A biosolids, the requirement is to reduce pathogens below detection levels as defined in the 1992 US EPA regulation. More specifically, the microbiological requirement for Class A is as follows (with either of the requirements to be met):-

Salmonella <3 MPN per 4 g dry weight basis

Faecal coliforms 1000 MPN per g (dry weight basis).

In addition to meeting the above criteria, a vector reduction criterion (reduction of volatile solids by 38%) needs to be met to conform to Class A.

In the Class B biosolids, the requirement is to reduce pathogens to levels that are unlikely to pose a threat to public health and the environment under the specific conditions of use. Specifically, the faecal coliform density in the treated sewage sludge (biosolids) should be 2 million MPN or CFU per gram (dry weight basis).

Unlike Class A biosolids, which are essentially pathogen free, Class B biosolids may contain some pathogens. Site use restrictions that restrict crop harvesting, animal grazing and public access for a certain period of time are required. This allows environmental factors e.g. sunlight and desiccation) to play a role in further reducing pathogen levels.

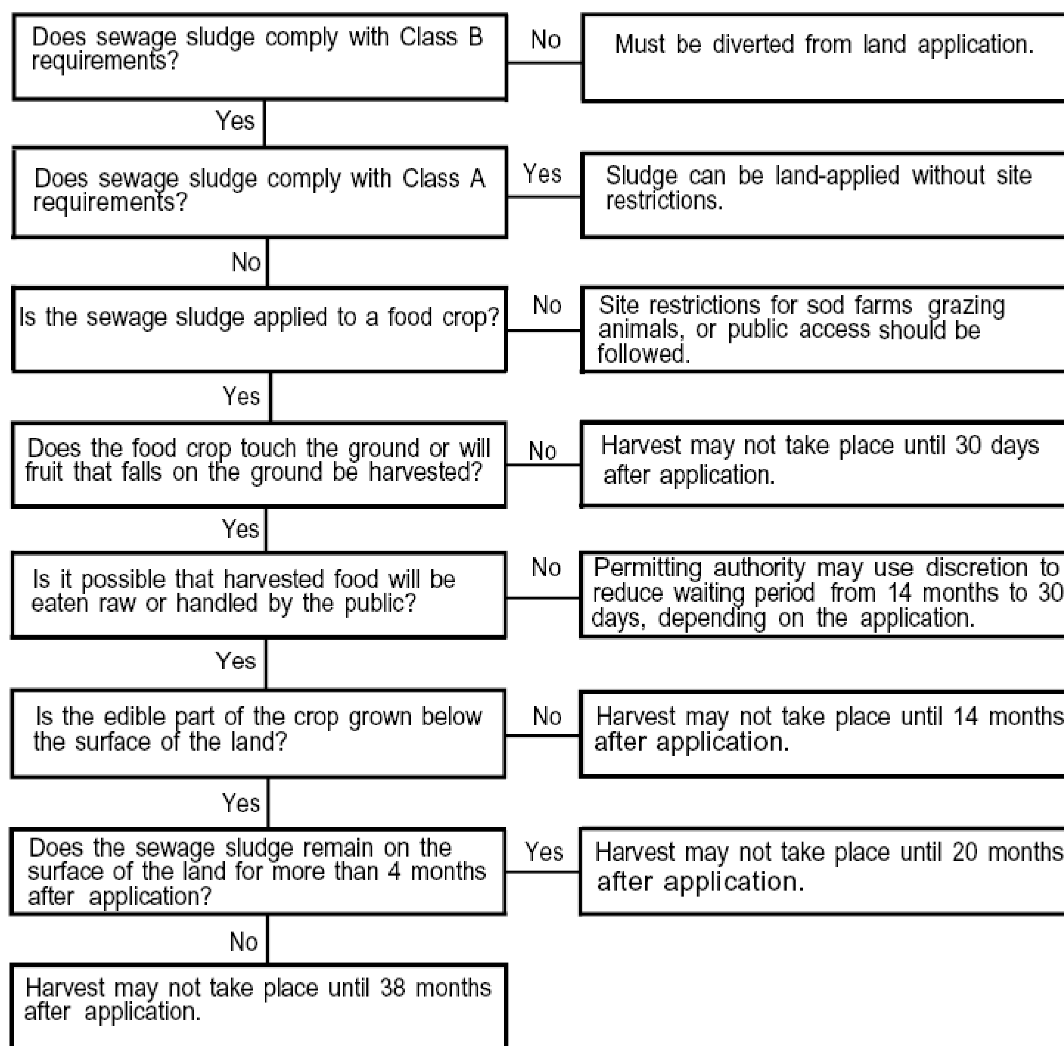
Since the application of Class B biosolids must be conducted in compliance with site restrictions and because it is not possible for regulators to follow the land application of biosolids applied on lawns and home gardens, Class B biosolids cannot be sold or given away in bags or other containers or applied on lawns and home gardens.

In the USA EPA guidelines, public health is protected by limiting the potential for public exposure to pathogens. This is accomplished through treatment of the sewage sludge or through a combination of sewage sludge treatment and restrictions on the land application site that prevent exposure to the pathogens in the biosolids and allow time for the environment to reduce the pathogens to below detectable levels. The Part 503 vector attraction reduction requirements also help reduce the spread of pathogens by birds, insects, and other disease carriers (i.e. vectors) by requiring that all sewage sludge that is to be land applied undergo vector attraction reduction.

One of the approaches for controlling pathogens (and relevant to this review) is composting that uses biological processes to generate heat to control pathogens. Vector reduction is accomplished either by treating the waste that it is no longer attractive to the vectors such as (insects, birds, rodents and domestic animals) or placing barriers. Technological as well as management options such as volatiles (solids) which attract vectors.

The control of possible pathogen transfer pathways due to agricultural use (or animal grazing) is achieved, using a decision tree (see Figure 9.1) that is provided in the guideline. The use of this decision tree requires specific decision making to enable the use of the compost based on the risk attributed to the specific application.

Figure 9.1 Decision tree for the land application of biosolids as in United States Environmental Protection Agency guidelines (2003)



The guideline also deals with restriction to animals and stipulates the following:

Animal Grazing

"503.32(b)(5)(v): Animals shall not be allowed to graze on the land for 30 days after application of sewage sludge. Biosolids can adhere to animals that walk on biosolids amended land and thereby be brought into potential contact with humans who come in contact with the animals (for example, horses and milking cows allowed to graze on a biosolids amended pasture). Thirty days is sufficient to substantially reduce the

pathogens in surface applied biosolids, thereby significantly reducing the risk of human and animal contamination".

There is also a 1 year waiting period for turf that has to be harvested under the same conditions.

The Guidelines also adopt "process controls" to manage pathogens as detailed below

1. Processes to Further Reduce Pathogens (PFRPs) which are linked to Class A (40 CFR Part 257). PFRP can be used to meet Class A requirement. The specific description of PFRPS is as follows:

"Using either the within-vessel composting method or the static aerated pile composting method, the temperature of sewage sludge is maintained at 55°C (131°F) or higher for 3 consecutive days. Using the windrow composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 15 consecutive days or longer. During the period when the compost is maintained at 55°C (131°F) or higher, there shall be a minimum of five turnings of the windrow

2. Processes to Significantly Reduce Pathogens (PSRPs) which are linked to Class B (40 CFR Part 257 regulation). PSRP can be used to achieve Class B requirements and are described as follows:

"For aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40°C (104°F) or higher and remains at 40°C (104°F) or higher for 5 days. For 4 hours during the 5 day period, the temperature in the compost pile exceeds 55°C (131°F)."

All specific operational details, microbiological controls, process controls and all laboratory methodology are discussed in the original document in detail.

9.2.2 Queensland Stock Act

In Queensland the Stock Regulation Act 1988 deals with stock access to animal matter or animal-contaminated matter applied to pasture and states the following:

"57 Restrictions for animal and animal-contaminated matter

(c) allow, or fail to take every reasonable measure to prevent, stock access to animal matter or animal-contaminated matter;
"1 applying fertilizer containing animal matter to a stock grazing pasture for the sole purpose of fertilizing the pasture and, before and during the application of the fertilizer, restricting access by stock to the fertilizer
2 lawfully discharging cattle feedlot effluent on a stock grazing pasture to dispose of the effluent
3 disposing of animal matter or animal-contaminated matter at a dump site that is suitably fenced, or placing the matter in suitable bins or burying the matter in a deep pit on the site, to prevent access to the matter by feral cattle, feral pigs and feral goats"

However the duration of preventing animal access to pasture is not specified. With this exception, there is no specific legislation – at either the national or state level – addressing the issue of ruminant exposure via grazing to animal matter or animal-contaminated matter applied to pasture.

9.2.3 England, Scotland. Wales and Northern Ireland

The relevant UK legislation (Advisory committee on the microbiological safety of food ad hoc group 2006) has been described in the following words (note direct reference to chicken carcasses):-

“The Animal By-Products Regulation (Regulation (EC) No 1774/2002) is enforced through the Animal By-Products Regulations 2003 in England, and equivalent legislation in Scotland, Wales and Northern Ireland. The Regulation prohibits the composting of **poultry carcases** or the spreading of litter or manure containing carcase material. Offences under the Regulations should be reported to the Local Authority who are responsible for enforcing the Regulations. Local Authorities have the right of access to farms for enforcement purposes. In Northern Ireland, offences under the Animal By-Products Regulations (NI) 2003 should be reported to DARDNI which is responsible for enforcing this aspect of the Regulations. DARDNI officials have a right of access to farms for enforcement purposes and have the power to serve Notices requiring the correct disposal of animal by-products.”

9.2.4 Other Australian Guidelines

In this section, the guidelines identified by this review are listed, along with a few key relevant comments.

a) AS 4454—2003 Australian Standard Composts, soil conditioners and mulches.

Describes general composting but does not deal with pathogens.

b) "Environmental guidelines for composting and other organic recycling facilities - Recycling organic material to benefit the environment". Publication 508 June 1996 – EPA Victoria

This guideline has a component based on composting and includes animal sourced materials.

c) Guidelines for environmental management biosolids land application EPA Victoria Publication 943 ISBN 0 7306 7641 2 EPA Victoria, April 2004

This guideline is principally derived from the US EPA 503 requirements including Processes to Further Reduce Pathogens (PFRP) (United States Environmental Protection Agency 2003). Animal waste is not directly addressed in these guidelines. However the principles described may serve as a useful basis for the management of the composting process. This guideline has a comprehensive description of pathogens, *Salmonella* and *E. coli.* Issues such as post process re-growth by *Salmonella* are addressed within these guidelines.

d) "South Australian Biosolids Guidelines - for the safe handling, reuse or disposal of biosolids" - Department of Environment and Natural resources – South Australia

These guidelines were developed for application to bio-solids produced from sewage.

e) “Western Australian Guidelines for direct land application of biosolids and biosolid products” February 2002 – Department of Environmental Protection, Water and Rivers Commission and Department of Health.

These guidelines were developed for application to bio-solids produced sewage and excludes piggeries.

9.2.5 US FDA Guideline for Fruits and Vegetables

The US FDA document “Guidance for Industry, Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables” (US Food and Drug Administration 1998) is a comprehensive overview of the relationships between food crop production systems and risk activities such as composting and use of animal manures and animal sourced composts as fertilisers.

9.3 Specific details within relevant existing Guidelines

In this section, specific details on the microbiological criteria mentioned in the guidelines listed in the previous section are summarised.

Victorian Guidelines

Guidelines	**Nature of Material**	***Salmonella***	***E. coli* /Thermotolerant coliforms**
Environmental guidelines for composting and other organic recycling facilities. Vic EPA	Compost – especially if derived from sewage sludge, **animal excreta, offal**, grease trap sludge, food residues, or unsegregated municipal solid waste – needs effective treatment to reduce the risk of pathogen transmission.	< 1 MPN in 50 grams*	< 100 MPN per gram of solids*

* Maximum Levels (dry weight basis)

Guidelines	**Nature**	***Salmonella***	***E. coli* /Thermotolerant coliforms**
Guidelines for environmental management – Bio-solids Land Application EPA Victoria	T2/C2	<10 *Salmonella* per 50 g dw	<1000 *E. coli* MPN per g dw

T2 is Treatment Grade 2. One method to achieve T2 is composting where the temperature of all compost material to be ≥53°C for ≥5 continuous days or ≥55°C for ≥3 continuous days. C2 is contamination grade 2 (fully detailed in the Guidelines).

Bio-solids coming from layer waste composts would typically be regarded as T2 treatment grade and the C2 contamination grade. The guidelines then provide details of the management strategy required for use of this material on pasture or fodder as shown below.

Category of use	***Minimum Grade***	***Management controls – animal withholding periods and food safety 1***
Pasture/Fodder for cattle (dairy and beef) and poultry (but not pigs)	**T2 / C2**	30 day or until pasture re-established for grazing (and forage) withholding period post soil incorporation or post surface spreading with 'watering in' / rainfall. Otherwise, a 60-day withholding period for surface application applies. Application site EIP focused on any contaminant risks, including ensuring soil limits are not exceeded and preferably incorporation of bio-solids into soil matrix. Surface application requires assessment of risks to food standards.

<u>West Australian Guidelines</u>

These guidelines generally deal with bio-solids. However, the following criteria deal with a composted product.

Pathogen Grade	**Maximum Pathogen Levels**	**Treatment Methods That Typically Achieve the Requisite Pathogen Levels***
Grade P2	<10 Salmonella per 50 g of dry product **AND** Thermo-tolerant Coliforms - < 1000 per g of dry product	Composted at >53 °C for a 5 day period. OR Composted at >55 °C for a 3 day period. OR Heated to 70 °C for 1 hour and then dried to >90% solids. OR Digested, heated to 70 °C for 1 hour and then dried to >75% solids. OR Aerobic thermophilic digestion (55-60 °C for a 10 day period), with a volatile solids reduction of >38% and total solids reduction of >50%.

<u>South Australian Guidelines</u>

Under these guidelines, compost with < 1 *Salmonella* in 50 g are classified as Stabilisation Grade A. These products have the following permitted uses without EPA approval - Home Garden, Urban Landscaping, Agriculture (non-irrigated) and Forestry Site Rehabilitation.

9.4 Possible criteria relevant for chicken waste composts

Criteria for layer waste composting need to be developed encompassing the three major risk areas.

- Human health
- Cattle health
- Environmental aspects that can have both a direct or in direct impact on the above two criteria

The issues surrounding *Cl. botulinum* are complex. As discussed in the review many factors need to be taken into account:

- composting of layer wastes is widely practiced in the USA
- carcass composting is not permitted in some countries for e.g. the UK because of the risk of animal botulism
- as listed in the review the key situation is that the composting is carried out per best practice, with turning and aeration that are conducive for carcass breakdown and not creating anaerobic conditions suitable for proliferation of *Cl botulinum*. The toxins are suggested to be heat labile and extended heating at 55°C may denature the toxins (if present).
- the issue of spores and the environment is a hypothetical situation but nevertheless needs to be taken into consideration. If spores are present and do survive the composting process they are likely to transfer in to the environment. If the waste is continuously disposed in the same area, a build up of spores is possible. This can be balanced by subsequent dilution and dispersion over time.

- The key is high composting standards such as contained compost operations that control issues such as run-off and vectors to protect the production system.
- Cattle botulism outbreaks in Queensland occurred within a totally different context i.e. the use of chicken litter as a feed component. Nevertheless the possible presence of Types C and D in Australian (Queensland) environments needs to be taken into account.
- Another measure which can be adopted is to strictly limit/prevent cattle access to areas where composted carcass waste has been spread (the rational behind the British guidelines)
- A HACCP plan can be developed to have documented process controls during each compost operation. This would allow the history of the process can be monitored and the possibility of taking corrective action
- Above all, the education of all personnel and farmers dealing with the composting operation is vital
- The limited Australian study has recovered botulinum toxin from one sample. This sample was a poorly composted sample which contained a rotting chicken carcass. The positive toxin result indicated the presence of the organism and confirmed that the conditions for toxin production existed within that compost site.

As previously cited in the USEPA document, PFRP type process controls for composting have the possibility of achieving Class A compost. This requires temperatures of 55°C for 15 days for windrows and 55°C for 3 days for static

aerated piles. The windrows require a minimum of 5 turns. This process is recognised by the guidelines to be able to reduce the non-spore forming bacteria like *Salmonella.*

Thus the following recommendations are made

a) Composting should be performed using all the standard recommended procedures for best practice composting (includes aeration, management of run-off, and control of wild animals). The aim of this composting should be to achieve a temperature of 55°C for 15 days for windrows with a minimum of 5 turns. For piles or bin contained composting the aim should be to achieve a temperature of above 55°C for 3 days.

b) Microbiological criteria should be focussed on *Salmonella* levels and reflect the end-use of the product. For end-uses such as vegetables that undergo minimal processing, the *Salmonella* level should be set at absence in 50 g (dry weight). For use on pasture, the existing generally used criterion of < 10 *Salmonella* per 50 g (dry weight) should be used and supported by a with-holding period based on pasture re-establishment, soil incorporation and so on (30 to 60 days (depending upon local management and environmental conditions along the lines suggested by the Victorian EPA guidelines.

c) To manage the issue of the risk of animal botulism a decision tree to guide compost producers and end users needs to be developed. Additionally a HACCP approach is required to support this decision tree

9.5 Summary

- A range of guidelines on the methods of processing and the microbiological quality of bio-solids (and other) exist which can form the basis for a set of criteria to ensure microbiological safety of compost
- Based on the (United States Environmental Protection Agency 2003) guideline the safety of the product could be adopted (a) via a process control and

- Specific possible criteria for layer waste composts are provided and focus on use of best practice composting, level of *Salmonella* (not *E. coli*) and the use of decision tree / HACCP plans to mange the animal botulism risk

Chapter 10

References

Advisory committee on the microbiological safety of food ad hoc group (2006) *BOTULISM IN CATTLE.*

Allison, M.J., Maloy, S.E. and Matson, R.R. (1976) Inactivation of Clostridium botulinum toxin by ruminal microbes from cattle and sheep. *Appl Environ Microbiol* 32, 685-688.

Anderson, G.L., Kenney, S.J., Millner, P.D., Beuchat, L.R. and Williams, P.L. (2006a) Shedding of foodborne pathogens by *Caenorhabditis elegans* in compost-amended and unamended soil. *Food Microbiol* 23, 146-153.

Anderson, L.A., Miller, D.A. and Trampel, D.W. (2006b) Epidemiological investigation, cleanup, and eradication of pullorum disease in adult chickens and ducks in two small-farm flocks. *Avian Dis* 50, 142-147.

Anon (2001) *Composting Poultry Mortality - Alabama Guide Sheet No. AL 317*: US Department of Agriculture

Appleyard, W.T. and Mollison, A. (1985) Suspected bovine botulism associated with broiler litter waste. *Vet Rec* 116, 522.

Arsenault, J., Letellier, A., Quessy, S., Normand, V. and Boulianne, M. (2007) Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. *Prev Vet Med* 81, 250-264.

Australian Salmonella Reference Centre (2006) *2006 Annual Report*. Adelaide: Institute for Medical and Veterinary Science.

Bailey, J.S. and Cosby, D.E. (2005) *Salmonella* prevalence in free-range and certified organic chickens. *J Food Protect* 68, 2451-2453.

Baker, R.C., Paredes, M.D. and Qureshi, R.A. (1987) Prevalence of *Campylobacter jejuni* in eggs and poultry meat in New York State. *Poult Sci* 66, 1766-1770.

Barbour, E.K. and Nabbut, N.H. (1982) Isolation of salmonella and some other potential pathogens from two chicken breeding farms in Saudi Arabia. *Avian Dis* 26, 234-244.

Bendfeldt, E.S., Peer, R.W. and Flory, G.A. (2006) In-house composting as a rapid response to Avian influenza. *BioCycle* 47, 38-42.

Blake, J.P. (2004) Methods and technologies for handling mortality losses. *World's Poult Sci J* 60, 489-499.

Böhnel, H. (2002) Household biowaste containers (bio-bins) - Potential incubators for *Clostridium botulinum* and botulinum neurotoxins. *Wat Air Soil Poll* 140, 335-341.

Böhnel, H., Briese, B.H. and Gessler, F. (2002) Methods for health risk assessment by *Clostridium botulinum* in biocompost. In *Microbiology of Composting* ed. H, I., N. Riddech, S. Klammer pp.517-526. Berlin-Heidelberg: Springer-Verlag.

Böhnel, H. and Lube, K. (2000) *Clostridium botulinum* and bio-compost. A contribution to the analysis of potential health hazards caused by bio-waste recycling. *J Vet Med B Infect Dis Vet Public Health* 47, 785-795.

Böhnel, H., Wernery, U. and Gessler, F. (2003) Two Cases of Equine Grass Sickness with Evidence for Soil-borne Origin Involving Botulinum Neurotoxin. *J Vet Med B* 50, 178-182.

Bonhotal, J. (2006) Poultry Waste Composting at Brey Egg Farm: Case Study.

Brodie, H.L., Carr, L.E. and Condon, P. (2000) A comparison of static pile and turned windrow methods for poultry litter compost production. *Compost Sci Utilization* 8, 178-189.

Burge, W.D., Enkiri, N.K. and Hussong, D. (1987) *Salmonella* regrowth in compost as influenced by substrate. *Microb Ecol* 14, 243-253.

Caldwell, K.N., Anderson, G.L., Williams, P.L. and Beuchat, L.R. (2003) Attraction of a free-living nematode, *Caenorhabditis elegans*, to foodborne pathogenic bacteria and its potential as a vector of *Salmonella poona* for preharvest contamination of cantaloupe. *J Food Protect* 66, 1964-1971.

Campanella, O.H. and Peleg, M. (2001) Theoretical comparison of a new and the traditional method to calculate *Clostridium botulinum* survival during thermal inactivation. *J Sci Food Agri* 81, 1069-1076.

Carrillo, C.M.L., Connerton, P.L., Pearson, T. and Connerton, I.F. (2007) Free-range layer chickens as a source of *Campylobacter* bacteriophage. *Antonie Van Leeuwenhoek Inter J Gen Mol Microbiol* 92, 275-284.

Carter, T.A., Anderson, K.E., Arends, J., Barker, J.C., Bunton, K., Hawkins, B., Parsons, J., Rives, D.V., Scheideler, S.E., Stringham, S.M. and Wineland, M.J. (1996) *Composting Poultry Mortality*: North Carolina State University, 10/89, PS&T Guide #47

Cekmecelioglu, D., Demirci, A. and Graves, R.E. (2005a) Feedstock optimization of in-vessel food waste composting systems for inactivation of pathogenic microorganisms. *J Food Prot* 68, 589-596.

Cekmecelioglu, D., Demirci, A., Graves, R.E. and Davitt, N.H. (2004) Optimization of windrow food waste composting to inactivate pathogenic microorganisms. In *ASAE Annual International Meeting 2004*. pp.7545-7562.

Cekmecelioglu, D., Demirci, A., Graves, R.E. and Davitt, N.H. (2005b) Optimization of windrow food waste composting to inactivate pathogenic microorganisms. *Transactions of the ASAE* 48, 2023-2032.

Cekmecelioglu, D., Heinemann, P.H., Demirci, A. and Graves, R.E. (2005c) Modeling of compost temperature and inactivation of *Salmonella* and *E. coli* O157:H7 during windrow food waste composting. *Trans Am Soc Agric Engin* 48, 849-858.

Ceustermans, A., De Clercq, D., Aertsen, A., Michiels, C., Coosemans, J. and Ryckeboer, J. (2007) Inactivation of *Salmonella* Senftenberg strain W 775 during composting of biowastes and garden wastes. *J Appl Microbiol* **103**, 53-64.

Chaffer, M., Baum, M., Grinberg, K., Molad, T. and Elad, D. (2006) Application of PCR for Detection of *Clostridium botulinum* Type D in Bovine Samples. *J Vet Med B* 53, 45-47.

Chalmers, G., Martin, S.W., Hunter, D.B., Prescott, J.F., Weber, L.J. and Boerlin, P. (2008) Genetic Diversity of *Clostridium perfringens* Isolated from Healthy Broiler Chickens at a Commercial Farm. *Vet Microbiol* 127, 116-127.

Chinivasagam, H.N., Redding, M., Runge, G.A. and Blackall, P.J. (2006) A survey on the presence and levels of food-borne pathogens in Australian broiler litter. In *Australian Veterinary Poultry Alliance – Scientific meeting*. Gold Coast.

Chinivasagam, H.N., Thomas, R.J., Casey, K., McGahan, E., Gardner, E.A., Rafiee, M. and Blackall, P.J. (2004) Survey of piggery effluent ponds for selected pathogens and indicator organisms. *J Appl Microbiol* 97, 883-891.

Christensen, K.K., Carlsbaek, M. and Kron, E. (2002) Strategies for evaluating the sanitary quality of composting. *J Appl Microbiol* 92, 1143-1158.

Collins, E.R. (1996) *Composting Dead Poultry* Virginia Coorporative Extention - Publication Number 442-037.

Conner, D.E., Blake, J.P. and Donald, J.O. (1991) Microbiological safety of composted poultry farm mortalities. . In *ASAE/ CSAE Meeting*. pp.Paper No. 91-4053. St Joseph, MO: The American Society of Agricultural Engineers.

Cooperband, L.R. and Middleton, J.H. (1996) Changes in chemical, physical and biological properties of passively-aerated cocomposted poultry litter and municipal solid waste compost. *Compost Sci & Utilization* 4, 24-34.

Cox, J.M., Woolcock, J.B. and Sartor, A.L. (2002) The significance of *Salmonella,* particularly *S.* Infantis, to the Australian egg industry. RIRDC Web Publication No W02/028: Rural Industries Research and Development Corporation.

Crawshaw, T. and Young, S. (2003) Increased mortality on a free-range layer site [5]. *Vet Record* 153, 664.

Critchley, E.M. (1991) A comparison of human and animal botulism: a review. *J R Soc Med* 84, 295-298.

Curci, V.C.L.M., Dutra, I.S., Dobereiner, J. and Lucas Jr, J. (2007) The effect of composting cattle carcasses on botulinum spores and toxin content. *Pesquisa Vet Brasileira* 27, 157-161.

Das, K.C., Minkara, M.Y., Melear, N.D. and Tollner, E.W. (2002) Effect of poultry litter amendment on hatchery waste composting. *J Appl Poult Res* 11, 282-290.

Davies, R. and Breslin, M. (2001) Environmental contamination and detection of *Salmonella enterica* serovar enteritidis in laying flocks. *Vet Rec*149, 699-704.

Davies, R. and Breslin, M. (2003a) Observations on *Salmonella* contamination of commercial laying farms before and after cleaning and disinfection. *Vet Rec* 152, 283-287.

Davies, R.H. and Breslin, M. (2003b) Persistence of *Salmonella* Enteritidis Phage Type 4 in the environment and arthropod vectors on an empty free-range chicken farm. *Environ Microbiol* 5, 79-84.

Davies, R.H. and Wray, C. (1995) Mice as carriers of *Salmonella enteritidis* on persistently infected poultry units. *Vet Rec* 137, 337-341.

Déportes, Benoit-Guyod, Zmirou and Bouvier (1998) Microbial disinfection capacity of municipal solid waste (MSW) composting. *J Appl Microbiol* 85, 238-246.

Déportes, I., Benoit-Guyod, J. and Zmirou, D. (1995) Hazard to man and the environment posed by the use of urban waste compost: a review. *Science of the Total Environment* 172, 197-222.

DeRouchey, J.M., Harner, J.P. and Murphy, J.P. (2005) Catastrophic mortality composting: Is it safe and effective? *J Appl Poult Res* 14, 414-416.

Desmarais, T.R., Solo-Gabriele, H.M. and Palmer, C.J. (2002) Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl Environ Microbiol* 68, 1165-1172.

Dohms, J.E. (2003) Botulism. In *Diseases of Poultry* eds. Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R. and Swayne, D.E. pp.785-791. Ames: Iowa State University Press.

Dohms, J.E., Allen, P.H. and Rosenberger, J.K. (1982) Cases of type C botulism in broiler chickens. *Avian Dis* 26, 204-210.

Dohms, J.E. and Cloud, S.S. (1982) Susceptibility of broiler chickens to Clostridium botulinum type C toxin. *Avian Dis* 26, 89-96.

Douglas, I. (2001) Chicken litter – a botulism risk to stock: Department of Primary Industries and Fisheries, Queensland.

Droffner, M.L. and Brinton, W.F. (1995) Survival of *E. coli* and *Salmonella* populations in aerobic thermophilic composts as measured with DNA gene probes. *Zentral Hyg Umweltmed* 197, 387-397.

Eales, C.E. and Turner, A.W. (1952) Description of *Clostridium botulinum* type D recovered from soil in South Australia. *Aust J Exp Biol Med Sci* 30, 295-300.

Elwell, D.L., Keener, H.M., Carey, D.S. and Schlak, P.P. (1998) Composting unamended chicken manure. *Compost Sci Utilization* 6, 22-35.

Elwell, D.L., Keener, H.M. and Hansen, R.C. (1996) Controlled, high rate composting of mixtures of food residuals, yard trimmings and chicken manure. *Compost Sci Utilization* 4, 6-15.

Eriksson De Rezende, C.L., Mallinson, E.T., Gupte, A. and Joseph, S.W. (2001) Salmonella spp. are affected by different levels of water activity in closed microcosms. *J Ind Microbiol Biotechnol* 26, 222-225.

Esteban, J.I., Oporto, B., Aduriz, G., Juste, R.A. and Hurtado, A. (2008) A survey of food-borne pathogens in free-range poultry farms. *Int J Food Microbiol* In Press, Corrected Proof.

Etzel, T. ACE POULTRY WASTE COMPOSTING. In *Compost fact sheet*.

Fitzpatrick, S. (2006) Botulism Poisoning in Cattle in the Northern Territory. In *Agnote No: K29*: Department of Primary Industries, Fisheries and Mines, Northern Territory Government.

Flory, G.A., Peer, R.W. and Bendfeldt, E.S. (2006) Composting wild birds, game birds and backyard poultry. *BioCycle* 47, 52-54.

Freeman, P. and Bevan, P. (2007) *Botulism in cattle* No. PRIMEFACT 596: NSW Department of Primary Industries.

Garcia-Siera, J., Rozeboom, D.W., Straw, B.E., Thacker, B.J., Granger, L.M., Fedorka-Cray, P.J. and Gray, J.T. (2001) Studies on survival of pseudorabies virus, *Actinobacillus pleuropneumoniae*, and *Salmonella* serovar Choleraesuis in composted swine carcasses. *J Swine Health Prod* 9, 225-231.

Gast, R.K. and Beard, C.W. (1993) Research to understand and control *Salmonella enteritidis* in chickens and eggs. *Poultry Sci* 72, 1157-1163.

Gerba, C.P., Goyal, S.M., La Bell, R.L., Cech, I. and Bodgan, G.F. (1979) Failure of indicator bacteia to reflect the occurrence of enteroviruses in marine waters. *Am J Public Health* 69, 1116-1119.

Gerba, C.P. and Smith, J.E. (2005) Sources of pathogenic microorganisms and their fate during land application of wastes. *J Environ Qual* 34, 42-48.

Gessler, F. and Böhnel, H. (2006a) Persistence and mobility of a *Clostridium botulinum* spore population introduced to soil with spiked compost. *FEMS Microbiol Ecol* 58, 384-393.

Gessler, F. and Böhnel, H. (2006b) Persistence and mobility of a *Clostridium botulinum* spore population introduced to soil with spiked compost. *FEMS Microbiology Ecology* 58, 384-393.

Gibbs, D.S., Anderson, G.L., Beuchat, L.R., Carta, L.K. and Williams, P.L. (2005) Potential role of *Diploscapter* sp. strain LKC25, a bacterivorous nematode from soil, as a vector of food-borne pathogenic bacteria to preharvest fruits and vegetables. *Appl Environ Microbiol* 71, 2433-2437.

Girardin, H., Morris, C.E., Albagnac, C., Dreux, N., Glaux, C. and Nguyen-The, C. (2005) Behaviour of the pathogen surrogates *Listeria innocua* and *Clostridium sporogenes* during production of parsley in fields fertilized with contaminated amendments. *FEMS Microbiol Ecol* 54, 287-295.

Glanville, T.D. and Trampel, D.W. (1997) Composting alternative for animal carcass disposal. *JAVMA* 210, 1116-1120.

Gong, C.M., Koichi, I., Shunji, I. and Takashi, S. (2005) Survival of pathogenic bacteria in compost with special reference to *Escherichia coli. J Environ Sci (China)* 17, 770-774.

González, J.L. and Sánchez, M. (2005) Treatment of poultry mortalities on poultry farms. *Compost Sci Utilization* 13, 136-140.

Goss, M. and Richards, C. (2007) Development of a risk-based index for source water protection planning, which supports the reduction of pathogens from agricultural activity entering water resources. *J Environ Man* In Press, Corrected Proof.

Governo, J., Gaskin, J., Faucette, B. and Borden, D. (2003) Large-Scale Composting in Georgia*, The Compost White Paper*. Atlanta,: Department of Natural Resources, Georgia.

Graham, J.M., Smith, G.R., Borland, E.D. and MacDonald, J.W. (1978) Avian botulism in winter and spring and the stability of *Clostridium botulinum* type C toxin. *Vet Rec* 102, 40-41.

Gregory, A.R., Ellis, T.M., Jubb, T.F., Nickels, R.J. and Cousins, D.V. (1996) Use of enzyme-linked immunoassays for antibody to types C and D botulinum toxins for investigations of botulism in cattle. *Aust Vet J* 73, 55-61.

Gregory, E., Barnhart, H., Dreesen, D.W., Stern, N.J. and Corn, J.L. (1997) Epidemiological study of *Campylobacter* spp. in broilers: Source, time of colonization, and prevalence. *Avian Dis* 41, 890-898.

Grimes, T. and Jackson, C. (2001) *Code of Practice for biosecurity in the egg industry*: Research and Development Corporation.

Guillouais, S. and Couronne, M.P. (2003) Studies of rustic composting of chicken dung; interests and limits. *Essais de compostage rustique des fumiers de volailles; intérêts et limites*, 27-34.

Haque, A.K.M.A. and Vandepopuliere, J.M. (1994) Composting Cage Layer Manure with Poultry Litter. *J Appl Poult Res* 3, 268-273.

Hassen, A., Belguith, K., Jedidi, N., Cherif, A., Cherif, M. and Boudabous, A. (2001) Microbial characterization during composting of municipal solid waste. *Bioresour Technol* 80, 217-225.

Haug, R.T. (1993) Composting systems. In *The Practical Handbook of Compost Engineering*. pp.21-93. Cited by Kalbasi, A., S. Mukhtar, S. E. Hawkins, and B. W. Auvermann, 2005: Carcass composting for management of farm mortalities: A review. Compost Science and Utilization, 2013, 2180-2193.: Lewis Publishers, CRC Press Inc.

Heinonen-Tanski, H., Mohaibes, M., Karinen, P. and Koivunen, J. (2006) Methods to reduce pathogen microorganisms in manure. *Livestock Science Biosecurity of Livestock Effluents* 102, 248-255.

Herrmann, R.F. and Shann, J.F. (1997) Microbial Community Changes During the Composting of Municipal Solid Waste. *Microb Ecol* 33, 78-85.

Himathongkham, S. and Riemann, H. (1999) Destruction of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in chicken manure by drying and/or gassing with ammonia. *FEMS Microbiol Lett* 171, 179-182.

Hog Producers Sustainable Farming Group (1996) *Managing poultry mortality composting systems*: Ministry of Agriculture and Food, British Columbia.

Hogg, R.A., White, V.J. and Smith, G.R. (1990) Suspected botulism in cattle associated with poultry litter. *Vet Rec* 126, 476-479.

Holdeman, L.V. (1970) The ecology and natural history of *Clostridium botulinum*. *J Wildlife Dis* 6, 205-210.

Hubalek, Z. and Halouzka, J. (1988) Thermal sensitivity of *Clostridium botulinum* type C toxin. *Epidemiol Infect* 101, 321-325.

Humphrey, T. (2004) *Salmonella*, stress responses and food safety. *Nature Reviews Microbiology* 2, 504-509.

Humphrey, T., O'Brien, S. and Madsen, M. (2007) Campylobacters as zoonotic pathogens: A food production perspective. *Int J Food Microbiol* 117, 237-257.

Humphrey, T.J., Martin, K.W., Slader, J. and Durham, K. (2001) *Campylobacter* spp. in the kitchen: spread and persistence. *J Appl Microbiol* 90, 115S-120S.

Huss, H.H. (1980) Distribution of *Clostridium botulinum*. *Appl Environ Microbiol* 39, 764-769.

Hutchison, M.L., Walters, L.D., Avery, S.M. and Moore, A. (2005a) Decline of zoonotic agents in livestock waste and bedding heaps. *J Appl Microbiol* 99, 354-362.

Hutchison, M.L., Walters, L.D., Moore, A. and Avery, S.M. (2005b) Declines of zoonotic agents in liquid livestock wastes stored in batches on-farm. *J Appl Microbiol* 99, 58-65.

Hutchison, M.L., Walters, L.D., Moore, A., Crookes, K.M. and Avery, S.M. (2004) Effect of Length of Time before Incorporation on Survival of Pathogenic Bacteria Present in Livestock Wastes Applied to Agricultural Soil. *Appl Environ Microbiol* 70, 5111-5118.

Hutchison, M.L., Walters, L.D., Moore, T., Thomas, D.J. and Avery, S.M. (2005c) Fate of pathogens present in livestock wastes spread onto fescue plots. *Appl Environ Microbiol* 71, 691-696.

Imbeah, M. (1998) Composting piggery waste: A review. *Bio Technol* 63, 197-203.

Jay, J. (1978) *Modern Food Microbiology*. New York: Van Nostrand Reinhold Company.

Jay, S., Davos, D., Dundas, D., Frankish, F. and Lightfoot, D. (2003) Salmonella. In *Foodborne Microorganisms of Public Health Significance* ed. Hocking, A.D. pp.207-266. Sydney: Australian Institute of Food Science and Technology.

Jean, D., Fecteau, G., Scott, D., Higgins, R. and Quessy, S. (1995) *Clostridium botulinum* type C intoxication in feedlot steers being fed ensiled poultry litter. *Can Vet J* 36, 626-628.

Jeffrey, J.S. (2001) Guidelines to curb bacteria in stacked poultry litter. *World Poultry* 17, 45.

Johnson, E.A., Summanen, P. and Finegold, S.M. (2007) Clostridium. In *Manual of Clinical Microbiology, Vol 1* eds. Murray, P.R., Baron, E.J., Landry, M.L., Jorgensen, J.H. and Pfaller, M.A. Washington: American Society of Microbiology.

Kalbasi, A., Mukhtar, S., Hawkins, S.E. and Auvermann, B.W. (2005) Carcass composting for management of farm mortalities: A review. *Compost Sci Utilization* 13, 180-193.

Kalbasi, A., Mukhtar, S., Hawkins, S.E. and Auvermann, B.W. (2006) Design, utilization, biosecurity, environmental and economic considerations of carcass composting. *Compost Sci Utilization* 14, 90-102.

Karasawa, T., Wang, X., Maegawa, T., Nakamura, S., Hang'ombe, B.M. and Isogai, E. (2000) Demonstration of botulinum toxins of types B and D in soil samples from Zambia. *Ann Trop Med Parasitol* 94, 409-411.

Keener, H.M., Elwell, D.L. and Monnin, M.J. (2000) Procedures and equations for sizing of structures and windrows for composting animal mortalities. *Applied Engineering in Agriculture* 16, 681-692.

Kelleher, B.P., Leahy, J.J., Henihan, A.M., O'Dwyer, T.F., Sutton, D. and Leahy, M.J. (2002) Advances in poultry litter disposal technology - a review. *Biores Technol* 83, 27-36.

Kenney, S.J., Anderson, G.L., Williams, P.L., Millner, P.D. and Beuchat, L.R. (2005) Persistence of *Escherichia coli* O157:H7, *Salmonella Newport*, and *Salmonella Poona* in the gut of a free-living nematode, *Caenorhabditis elegans*, and transmission to progeny and uninfected nematodes. *Int J Food Microbiol* 101, 227-236.

Kenney, S.J., Anderson, G.L., Williams, P.L., Millner, P.D. and Beuchat, L.R. (2006) Migration of *Caenorhabditis elegans* to manure and manure compost and potential for transport of *Salmonella newport* to fruits and vegetables. *Int J Food Microbiol* 106, 61-68.

Kinde, H., Castellan, D.M., Kerr, D., Campbell, J., Breitmeyer, R. and Ardans, A. (2005) Longitudinal monitoring of two commercial layer flocks and their environments for *Salmonella enterica* serovar enteritidis and other salmonellae. *Avian Dis* 49, 189-194.

Knoll, K.H. (1961) Public health and refuse disposal. *Compost Sci* 2, 35-40.

Kozaki, S. and Notermans, S. (1980) Stabilities of *Clostridium botulinum* type B and C toxins in ruminal contents of cattle. *Appl Environ Microbiol* 40, 161-162.

Kreher, H. Demonstrating poultry waste drying and composting. *Fact sheet* (http://cwmi.css.cornell.edu/krehercasestudy.pdf - checked 2/03/08).

Krogstad, O. and Gudding, R. (1975) The survival of some pathogenic micro-organisms during reactor composting. *Acta Agric Scand* 25, 281-284.

Kwak, W.S., Huh, J.W. and McCaskey, T.A. (2005) Effect of processing time on enteric bacteria survival and on temperature and chemical composition of broiler poultry litter processed by two methods. *Biores Technol* 96, 1529-1536.

Lapuz, R., Tani, H., Sasai, K., Shirota, K., Katoh, H. and Baba, E. (2007) An epidemiological analysis of *Salmonella enteritidis* contamination in a rat-infested chicken layer farm, an egg processing facility, and liquid egg samples by pulsed-field gel electrophoresis. *J Vet Med Sci* 69, 649-652.

Lasaridi, K., Protopapa, I., Kotsou, M., Pilidis, G., Manios, T. and Kyriacou, A. (2006) Quality assessment of composts in the Greek market: The need for standards and quality assurance. *J Environ Management* 80, 58-65.

Lau, A.K., Lo, K.V., Liao, P.H. and Yu, J.C. (1992) Aeration experiments for swine waste composting. *Biores Technol* 41, 145-152.

Lawson, M.J. and Keeling, A.A. (1999) Production and physical characteristics of composted poultry carcases. *British Poult Sci* 40, 706-708.

Lemunier, M., Francou, C., Rousseaux, S., Houot, S., Dantigny, P., Piveteau, P. and Guzzo, J. (2005) Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts. *Appl Environ Microbiol* 71, 5779-5786.

Leon-Velarde, C.G., Cai, H.Y., Larkin, C., Bell-Rogers, P., Stevens, R.W.C. and Odumeru, J.A. (2004) Evaluation of methods for the identification of *Salmonella enterica* serotype Typhimurium [*S. typhimurium*] DT104 from poultry environmental samples. *J Microbiol Methods* 58, 79-86.

Liebana, E., Garcia-Migura, L., Clouting, C., Clifton-Hadley, F.A., Breslin, M. and Davies, R.H. (2003) Molecular fingerprinting evidence of the contribution of wildlife vectors in the maintenance of *Salmonella* Enteritidis infection in layer farms. *J Appl Microbiol* 94, 1024-1029.

Limawongpranee, S., Hayashidani, H., Okatani, A.T., Hirota, C., Kaneko, K. and Ogawa, M. (1999) Contamination of Salmonella blockley in the environment of a poultry farm. *Avian Dis* **43**, 302-309.

Livesey, C.T., Sharpe, R.T. and Hogg, R.A. (2004) Recent association of cattle botulism with poultry litter. *Vet Rec* 154, 734-735.

Logsdon, G. (1993) Composting chicken litter in indoor windrows. *BioCycle* 34, 62-63.

Long, S.C. and Tauscher, T. (2006) Watershed issues associated with Clostridium botulinum: A literature review. *J Water Health* 4, 277-288.

Losikoff, M.E. (1978) Establishment of a heat inactivation curve for Clostridium botulinum 62A toxin in beef broth. *Appl Environ Microbiol* 36, 386-388.

Lung, A.J., Lin, C.M., Kim, J.M., Marshall, M.R., Nordstedt, R., Thompson, N.P. and Wei, C.I. (2001) Destruction of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in cow manure composting. *J Food Prot* 64, 1309-1314.

Luquez, C., Bianco, M.I., de Jong, L.I.T., Sagua, M.D., Arenas, G.N., Ciccarelli, A.S. and Fernandez, R.A. (2005) Distribution of botulinum toxin-producing clostridia in soils of Argentina. *Appl Environ Microbiol* 71, 4137-4139.

Martin, S. (2003) *Clostridium botulinum* type D intoxication in a dairy herd in Ontario. *Can Vet J* 44, 493-495.

McCrea, B.A. and Macklin, K.S. (2006) Effect of different cleaning regimens on recovery of *Clostridium perfringens* on poultry live haul containers. *Poult Sci* 85, 909-913.

McLoughlin, M.F., McLlroy, S.G. and Neill, S.D. (1988) A major outbreak of botulism in cattle being fed ensiled poultry litter. *Vet Rec* 122, 579-581.

Methner, U., Diller, R., Reiche, R. and Bohland, K. (2006) [Occurence of salmonellae in laying hens in different housing systems and inferences for control]. *Berl Munch Tierarztl Wochenschr* 119, 467-473.

Millner, P.D., Powers, K.E., Enkiri, N.K. and Burge, W.D. (1987) Microbially mediated growth suppression and death of salmonella in composted sewage sludge. *Micro Ecol* 14, 255-265.

Moore, J.E., Watabe, M., Stewart, A., Cherie Millar, B. and Rao, J.R. (2007) A novel challenge test incorporating irradiation (^{60}Co) of compost sub-samples to validate thermal lethality towards pathogenic bacteria. *Ecotoxicology and Environmental Safety* doi:10.1016/j.ecoenv.2007.08.024

Namata, H., Meroc, E., Aerts, M., Faes, C., Abrahantes, J.C., Imberechts, H. and Mintiens, K. (2007) *Salmonella* in Belgian laying hens: An identification of risk factors. *Preventive Veterinary Medicine* doi:10.1016/j.prevetmed.2007.09.002

Neill, S.D., McLoughlin, M.F. and McIlroy, S.G. (1989) Type C botulism in cattle being fed ensiled poultry litter. *Vet Rec* 124, 558-560.

Neubauer, C., Bibl, D., Szolgyenyi, W., Jauk, V., Schmidt, M., Gabler, C. and Vasicek, L. (2005) Epidemiological investigation of *Campylobacter* spp. in Austrian broiler flocks: Prevalence and risk factors. *Wiener Tierarztliche Monatsschrift* 92, 4-10.

Nicholson, F.A., Groves, S.J. and Chambers, B.J. (2005a) Pathogen survival during livestock manure storage and following land application. *Biores Technol* 96, 135-143.

Nicholson, F.A., Groves, S.J. and Chambers, B.J. (2005b) Pathogen survival during livestock manure storage and following land application. *Biores Technol* 96, 135-143.

Niilo, L. (1980) *Clostridium perfringens* in animal disease: a review of current knowledge. *Can Vet J* 21, 141-148.

Notermans, S., Breukink, H.J., Wensing, T. and Wagenaar, G. (1978) [Incidence of *Clostridium botulinum* in the rumen contents and faeces of cattle fed brewers' grains naturally contaminated with Clostridium botulinum (author's transl)]. *Tijdschr Diergeneeskd* 103, 1327-1333.

Notermans, S., Dufrenne, J. and Oosterom, J. (1981) Persistence of *Clostridium botulinum* type B on a cattle farm after an outbreak of botulism. *Appl Environ Microbiol* 41, 179-183.

Notermans, S., Kozaki, S. and van Schothorst, M. (1979) Toxin production by *Clostridium botulinum* in grass. *Appl Environ Microbiol* 38, 767-771.

Olsen, A.R. and Hammack, T.S. (2000) Isolation of Salmonella spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (*Diptera*: *Muscidae*), at caged-layer houses. *J Food Protect* 63, 958-960.

Omeira, N., Barbour, E.K., Nehme, P.A., Hamadeh, S.K., Zurayk, R. and Bashour, I. (2006) Microbiological and chemical properties of litter from different chicken types and production systems. *Sci Total Environ* 367, 156-162.

Ortolani, E.L., Brito, L.A., Mori, C.S., Schalch, U., Pacheco, J. and Baldacci, L. (1997) Botulism outbreak associated with poultry litter consumption in three Brazilian cattle herds. *Vet Hum Toxicol* 39, 89-92.

Otter, A., Livesey, C., Hogg, R., Sharpe, R. and Gray, D. (2006) Risk of botulism in cattle and sheep arising from contact with broiler litter. *Vet Rec* 159, 186-187.

Owen, R., Roche, P.W., Hope, K., Yohannes, K., Roberts, A., Liu, C., Stirzaker, S., Kong, F., Bartlett, M., Donovan, B., East, I., Fitzsimmons, G., McDonald, A., McIntyre, P.B. and Menzies, R.I. (2007) Australia's notifiable diseases status, 2005: annual report of the National Notifiable Diseases Surveillance System. *Communicable Diseases Intelligence (Quarterly report)* 31,1.

Paluszak, Z., Bauza-Kaszewska, J., Ligocka, A., Olszewska, H. and Philipp, W. (2003) Microbiological studies of composted sewage sludge for agricultural usage. *Tierarztliche Umschau* 58, 297-303.

Park, S.F. (2002) The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *Int J Food Microbiol* 74, 177-188.

Pecelunas, K.S., Wages, D.P. and Helm, J.D. (1999) Botulism in chickens associated with elevated iron levels. *Avian Dis* 43, 783-787.

Pietronave, S., Fracchia, L., Rinaldi, M. and Martinotti, M.G. (2004) Influence of biotic and abiotic factors on human pathogens in a finished compost. *Water Res* 38, 1963-1970.

Pourcher, A.M., Morand, P., Picard-Bonnaud, F., Billaudel, S., Monpoeho, S., Federighi, M., Ferre, V. and Moguedet, G. (2005) Decrease of enteric micro-organisms from rural sewage sludge during their composting in straw mixture. *J Appl Microbiol* 99, 528-539.

Ranalli, G., Bottura, G., Taddei, P., Garavani, M., Marchetti, R. and Sorlini, C. (2001) Composting of solid and sludge residues from agricultural and food industries. Bioindicators of monitoring and compost maturity. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 36, 415-436.

Rasschaert, G., Houf, K., Van Hende, J. and De Zutter, L. Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in poultry flocks and assessment of the sampling site for status determination at slaughter. *Vet Microbiol* doi:10.1016/j.vetmic.2007.03.011

Rendos, J.J., Eberhart, R.J. and Kesler, E.M. (1975) Microbial populations of teat ends of dairy cows and bedding materials. *Journal of Dairy Science* 58, 1492. Cited by Nicholson *et al.* 1999: A study on farm manure applications to agricultural land and an assessment of the risks of pathogen transfer into the food chain. A report to The Ministry of Agriculture Fisheries and Food.

Ritz, C.W. and Worley, J.W. (2005) *Poultry Mortality Composting Management Guide*: The University of Georgia College of Agricultural and Environmental Sciences

Rivoal, K., Ragimbeau, C., Salvat, G., Colin, P. and Ermel, G. (2005) Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from free-range broiler farms and comparison with isolates of various origins. *Appl Environ Microbiol* 71, 6216-6227.

Roberts, T.A. and Collings, D.F. (1973) An outbreak of type-C botulism in broiler chicken. *Avian Dis* 17, 650-658.

Roberts, T.A., Thomas, A.I. and Gilbert, R.J. (1973) A third outbreak of type C botulism in broiler chickens. *Vet Rec* 92, 107-109.

Runge, G.A., Blackall, P.J. and Casey, K.D. (2007) *Chicken Litter Issues Associated with Sourcing and Use*. Canberra: Rural Industries Research and Development Corporation.

Russ, C.F. and Yanko, W.A. (1981) Factors affecting *Salmonella* repopulation in composted sludges. *Appl Environ Microbiol* 41/3, 597-602.

Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K. and Swings, J. (2003) Microbiological aspects of biowaste during composting in a monitored compost bin. *J Appl Microbiol* 94, 127-137.

Sahin, O., Kobalka, P. and Zhang, Q. (2003) Detection and survival of *Campylobacter* in chicken eggs. *J Appl Microbiol* 95, 1070-1079.

Segner, W.P., Schmidt, C.F. and Boltz, J.K. (1971a) Enrichment, isolation, and cultural characteristics of marine strains of *Clostridium botulinum* type C. *Appl Microbiol* 22, 1017-1024.

Segner, W.P., Schmidt, C.F. and Boltz, J.K. (1971b) Minimal growth temperature, sodium chloride tolerance, pH sensitivity, and toxin production of marine and terrestrial strains of *Clostridium botulinum* type C. *Appl Microbiol* 22, 1025-1029.

Sergeant, E., Grimes, T., Jackson, C., Baldock, F. and Whan, I. (2003) *Salmonella enteritidis surveillance and response options* No. RIRDC Publication No 03/006: Rural Industries Research and Development Corporation.

Seo, K.H., Holt, P.S., Gast, R.K. and Hofacre, C.L. (2000) Combined effect of antibiotic and competitive exclusion treatment on *Salmonella enteritidis* fecal shedding in molted laying hens. *J Food Protect* **63**, 545-548.

Shane, S.M. (1992) The significance of *Campylobacter jejuni* infection in poultry: a review. *Avian Pathol* 21, 189-213.

Shane, S.M. (2006) Emergency depletion and disposal of poultry flocks. *World Poult* 22, 42-44.

Sidhu, J., Gibbs, R.A., Ho, G.E. and Unkovich, I. (1999) Selection of *Salmonella typhimurium* as an indicator for pathogen regrowth potential in composted biosolids. *Lett Appl Microbiol* 29, 303-307.

Sidhu, J., Gibbs, R.A., Ho, G.E. and Unkovich, I. (2001) The role of indigenous microorganisms in suppression of *Salmonella* regrowth in composted biosolids. *Water Res* 35, 913-920.

Skov, M.N., Spencer, A.G., Hald, B., Petersen, L., Nauerby, B., Carstensen, B. and Madsen, M. (2004) The role of litter beetles as potential reservoir for *Salmonella enterica* and thermophilic *Campylobacter* spp. between broiler flocks. *Avian Dis* 48, 9-18.

Smart, J.L., Jones, T.O., Clegg, F.G. and McMurtry, M.J. (1987) Poultry waste associated type C botulism in cattle. *Epidemiol Infect* 98, 73-79.

Smith, L.D. (1975a) Common mesophilic anaerobes, including *Clostridium botulinum* and *Clostridium tetani*, in 21 soil specimens. *Appl Microbiol* 29, 590-594.

Smith, L.D. (1975b) Inhibition of *Clostridium botulinum* by strains of *Clostridium perfringens* isolated from soil. *Appl Microbiol* 30, 319-323.

Sobratee, N., Mohee, R., Driver, M.F. and Mudhoo, A. (2008) Survival kinetics of faecal bacterial indicators in spent broiler litter composting. *J Appl Microbiol* 104, 204-214.

Someya, A., Otsuki, K. and Murase, T. (2005) Antimicrobial susceptibilities of *Salmonella* isolates obtained from layer chicken houses on a commercial egg-producing farm in Japan, 1997 to 2002. *J Food Protect* 68, 2030-2034.

Souza, A.M., Marques, D.F., Do?bereiner, J. and Dutra, I.S. (2006) Spores and toxins of Clostridium botulinum in raining ponds used as water supply for cattle in pastures in the Araguaia Valley, State of Goiás, Brazil. *Pesq Vet Bras* 26, 133-138.

Stephens, N., Sault, C., Firestone, S.M., Lightfoot, D. and Bell, C. (2007) Large outbreaks of *Salmonella* Typhimurium phage type 135 infections associated with the consumption of products containing raw egg in Tasmania. *Communicable Diseases Intelligence (Quarterly report)* 31, 118.

Sutherland, P.S., Miles, D.W. and Laboyrie, D.A. (2003) *Listeria monocytogenes*. In *Foodborne Microorganisms of Public Health Significance* ed. Hocking, A.D. pp.381-444. Sydney: Australian Institute of Food Science and Technology.

Szabo, E.A. and Gibson, A.M. (2003) *Clostridium botulinum*. In *Foodborne Microorganisms of Public Health Significance* ed. Hocking, A.D. pp.505 - 542: Sydney: Australian Institute of Food Science and Technology.

Takeda, M., Kasai, H., Torii, Y., Mukamoto, M., Kohda, T., Tsukamoto, K. and Kozaki, S. (2006) Protective effect of botulinum C/D mosaic toxoid against avian botulism. *J Vet Med Sci* 68, 325-330.

Taylor, L. (2004) Botulism: a potential disease problem in intensively fed beef and dairy cattle. DPI note - File No: APH162: Department of Primary Industries and Fisheries, Queensland.

Thomas, R.J. (1991) Detection of *Clostridium botulinum* types C and D toxin by ELISA. *Aust Vet J* 68, 111-113.

Tiquia, S.M. and Tam, N.F.Y. (2002) Characterization and composting of poultry litter in forced-aeration piles. *Process Biochem* 37, 869–880.

Tiquia, S.M., Yann, N.F.Y. and Hodgkiss, I.J. (1998) *Salmonella* elimination during composting of pig litter. *Biores Technol* 63, 193-196.

Trueman, K.F., Bock, R.E., Thomas, R.J., Taylor, J.D., Green, P.A., Roeger, H.M. and Ketterer, P.J. (1992) Suspected botulism in three intensively managed Australian cattle herds. *Vet Rec* 130, 398-400.

Turner, C. (2002) The thermal inactivation of E. coli in straw and pig manure. *Biores Technol* 84, 57-61.

United States Environmental Protection Agency (2003) Environmental Regulations and TechnologyControl of Pathogens and Vector Attraction in Sewage Sludge, EPA/625/R-92/013: U.S. Environmental Protection Agency Office of Research and Development.

US Food and Drug Administration (1998) *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. Washington: US Food and Drug Administration.

USEPA (1993) Modeling the final rules. 40 CFR Part 503 Subpart D. *Federal Register* 58, 9398–9400.

van Asselt, E.D. and Zwietering, M.H. (2006) A systematic approach to determine global thermal inactivation parameters for various food pathogens. *Int J Food Microbiol* 107, 73-82.

Wales, A., Breslin, M., Carter, B., Sayers, R. and Davies, R. (2007) A longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks. *Avian Pathol* 36, 187-197.

Wales, A., Breslin, M. and Davies, R. (2006) Assessment of cleaning and disinfection in *Salmonella*-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques. *Vet Microbiol* 116, 283-293.

Wallace, R.B. (2003) Campylobacter. In *Foodborne Microorganisms of Public Health Significance* ed. Hocking, A.D. pp.311-323. Sydney: Australian Institute of Food Science and Technology.

Wesley, I.V. and Baetz, A.L. (1999) Natural and experimental infections of *Arcobacter* in poultry. *Poult Sci* 78, 536-545.

Wiley, B.B. and Westerberg, S.C. (1969) survival of human pathogens in composted sewage. *Appl Microbiol* 18/6, 994-1001.

Wilkinson, K.G. (2007) The biosecurity of on-farm mortality composting *J Appl Microbiol* 102, 609-618.

Wobeser, G. and Galmut, E.A. (1984) Internal temperature of decomposing duck carcasses in relation to botulism. *J Wildl Dis* 20, 267-271.

Wobeser, G., Marsden, S. and MacFarlane, R.J. (1987) Occurrence of toxigenic *Clostridium botulinum* type C in the soil of wetlands in Saskatchewan. *J Wildl Dis* 23, 67-76.

Wong, D.M., Young-Perkins, K.E. and Merson, R.L. (1988) Factors influencing *Clostridium botulinum* spore germination, outgrowth, and toxin formation in acidified media. *Appl Environ Microbiol* 54, 1446-1450.

You, Y.W., Rankin, S.C., Aceto, H.W., Benson, C.E., Toth, J.D. and Dou, Z.X. (2006) Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. *Appl Environ Microbiol* 72, 5777-5783.

Zaleski, K.J., Josephson, K.L., Gerba, C.P. and Pepper, I.L. (2005) Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil. *Appl Environ Microbiol* 71, 3701-3708.

Zechmeister, T.C., Kirschner, A.K., Fuchsberger, M., Gruber, S.G., Suess, B., Rosengarten, R., Pittner, F., Mach, R.L., Herzig, A. and Farnleitner, A.H. (2005) Prevalence of botulinum neurotoxin C1 and its corresponding gene in environmental samples from low and high risk avian botulism areas. *Altex* 22, 185-195.

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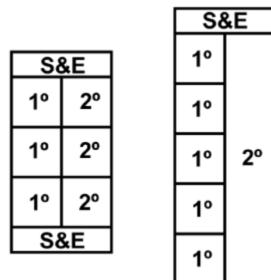
Appendix 1

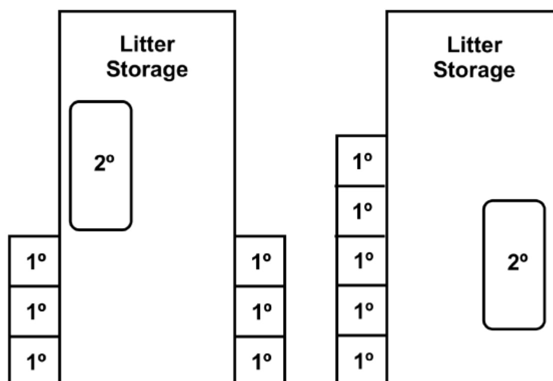
Details of selected composting methodologies

(1) Georgia method (Ritz and Worley 2005)

The overall layout:



Stand-alone composters with primary (1º), secondary (2º) and storage space (S&E) for bulk materials and equipment. Bin width and depth typically 6-8 feet.



Compost bins incorporated within a litter storage facility. Space for secondary compost is provided within the storage facility. Stack house width and length typically 40 x 100 feet, though based on specific storage needs.

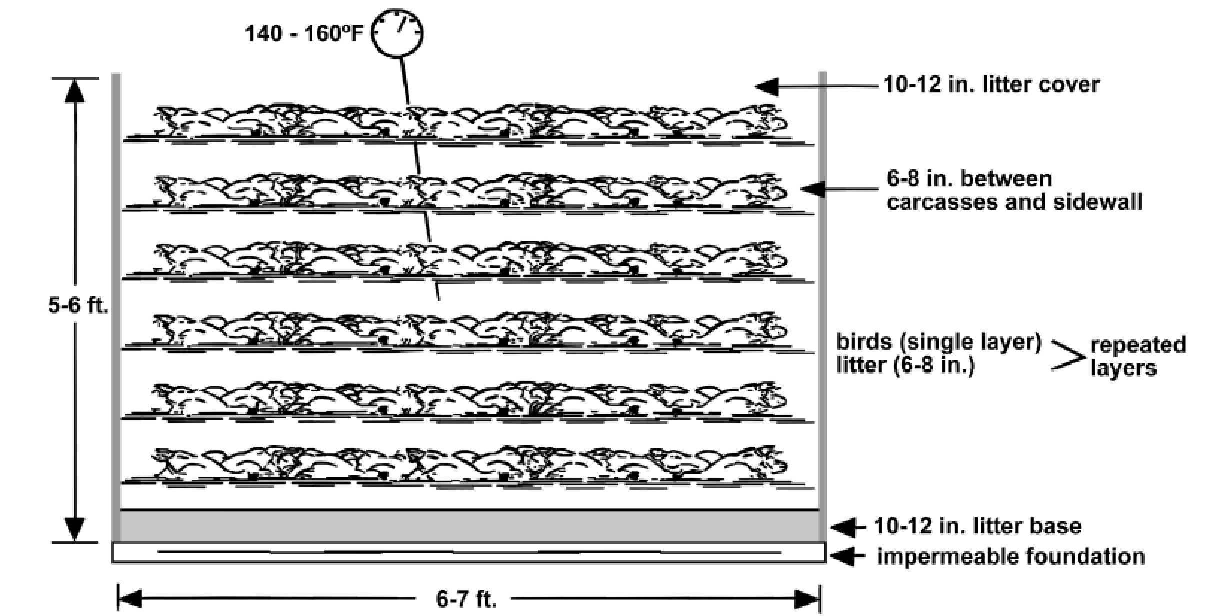
The nutrients

A good C:N ratio and moisture is essential for composting

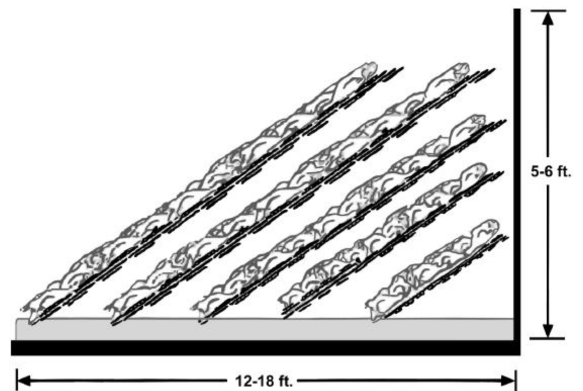
Birds (C:N, 5:1); litter (C:N, 7:1 to 25:1); straw (C:N, 80:1);

peanut hulls (C:N, 50:1); wood shavings (C:N, 300 - 700:1);

The bin layout – normal design



Alternative design



The process

- Place and initial layer of fresh litter (8-12 inches)
- Place thin layer of bulking material, peanut hulls, coarse shavings, straw
- Place bird carcases, single layer, side by side and 6 inches from wall
- Added small amount of water if necessary
- Add layer of litter, twice a thick as the layer of carcasses
- Repeat for additional carcasses, not exceeding a height of 5-6 feet
- Last layer litter (8-10 inches)

North Carolina method (Carter *et al.* 1996)

The authors suggest that the factors that need to be considered in developing a composting system for commercial egg pullets, is the total weight of the carcasses near maturity where as for layers the average daily mortality should be considered because the weights and mortality are more uniform throughout the flock cycle for these types of poultry.

The recipe suggested is as follows

Material

Bottom Layer

12 inches of litter or shavings on concrete

Middle Layer

1/10 part (by weight) of straw or peanut hulls

1 part (by weight) of dead birds
(one bird deep, 6 inches from edge)

2 parts litter

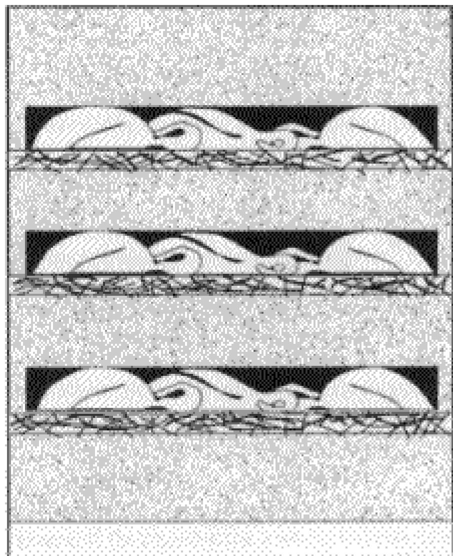
Top Layer

6 inches of litter

Example

5 pounds of straw or peanut hulls
50 pounds of dead birds
100 pounds of litter

The compost bin



Appendix 2

Summary of botulism cattle outbreaks (Advisory committee on the microbiological safety of food ad hoc group 2006)

: Summary of botulism in cattle outbreaks (England and Wales 2003-2005)[2]

Incident No.[1]	No. Clinical Cases[2]	Herd Size	Enter prise	Age	Related Outbreaks	Previous Outbreak on Farm	Suspected Source	Exposure	How Exposed
2003/01	7	300	Dairy	>24 months			Poultry Litter	Direct	Stacked
2003/05			Beef	13 - 24 months		Y	Poultry Litter	Direct	Broiler unit on farm
2003/16	10	50	Beef	13 - 24 months			Poultry Litter	Direct	Spread on fields
2003/23	3	15	Beef	13 - 24 months			None identified	Not Known	
2003/26	3	18	Beef	13 - 24 months			Poultry Litter	Indirect	Used on neighbouring farm
2003/37	17	49	Dairy	13 - 24 months	2003/43, 2003/44, 2003/45		Poultry Litter	Direct	Stacked/spread on fields
2003/42	2	40	Beef	13 - 24 months	2003/46		Poultry Litter	Direct	Stacked in field
2003/43			Beef	> 24 months	2003/37, 2003/44, 2003/45		Poultry Litter	Indirect	Spread on neighbouring farm
2003/44			Beef	13 - 24 months	2003/37, 2003/43, 2003/45		Poultry Litter	Indirect	Spread on neighbouring farm
2003/45			Beef	> 24 months	2003/37, 2003/43, 2003/44		Poultry Litter	Indirect	Spread on neighbouring farm
2003/46	1	8	Beef	13 - 24 months	2003/42		Poultry Litter	Direct	Stacked in field
2003/47	2		Dairy	> 24 months			Poultry Litter	Direct	Used as bedding
2003/50	7	30	Beef	13 - 24 months			Poultry Litter	Indirect	Spread on adjacent field
2003/51	5	70	Dairy	> 24 months		Y	Poultry Litter	Direct	Poultry litter spread on silage field
2003/56	2	48	Dairy	> 24 months	2003/59		Poultry Litter	Indirect	Spread on adjacent field
2003/57	16		Beef	7 - 12 months			Vegetable Waste	Direct	
2003/59	3	16	Beef	> 24 months	2003/56		Poultry Litter		
2003/60	4		Beef	> 24 months			Poultry Litter	Indirect	Adjacent to farm with botulism

[2] Data on toxin types not reported

Incident No.[1]	No. Clinical Cases[2]	Herd Size	Enter prise	Age	Related Outbreaks	Previous Outbreak on Farm	Suspected Source	Exposure	How Exposed
2003/65	16	29	Dairy	13 - 24 months			Poultry Litter	Direct	Stacked in field
2003/72	1	45	Beef	13 - 24 months			Poultry Litter	Indirect	Stacked next to housing
2004/02	1		Beef	13 - 24 months			Poultry Litter	Indirect	Stacked in adjacent field
2004/05	3	31	Beef	> 24 months			Poultry Litter	Direct	Used as bedding
2004/30	5		Dairy	> 24 months	2004/31, 2004/35		Poultry Litter	Indirect	Spread on adjacent field
2004/31	2		Beef	> 24 months	2004/30, 2004/35		Poultry Litter	Direct	Stacked in field
2004/35	3		Beef	13 - 24 months	2004/30, 2004/31		Poultry Litter	Direct	Stacked in field
2004/39	8	106	Beef	13 - 24 months			Poultry Litter	Indirect	Adjacent to Poultry Farm/Litter stack
2004/46	2	20	Beef	13 - 24 months			Poultry Litter	Indirect	Stacked & spread on adjacent field
2004/47		11	Beef	13 - 24 months	2004/53		Poultry Litter	Indirect	Spread on adjacent field
2004/49	15	34	Beef	> 24 months			Poultry Litter	Direct	Stacked in field
2004/50		35	Beef	13 - 24 months			Poultry Litter	Direct	Spread on fields
2004/53			Dairy	> 24 months	2004/47		Poultry Litter	Indirect	Adjacent to poultry farm
2004/57	1		Beef	> 24 months			Pheasant carcases	Direct	In feed store/troughs
2004/58	4	24	Dairy	7 - 12 months			Not Established	Not known	
2004/60	2		Beef	13 - 24 months			Poultry Litter	Indirect	Stacked in adjacent field
2004/62			Beef	> 24 months			Poultry Litter	Direct	Stacked in field
2004/66	11		Dairy	13 - 24 months			Poultry Litter	Direct	Spread on Pasture
2004/73	4		Dairy	13 - 24 months			Poultry Litter	Indirect	
2004/75	2	40	Beef	7 - 12 months			None identified		
2004/77	2	39	Dairy	13 - 24 months			Chicken carcases	Direct	
2004/78	9	400	Beef	> 24 months			Bread Waste		
2005/02	8	50	Dairy	13 - 24 months			Poultry Litter	Indirect	Broiler unit on farm
2005/06	4	70	Dairy	> 24 months			Fox carcase	Direct	

Incident No.[1]	No. Clinical Cases[2]	Herd Size	Enter prise	Age	Related Outbreaks	Previous Outbreak on Farm	Suspected Source	Exposure	How Exposed
2005/15	5	70	Dairy	> 24 months	2005/58, 2005/61		Poultry Litter	Indirect	Stacked on neighbouring farm
2005/24	2	15	Beef	13 - 24 months			Poultry Litter	Direct	Spread on pasture cattle were grazing
2005/26	4	14	Beef	13 - 24 months			Poultry Litter	Direct	Spread on pasture cattle were grazing
2005/32	3	13	Beef	> 24 months			Silage		
2005/49	2	28	Beef	13 - 24 months		Y	Poultry Litter	Indirect	Spread in adjacent arable field
2005/50	5	22	Dairy	7 - 12 months			Poultry Litter	Direct	Stacked in field where cattle grazing
2005/58	2	48	Dairy	> 24 months	2005/15, 2005/61		Poultry Litter	Indirect	Stacked on neighbouring Farm
2005/60	2		Dairy	> 24 months			Pheasant carcases	Direct	
2005/61	5	34	Dairy	13 - 24 months	2005/15, 2005/58		Poultry Litter	Indirect	Stacked on neighbour's field
2005/62	6		Beef	> 24 months			Poultry Litter	Indirect	Spread on arable land
2005/65	3		Dairy	> 24 months			Poultry Litter	Indirect	Spread on neighbour's field
2005/66			Dairy	7 - 12 months			Poultry Litter	Indirect	Spread on Neighbour's Field
2005/67			Dairy	13 - 24 months			Poultry Litter	Indirect	Spread on Neighbour's Farm

[1] VLA incident number. Incident numbers not referred to did not involve botulism
[2] Approximate only, as the number of cattle affected may have increased after investigation of farm.