RISK PROFILE: *SALMONELLA* SPP. IN ANIMAL FEED

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EXECUTIVE SUMMARY

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management. Risk profiling may result in a range of activities e.g. immediate risk management action, a decision to conduct a quantitative risk assessment, or a programme to gather more data. Risk Profiles also provide information for ranking of food safety issues.

The food/hazard combination addressed by this Risk Profile is *Salmonella* spp. in animal feed. The NZFSA have commissioned this Risk Profile in order to address the following specific risk management questions:

- What is the risk of introduction of salmonellae into food-producing animals (poultry broilers and layers, cattle, sheep, pigs) by contamination of feed?
- What is the flow-on effect for human exposure?

Salmonellosis is a significant disease in New Zealand, with 1,000-2,000 notified cases per annum. The potential for human cases of salmonellosis to result from feed contamination in New Zealand was demonstrated by increases in notified human cases of infection with a specific *Salmonella* serotype subsequent to a known feed contamination incident.

No New Zealand risk assessments were found that directly assessed the risk of human salmonellosis due to contaminated animal feed. A European assessment concluded that in regions with low *Salmonella* prevalence in food-producing animals, *Salmonella* contaminated feed represents a major source for introduction of *Salmonella* into the food production chain. There is evidence to suggest that *Salmonella* prevalence in the meat of food-producing animals in New Zealand is low.

While few data are available, it is likely that when *Salmonella* is present in feed ingredients, concentrations will be low (<100 organisms/g). However, it is difficult to assess the impact of these low concentrations due to the shortage of information on *Salmonella* doses leading to colonisation in animal species. Processing of animal feed into a pelletised form includes a heat-processing step (conditioning) that would be expected to substantially reduce the already low levels of *Salmonella* in feed ingredients.

The fact that the most common *Salmonella* serotype in finished animal feed in New Zealand in recent years (*S.* Tennessee), based on industry data, occurs infrequently amongst human cases argues against animal feed as a major source of human salmonellosis in New Zealand. However, the available information on the *Salmonella* status of feed and feed ingredients in New Zealand is not sufficiently comprehensive to assess animal feed as a source of human salmonellosis cases.

The potential for introduction of novel *Salmonella* serotypes through imported feed ingredients cannot be discounted, although the similarity in serotypes present in feed ingredients in New Zealand and internationally suggests that the risk of this occurring is probably quite low.

Control of *Salmonella* contamination in the animal feed industry is complicated by the diversity of products, with some receiving heat treatment and some not. Further complication is introduced through the wide diversity of materials that may be used for or in animal feed. Information from New Zealand and overseas suggests that none of these source materials can be assumed to be free of *Salmonella*.

Data from the New Zealand feed industry indicate that animal protein products (mainly domestically sourced) and plant protein products (mainly imported) are feed ingredients from which *Salmonella* is isolated. However, information on the frequency of testing and prevalence in various types of feed is lacking, so this information could be skewed by the product types most commonly selected for testing.

Application of HACCP principles, including good manufacturing practices and general hygiene procedures are recognised as important measures for *Salmonella* control. Such measures are either in place or under development in the New Zealand feed industry, although it is uncertain what level of application of these principles is achieved outside the membership of the New Zealand Feed Manufacturers' Association. The members of the association are responsible for the production of more than 85% of the animal feed produced in New Zealand. It is recognised that the application of HACCP principles should ideally extend to rendering and crushing plants supplying ingredients to the animal feed industry.

The heterogeneous distribution of *Salmonella* contamination of animal feed ingredients means that testing of material is unlikely to be an effective control measure. However, application of well-structured testing programmes would provide a measure of the effectiveness of control measures and allow assessment of any emerging trends. The EFSA review concluded that establishment of microbiological criteria for *Salmonella* in the feed production chain was appropriate, but should be based on one or more hygiene criteria at critical stages of the production chain, rather than be based on end product testing.

Suitable application of heat treatment is likely to be the best measure for decontamination of animal feed. However, the residual protection provided by chemical treatments, such as organic acids and formaldehyde, may also contribute to reducing *Salmonella* contamination in feed and in the feed mill environment and *Salmonella* colonisation in food-producing animals.

1 STATEMENT OF PURPOSE

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles are part of the Risk Management Framework (RMF; http://www.nzfsa.govt.nz/about-us/risk-management-framework/index.htm) approach taken by the New Zealand Food Safety Authority (NZFSA). The Framework consists of a four step process, as shown in Figure 1.

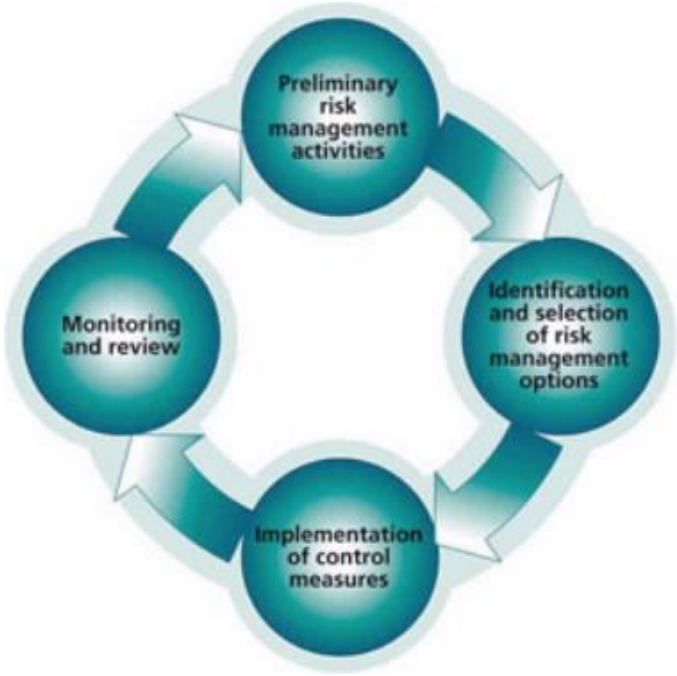


Figure 1: The four steps of the Risk Management Framework

This initial step in the RMF, Preliminary Risk Management Activities, includes a number of tasks:

- Identification of food safety issues;
- Risk profiling;
- Establishing broad risk management goals;
- Deciding on the need for a risk assessment;
- If needed, setting risk assessment policy and commissioning of the risk assessment;
- Considering the results of the risk assessment; and
- Ranking and prioritisation of the food safety issue for risk management action.

Risk profiling may be used directly by risk managers to guide identification and selection of risk management options, for example where:

- Rapid action is needed;
- There is sufficient scientific information for action; or
- Embarking on a risk assessment is impractical.

The sections in this Risk Profile are organised as much as possible as they would be for a conventional qualitative risk assessment, as defined by Codex, including hazard identification, hazard characterisation, exposure assessment and risk characterisation.

1.1 Food/Hazard Combination and Risk Management Questions

NZFSA has recognised *Salmonella* as one of the three most important foodborne pathogens in New Zealand. The organisation is taking a strategic approach to *Salmonella* risk management, with the ultimate aim of achieving a 30% reduction in foodborne salmonellosis after five years. Underpinning this strategy are a range of preliminary risk evaluation activities, including risk profiling to better understand the risk of *Salmonella* attributable to a range of food types[1].

The food/hazard combination addressed by this Risk Profile is *Salmonella* spp. in animal feed, with particular attention to the potential for *Salmonella* spp. in animal feed to be transmitted to humans via consumption of contaminated animal material.

The NZFSA have commissioned this Risk Profile in order to address the following specific risk management questions:

- What is the risk of introduction of salmonellae into food-producing animals (poultry broilers and layers, cattle, sheep, pigs) by contamination of feed?
- What is the flow-on effect for human exposure?

[1] http://www.nzfsa.govt.nz/foodborne-illness/salmonella/strategy.htm

2 HAZARD AND FOOD

2.1 *Salmonella*

The genus *Salmonella* is comprised of two species: *Salmonella enterica*, which is divided into 6 subspecies (*enterica, salamae, arizonae, diarizonae, houtanae and indica*), and *Salmonella bongori* (Jay *et al.*, 1997). Most isolates from humans and warm-blooded animals belong to subspecies I: *Salmonella enterica* subspecies *enterica*. Other *Salmonella enterica* subspecies and *Salmonella bongori* occur more commonly from ectothermic (cold blooded) animals and the environment, and are of lower pathogenicity.

Salmonella typing is primarily performed using serological identification of somatic (O), flagellar (H), and capsular (K) antigens.

Salmonella enterica serotypes are normally denoted in a shortened form that includes a non-italicised serotype name, e.g. *Salmonella enterica* subsp. *enterica* serovar Enteritidis becomes *Salmonella* Enteritidis. In older publications this may be represented as a full species name i.e. *Salmonella enteritidis*. Further subtyping may be performed using susceptibility to bacteriophages. These types are denoted as phage type (PT) or definitive phage type (DT) numbers. These two terms are interchangeable and both are used in the literature.

Molecular techniques, such as multiple-locus variable-number tandem repeat analysis (MLVA) and pulsed-field gel electrophoresis (PFGE), are being increasingly used for typing *Salmonella* strains in epidemiological investigations (Baggesen *et al.*, 2010).

Salmonella Typhi and *Salmonella* Paratyphi are serotypes which cause a serious enteric fever and are particularly well adapted to invasion and survival in human tissue. They have a particular antigen makeup and a differing ecology to other serotypes of *Salmonella*. They are not included in this Risk Profile.

2.2 Sources of *Salmonella*

Human: Person to person transmission of salmonellosis is well recognised, and secondary transmission of *Salmonella* in outbreaks has been demonstrated (Loewenstein, 1975). Carriage in faeces of convalescent cases can be quite substantial, with concentrations in the range of 10^6-10^7/g persisting up to 10 days after initial diagnosis. Reduction in numbers with time is variable although it appears that most people will have counts of less than 100 salmonellae/g after 35 to 40 days but a count of 6×10^3/g has been recorded in one patient 48 days post-illness (Pether and Scott, 1982).

Animal: Some *Salmonella* serotypes are largely confined to particular animal reservoirs, causing both systemic and enteric disease. For example *S.* Cholerae-suis is host restricted to pigs (Allison *et al.*, 1969), while other serotypes (for example *S.* Typhimurium) are frequently associated with intestinal infections in a wide range of animal species (Paulin *et al.*, 2002). *Salmonella* can be found in mammals, fish, reptiles, amphibians, insects and birds. Most *Salmonella* colonisations in animals produce no clinical signs. Animal feeds may be contaminated with salmonellae, although feeds that include animal products (e.g. meat and bone meal) should receive sufficient heat treatment to destroy the organism.

Food: Red and white meats, meat products, milk, cheese and eggs are considered the major food sources of human salmonellosis, although a wide variety of other foods have been associated with outbreaks (Jay *et al.*, 2003). Foods of non-animal origin in which *Salmonella* contamination has been reported include coconut, barley, cereal powder, yeast, cottonseed, chocolate, soybean sauce, cider, watermelon, watercress, white and black pepper. Tahini, a product made from crushed sesame seeds, has caused a number of *Salmonella* outbreaks worldwide, including New Zealand and Australia (Unicomb *et al.*, 2005). The absence in New Zealand of *S.* Enteritidis types that can penetrate into eggs means that this food type is likely to be of lower risk here.

Environment: Salmonellae in sewage effluents or dried animal faeces can contaminate pasture, soil and water. They can remain viable for months in soil. The organism may also be dispersed in dust and aerosols generated during the handling and processing of animals. Contamination in the environment can act as a source of colonisation for other animals i.e. spreading by rodents or wild bird populations.

Transmission Routes: May be transmitted to humans via contaminated food or water, animal contact, or from a contaminated environment. A simple overview is a cycle of events involving feedstuffs, animals, foodstuffs then humans.

2.3 Animal Feed

Food-producing animals are major reservoirs for many microorganisms of importance to human health, including serotypes of *Salmonella enterica* (Crump *et al.*, 2002). Pathogens are acquired through ingestion. Therefore, contaminated animal feed can contribute to colonisation and, in some cases, infection of food-producing animals with *Salmonella* and other pathogens.

2.3.1 The New Zealand animal feed manufacturing industry

The majority of animal feed manufactured in New Zealand is produced to meet the requirements of commercial animal production. More than 85% of manufactured animal feed in New Zealand is produced by members of the New Zealand Feed Manufacturers Association (NZFMA)[1].

Recent years have seen a trend towards consolidation within the New Zealand feed manufacturing and ingredient trading industries (Davidson and Pearson, 2009b).

Growth and increased intensification in the dairy industry has seen a large increase in the manufacture of dairy feeds in the period 2006-2008 (Davidson and Pearson, 2009b).

2.3.2 Types of animal feed in New Zealand

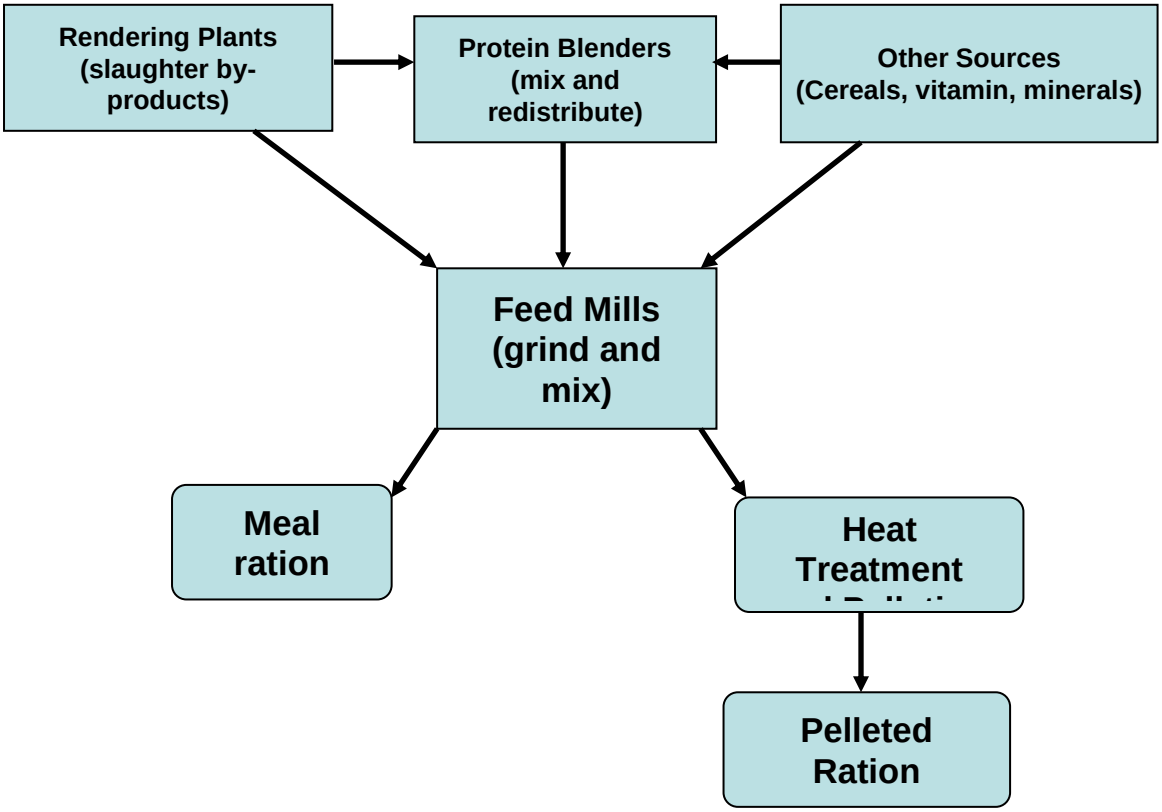
For the purpose of this risk profile, animal feed will mainly refer to manufactured compound feed. However, recent years have seen a diversification in feed ingredients and feeding practices in New Zealand's production animal industries. For this reason, the risk profile will consider a wider definition of feed, to include all non-pasture based feeds. Pasture based feeds include pasture grass, silage, haylage, etc.

[1] http://www.nzfma.org.nz/index.php

2.3.2.1 Compound animal feed

Crump *et al*. (2002) defined a high level overview of compound feed manufacture as outlined in Figure 2.

Figure 2: Outline of the steps in animal feed manufacture



Total compound feed production in New Zealand has risen steadily since 2006, with almost one million tonnes of compound feed manufactured in 2008 (Davidson and Pearson, 2009b). The major end users of compound feed are the broiler meat industry (41.6% in 2008), pork production (18.8%), dairy (14.5%) and the poultry layer industry (13.2%) (Davidson and Pearson, 2009a). This pattern of use represents a departure from traditional patterns in two areas:

- Increased use of compound feed in the dairy industry, reflecting the rapid growth and intensification in that sector; and
- Decreased volume of compound feed in the pork industry due to poor market conditions leading to a significant reduction in the national herd (Davidson and Pearson, 2009b).

Table 1 summarises the range of compound feeds used in animal production in New Zealand, as identified in a recent review of animal feed sources and feeding practices (Davidson and Pearson, 2009b).

Table 1: Compound feed types used in food animal product in New Zealand

Sector	Compound feed type	Use characteristics
Dairy – calves	Milk replacer Lower protein starter ration High protein starter ration	Birth to 6-12 weeks 4-16 weeks Birth to 10-12 weeks
Dairy – production	Springer (pre-calving) Early lactation ration Mid lactation ration Late lactation ration	2-3 weeks prior to calving Up to 120 days into lactation 100-200 days into lactation 200-300 days into lactation
Beef - production	Not commonly used, but some supplementary feeding of pelleted ration	40 days before slaughter
Sheep and goats	Rarely used	
Deer	Velvet ration	From beginning of August
Pigs	Creep Weaner 1 Weaner 2 Grower Finisher Dry sow Lactating sow Gilt replacer Boar	Birth to 3-5 weeks Weaning to 2 weeks post weaning From 2-12 weeks post weaning 12-16 weeks 16 weeks to market
Ostriches and Emus	Starter Grower Finisher In-lay Out-of-lay	Birth to 4-6 weeks 4 weeks to 1-2 months Top-up ration
Broilers – parent stock	Starter Rearer Grower Prelay Early Layer Late Layer	Hatching to 4 weeks 4-8 weeks Up to 16 weeks Up to about 21 weeks 21-40 weeks
Broilers – production	Starter Grower Finisher Withdrawal	Hatching to 7 days 7-20 days Up to 35 or 42 days Final 5-7 days pre-slaughter
Layers	Starter Grower Pre-lay Early Lay Late Lay	Hatching to 4 weeks 4-16 weeks 16-40 weeks 40-60 weeks Up to 60 weeks or longer
Finfish	Starter Grower Finisher	
Paua	Similar to finfish	

2.3.2.2 Blended dairy feeds

Supplementary feeding in the dairy industry has increased dramatically in recent years. According to NZFMA, there was a 77% increase in production of dairy feed from 2006 to 2007, followed by a further 53% increase from 2007 to 2008 (Davidson and Pearson, 2009b).

Blended feeds represent an intermediate product between single ingredient feeds and fully compounded feeds. They characteristically contain relatively few ingredients. These typically include palm kernel, tapioca, distiller's dried grains with solubles (DDGS) and premix (Davidson and Pearson, 2009b).

2.3.2.3 Plant and food processing by-products

Palm kernel

Palm kernel meal or palm kernel expeller (PKE) meal has become a significant feed item in New Zealand in recent years. This material is the waste left after oil extraction from palm kernel and is mainly used as supplemental feed in the dairy industry[1,2].

Imports of palm kernel increased by 142% from 2007 to 2008, with approximately 1.1 million tonnes imported in 2008 (Davidson and Pearson, 2009b). Palm kernel may be included as a component of compounded or blended feeds, but is more commonly fed directly to dairy cows.

Palm kernel has been implicated in one documented outbreak of *Salmonella* colonisation in cattle in England (Jones *et al.*, 1982).

Copra

Copra cake or copra meal is the product remaining after crushing of coconut to extract coconut oil. Use of copra has decreased in New Zealand since 2006, when Fonterra prohibited its direct feeding to dairy cows due to concerns over the presence of aflatoxin B1 in copra[3].

Distiller's Dried Grains with Solubles (DDGS)

DDGS is the dried residue left after fermentation of the starch fraction of maize to produce ethanol. Increases in the production of biofuels has resulted in increased availability of DDGS, with about 98% of DDGS now coming from production of oxygenated fuels and only 1-2% coming from alcoholic beverage production[4].

DDGS is commonly used in United States beef feedlots, where its abundance from the bourbon industry was the reason feedlots were set up. DDGS was first imported into New Zealand in 2008 (Davidson and Pearson, 2009b).

[1] http://www.ruralnews.co.nz/Default.asp?task=article&subtask=show&item=15186&pageno=1
[2] http://www.ravensdown.co.nz/NR/rdonlyres/D6AA696B-9146-4054-AF14-D8C61820B40A/0/palmkernelexpellermealtechsheet.pdf
[3] http://www.nzfarmersweekly.co.nz/article/6823.html
[4] http://www.ddgs.umn.edu/overview.htm

Dissolved Air Flotation Solids (DAFS)

Dissolved air flotation is used to separate insoluble fat and protein from liquid in waste streams. DAFS from the dairy industry have been used as pig feed, but more recently have been used as feed in the dairy industry (Davidson and Pearson, 2009b).

2.3.2.4 Food and industrial by-products

Feeding of food and industrial by-products results from practices that are price-driven at both the supplier and user end (Davidson and Pearson, 2009b). Materials that would normally cost the producer for disposal can be offloaded as animal feed at little or no cost. An example of this is food products that have been deemed to be unacceptable for human consumption.

Such materials have traditionally been used as feed sources in the pig industry, but 60-80% of this material is now used in the dairy industry (Davidson and Pearson, 2009b). Total use in 2008 was of the order of 200,000 tonnes (Davidson and Pearson, 2009b).

In terms of the current risk profile, this practice obviously raises concerns if the material was diverted to animal feed use due to the presence of *Salmonella*. However, no specific information was available on this possibility.

2.3.3 Potential impact of novel feeds on bacterial colonisation and shedding

Changes in the composition of animal feed have the potential to influence the probability of bacteria colonising the animal gut and the probability of the colonisation persisting. For example, fasting of ruminant animals has been shown to result in increased *Salmonella* population in the rumen of colonised animals (Brownlie and Grau, 1967). There is also evidence to suggest that changing the diet of ruminants from pasture feeding to a diet rich in starch (e.g. grain feeding) may increase populations of some bacteria in the gut and increase bacterial shedding (Callaway *et al.*, 2009).

Two contributing mechanisms have been suggested to explain this phenomenon:

- Pasture feeding results in formation of volatile fatty acids (VFAs) in the rumen. VFAs have bactericidal activity; and
- While ruminants are able to break down dietary starch, some starch reaches the colon where is provides a substrate for bacterial fermentation (Huntington, 1997).

There is evidence to suggest that feeding of DDGS to cattle increased shedding of *E. coli* O157:H7 (Callaway *et al.*, 2009). However, *Salmonella* prevalence in cattle was not affected by feeding of diets containing 25% DDGS (Jacob *et al.*, 2009). No information was found on the impact of other novel ruminant feed materials listed in section 2.3.2.3 on *Salmonella* shedding.

2.3.4 Composition of animal feeds

While individual feed formulations will vary in composition, the overall use of raw materials in the production of compound feeds in 2008 was (Davidson and Pearson, 2009b):

- Grains e.g. sorghum, wheat, barley, maize, oats 60%
- Plant proteins e.g. soy meal, canola meal 18%
- Animal proteins e.g. milk powders, meat and bone meal, fishmeal 7%
- Grain by-products e.g. wheat bran, broll, pollard, malt culms 6%
- Others e.g. molasses, salt, lime, vitamins, minerals 7%

The period 2003-2008 has seen a steady decline in the proportion of grains (66 to 60%), grain by-products (8 to 6%) and animal proteins used in compound feed manufacture and a concomitant increase in the proportion of plant proteins (11 to 18%) (Davidson and Pearson, 2009b). Typically, 25-35% of grains used in compound feed manufacture are imported, with wheat being the dominant grain import. Grain by-products and animal proteins are predominantly domestically sourced, with plant protein predominantly (>90%) imported (Davidson and Pearson, 2009b).

The contribution of various grain sources to compound feed production followed a general trend in the period 2003-2007, with wheat, as a proportion of total grain use, increasing from 43 to 53%, while maize decreased from 24 to 14% (Davidson and Pearson, 2009b). However, a global shortage of wheat in 2008 saw the use of wheat, as a proportion of total grain use, fall to 32%, while use of sorghum increased dramatically from 5% in 2007 to 29% in 2008 (Davidson and Pearson, 2009b).

Meat and bone meal has consistently been the main source of animal protein (75-90%) used in compound feed production (Davidson and Pearson, 2009b). Grain by-products used in compound feed manufacture have been consistently dominated by domestically sourced wheat by-products (bran, pollard, broll). While soy meal remains the main source of plant protein, the percentage of plant protein from this source used for compound feed manufacture has decreased from 95 to 62% during the period 2003-2008 (Davidson and Pearson, 2009b). In 2008, 19% of plant protein used in compound feed was in the form of palm kernel meal.

2.3.4.1 Imported feed ingredients

Approximately 35% of the raw materials for compound feed manufactured in New Zealand are imported, with wheat and soy meal usually being the major imported components. Sorghum, barley, canola meal, palm kernel meal and fishmeal may also be imported[1].

In 2008 the major imported ingredients used in compound feed product, by tonnage, were sorghum, soy meal, palm kernel, copra, wheat and fishmeal (Davidson and Pearson, 2009b). Prior to 2008, wheat was the dominant imported compound feed component. Imports of relevant products and sources for the 2008 year are given in Table 2.

[1] http://www.nzfma.org.nz/Nzfm/nzfm.php. Accessed 14 June 2010. Information presented is stated to be from the 2007 year

Table 2: Importations of key animal feed components and their use in the animal feed industry for 2008 year

Item	Import quantity (tonnes)§	Quantity used in the production of compound feed (tonnes)#	Major countries of origin
Cereals, grain sorghum	172,833	166,790	Australia (100%)
Oil-cake and other solid residues; whether or not ground or in the form of pellets, resulting from the extraction of soya-bean oil	137,994	108,225	Argentina (47%), Brazil (34%), USA (12%)
Oil-cake and other solid residues; whether or not ground or in the form of pellets, resulting from the extraction of palm nuts or kernels oils	1,104,184	33,086	Malaysia (53%), Indonesia (46%)
Copra+	8,436	9,133	Philippines (73%)
Cereals, meslin and wheat other than durum*	291,989	6,810	Australia (93%), Canada (7%)
Flours, meals and pellets; of fish or of crustaceans, molluscs or other aquatic invertebrates+	1,865	3,550	Peru (53%), Chile (18%)

§ from http://www.stats.govt.nz/infoshare/
# from (Davidson and Pearson, 2009a)
+ It is uncertain why reported import quantities are less than reported use quantities for these products, but may relate to carryover of product or differences in the reporting period
* Will include material for both human and animal consumption

The imports listed in Table 2 will include some material for human consumption (cereals, meslin and wheat other than durum) and material used in non-compounded feeds (palm kernel oil-cake). However, the available information does not allow determination of end use of imported material.

The large amount of palm kernel oil-cake imported during 2008 is due to increased use of this material as supplementary feed in the dairy industry. Imports increased dramatically during 2008 due to ongoing drought issues in some areas[1].

2.3.5 Issues and concerns in the New Zealand animal feed industry

A recent review of the New Zealand animal feed industry identified a number of issues of potential concern, with respect to the safety of animal feed (Davidson and Pearson, 2009b). Issues potentially relevant to the current risk profile include:

- Imported feeds. The importation of feed, feed ingredients and feed commodities has the potential to introduce pathogens or contaminants. The aquaculture industry is

[1] http://www.maf.govt.nz/mafnet/rural-nz/statistics-and-forecasts/farm-monitoring/2008/pastoral/part2.pdf

particular sensitive to this issue, as greater than 90% of aquaculture feed is imported in a compounded form.

- Food and industrial by-products. Ruminant animals can be fed almost any food or industrial by-product, provided it is not overtly toxic, and considerable growth in such practices has occurred, particularly in the dairy industry. There is potential for risk material to be included in animal feed via this route.

2.3.6 Sources of contamination of feed by the hazard

It is considered that the habitat of *Salmonella* spp. is limited to the digestive tract of animals and humans and that its presence in other environments is due to faecal contamination (Jay *et al.*, 2003). Two routes of contamination of animal feed are recognised; contamination of source materials and contamination or recontamination in the feedmill or during transport (Maciorowski *et al.*, 2004). While these contamination routes are relevant for all feed types, pelletised feed undergoes a heat treatment step with the potential to decontaminate or reduce contamination in the feed. Industry investigations carried out in New Zealand identified three potential mechanisms for *Salmonella* contamination of finished pelletised feed:

- Incomplete inactivation of *Salmonella* by heat during the pelleting process;
- Incidental contamination arising from cross contamination with dust from raw material; and
- Continuous recontamination from deposits of moist contaminated meal within the process, after the pelleting step.

However, it is not possible to say which route of contamination is most important for subsequent *Salmonella* colonisation of animals.

2.3.6.1 Routes of source material contamination

Source material of animal origin may carry salmonellae due to colonisation in the animal or due to contamination or recontamination during processing (e.g. rendering).

Source material of plant origin has the potential to become contaminated through direct deposition of *Salmonella*-containing animal faeces or through deposition of soil or dust previously contaminated with animal faecal material. There is also increasing evidence that *Salmonella* may be internalised in plant tissues in some circumstances (Heaton and Jones, 2008), although it is uncertain whether this is relevant to crops commonly used as components of animal feed.

There are a wide range of animals than may potentially come in contact with feed source material on the farm or during storage at the feedmill. *Salmonella* has been isolated from a number of animals common in the farm environment, including mice (Henzler and Opitz, 1992; Singer *et al.*, 1992; Weigel *et al.*, 2007; Whyte *et al.*, 2003), rats (Kinde *et al.*, 2005; Schnurrenberger *et al.*, 1968), wild birds (Craven *et al.*, 2000; Davies and Wray, 1996; Pangloli *et al.*, 2008; Pennycott *et al.*, 2006; Weigel *et al.*, 2007), insects (Hazeleger *et al.*, 2008; Kinde *et al.*, 2005; Pangloli *et al.*, 2008; Weigel *et al.*, 2007) and larger mammals (e.g. cats, raccoons, opossums) (Schnurrenberger *et al.*, 1968; Weigel *et al.*, 2007).

Soil/dust can be deposited on crops, be ingested by animals during feeding or enter feed source material storage areas through deposition. Salmonellae are able to survive in soils for extended periods following animal defecation or application of human or animal waste to

land (Thomason *et al.*, 1975; Zibilske and Weaver, 1978). Persistence of salmonellae in acid soils is facilitated by their ability to adapt to low pH environments (Foster, 1995). There is also some evidence that salmonellae may survive in soils in a viable but non-culturable state (Turpin *et al.*, 1993), although significance of this state is not yet understood.

2.3.6.2 Contamination at the feedmill

Inspection of a New Zealand feedmill by MIRINZ identified three circumstances likely to contribute to ongoing *Salmonella* contamination:

- Accumulation of moist feed in the conditioner and in the conditioner feed system during shutdown;
- Failure to divert and reprocess product that has not received full heat treatment; and
- Marginal heat treatment of feed during processing.

Salmonella was isolated from moist feed meal samples from the conditioner (the main heat treatment point in the process), the feed to the conditioner and product from the floor by the packing bin (post-conditioner) (P. D. Lowry, 1989, MIRINZ Confidential Report).

Davies and Wray (1997) found widespread contamination of mill environments with *Salmonella*, based on analysis of dust and aggregated fatty material. Mill locations most commonly contaminated with salmonellae were intake pits and augurs for raw ingredient receipt, the cooling system for pellets or mash, grinders and finished product bins. The mills with the highest overall prevalence of contamination were those where the inside of the cooling system had become colonised by salmonellae.

An isolate of the heat and desiccation resistant serotype *S.* Senftenberg was shown to have persisted in a Swedish feedmill through 1995 and 1996 (Löfström *et al.*, 2006). This serotype can be particularly problematic and has since been shown to have persisted on a poultry farm for more than two years, despite cleaning, disinfection, desiccation and depopulation (Broennum Pedersen *et al.*, 2008).

Rodent and wild bird faeces collected in and around feedmills have also been shown to contain *Salmonella* (Davies *et al.*, 1997; Davies and Wray, 1997; Whyte *et al.*, 2003). While no information on *Salmonella* carriage by wild animals around New Zealand feedmills was identified, *Salmonella* has been isolated from rats and wild birds in New Zealand (Alley *et al.*, 2002; Clark *et al.*, 2002; Robinson and Daniel, 1968).

2.3.7 Behaviour of the hazard in animal feed

2.3.7.1 Survival

Four different serotypes of *Salmonella* (*S.* Enteritidis PT4, *S.* Typhimurium – specific type not stated, *S.* Mbandaka and *S.* Senftenberg) were shown to survive for at least 26 months in commercial poultry meal after storage under ambient temperatures and normal atmosphere (Davies and Wray, 1996), although the initial contamination was high (approximately 10^5 MPN/100 g; MPN = most probable number) and had reduced to between 1 and 4 MPN/100 g after 26 months.

Pelletised poultry feed was inoculated with broth culture (6 ml /70 g of feed) containing high concentrations (>10^8 organisms/ml) of *S.* Typhimurium (Williams and Benson, 1978).

Samples were stored at 11, 25 or 38ºC. *S.* Typhimurium was found to survive longer at lower temperatures; at least 18 months at 11^{o}C, 16 months at 25^{o}C and 40 days at 38^{o}C, although the relative humidity at which each sample was stored was inconsistent. For *S.* Senftenberg, inoculated into chick starter feed and stored at 4°C, there was an initial decline in numbers followed by a period where the number of recoverable cells remained almost constant (Liu *et al.*, 1969).

Increasing the moisture content of feed has been shown to lead to a more rapid initial decline in *S.* Senftenberg, followed by lower stable concentrations (Liu *et al.*, 1969). Studies with *S.* Montevideo and *S.* Heidelberg in poultry feed and meat and bone meal demonstrated more rapid die off at higher water activities (a_w = 0.75) than lower water activities (a_w = 0.43 or 0.52) (Juven *et al.*, 1984). *S.* Montevideo counts decreased more slowly than *S.* Heidelberg at low a_w. Counts for both serotypes had generally reduced by 5-6 $\log_{10}$ after 14 weeks. This is consistent with earlier studies of *S.* Oranienburg and *S.* Senftenberg survival in fishmeal, which indicated increased survival at lower water activity, lower temperatures and under a nitrogen atmosphere (Doseburg *et al.*, 1970).

2.3.7.2 Growth

No reports of growth of salmonellae in animal feed were found, although it has been postulated that growth could occur if the feed were allowed to become wet (Jones, 2002). Additional of 40% water to meat and bone meal was sufficient to allow growth of *Salmonella* (Smyser and Snoeyenbos, 1979). High moisture content in feed is likely to result in a range of technological and quality issues. For example, meal moisture contents of 17% are sufficient to cause pellet press problems in the feedmill, while high feed water activity at the farm level is likely to result in feed quality issues (e.g. disintegration of pelleted feed, microbiological deterioration). High moisture content is very unlikely to occur in whole batches of commercial feed, but may occur in material 'hanging up' in uncleanable pockets within the processing environment.

2.3.7.3 Death

Heat treatment

The most common antimicrobial treatment applied to animal feed is heat treatment. The production of good quality pelleted feed includes a high-temperature conditioning, followed by passing of the feed through a die to form the pellet. The temperature increase during conditioning is achieved through steam injection. The friction involved in the pelleting process will instantly raise the temperature of the feed by a further few (3-6ºC) degrees (Cooney, 2010; Panel on Biological Hazards, 2008). Pelleting has been reported to involve temperatures between 70 and 90ºC (Panel on Biological Hazards, 2008). New Zealand feed mills have reported minimum meal temperatures during conditioning of 80ºC, with 90ºC achieved under optimum conditions (input feed moisture content, steam quality) (Lake *et al.*, 2005). New Zealand feed mills consulted also reported a conditioner residence time of 90 seconds. Although it was recognised that individual meal particles would have residence times normally distributed around this value, the reported range is plus or minus 12 seconds (Lake *et al.*, 2005).

The effectiveness of heat treatment may be influenced by the composition of the feed (e.g. fat). In general, variations in composition that decrease the availability of water will increase

the thermal resistance of the organism (Doyle and Mazzotta, 2000; Liu *et al.*, 1969). For example, Juneja and Eblen (2000) found that increasing the fat content of beef from 7 to 24% resulted in an increase in the lag time for thermal inactivation, but a decrease in the D-time. While no equivalent information on the impact of fat content on survival of *Salmonella* in animal feed was located, it should be noted that manufactured animal feeds usually have fat contents towards to bottom end of the range used by Juneja and Eblen[1,2,3]. The increased thermal resistance of microorganisms with increased product fat content has also been demonstrated for spore-forming organisms in meat and bone meal (Swan *et al.*, 1996)

The influence of water activity on heat resistance has been shown for *S.* Senftenberg (Liu *et al.*, 1969). For example, the D-time (the time required to achieve a 1 Log_{10} reduction in the microbial concentration at a defined temperature) at 60°C was 156 minutes with 5% moisture and 65.47 minutes at 10% moisture in meat and bone meal.

The effects of temperature and moisture on the inactivation of salmonellae in pelleted feed have been studied (Himathongkham *et al.*, 1996), but the data are not presented in the form of D-times and *z*-values (the temperature increase required to achieve a ten-fold decrease in D-time). Thermal inactivation increased with temperature and with increasing moisture content of the feed, as would be expected. Based on the data presented a heat treatment at 85°C for 90 seconds will produce a 10,000-fold (4 log_{10}) reduction in salmonellae where the moisture content of the feed is 15%. Using this model the optimum conditions achieved in New Zealand feedmills (90ºC for 90 seconds) would be expected to achieve an approximate 40,000-fold (log_{10} 4.6) reduction in *Salmonella* numbers at 15% moisture.

While no information is available on *Salmonella* counts in feed in New Zealand, studies overseas have reported maximum concentrations of approximately 80 CFU/g (see Appendix 2). A 10,000-fold reduction in such numbers would result in a *Salmonella* concentration of 0.008 CFU/g or approximately one organism per 100 g of feed. Hinton (1988) found no colonisation in 20 chickens consuming feed artificially contaminated with *S.* Kedougou at a concentration of 0.01 CFU/g. Little information is available on concentrations of *Salmonella* in feed causing colonisation in other animal species (see Appendix 3 for a summary).

Most studies on thermal inactivation of salmonellae in feed use artificially contaminated feed, but there is evidence to suggest that organisms in naturally contaminated feed may be more resistant to heat inactivation, probably due to selection of heat resistant salmonellae (Williams, 1981). This may be due to the fact that a range of biotic stresses (heat, cold, starvation, and desiccation) can increase the heat resistance of salmonellae (Kobayashi *et al.*, 2005; Wesche *et al.*, 2005). There is also evidence that *Salmonella* serotypes vary considerably in their heat resistance (Doyle and Mazzotta, 2000; Liu *et al.*, 1969; Ng *et al.*, 1969; Quintavalla *et al.*, 2001; Stopforth *et al.*, 2008; VanCauwenberge *et al.*, 1981) and resistance to desiccation (Broennum Pedersen *et al.*, 2008).

[1] http://www.nrm.co.nz/
[2] http://www.pclfeeds.co.nz/
[3] http://www.wan-nz.co.nz/

Chemical treatment

A number of chemicals may be added to feed to control *Salmonella* contamination, including organic acids (acetic, propionic, citric, formic) and their salts, ethanol, formaldehyde and isopropyl alcohol (Ha *et al.*, 1998; Martin *et al.*, 2005).

Organic acid-based treatments

It is considered that organic acids exert their antibacterial effects through disruption of pH gradients and intracellular pH regulation (Van Immerseel *et al.*, 2006). There is also evidence to suggest that organic acids interfere with expression of virulence genes, reducing intestinal invasion (de Jonge *et al.*, 2003; Van Immerseel *et al.*, 2006). However, there are also concerns that the use of organic acids may lead to the selection of acid tolerant strains, which may be better able to survive gastric acidity in humans (de Jonge *et al.*, 2003).

A propionic acid-based additive was added to mash poultry feed at 0.25, 0.5, 0.75 or 1% (Rouse *et al.*, 1988). It should be noted that the authors did not specify the manufacturer's recommended addition rate and whether than levels of addition were consistent with manufacturer's recommendations. Samples were then inoculated with *Salmonella* to a final concentration of approximately 10^2 or 10^6 CFU/g. At lower inoculation levels *Salmonella* was not detected in the feed after 24 hours at any propionic acid level and no *Salmonella* was detected in any samples after 72 hours. In the same study, broilers were colonised with *Salmonella* by feeding commercial poultry feed inoculated with *Salmonella* to a concentration of 10^3 CFU/g. Subsequent feeding of poultry feed containing 1 or 5% of the propionic acid–based additive, in addition to *Salmonella*-contaminated feed, resulted in broilers being free of *Salmonella* contamination by slaughter, as assessed by faecal and intestinal cultures.

Fishmeal or meat and bone meal was inoculated with *Salmonella* to a concentration of 10^4 (high), 10^3 (medium) or 10^2 (low) organisms/100 g and then treated with mixes of organic acids (propionic, formic, sorbic) at manufacturers recommended rates (10-15 kg/tonne of feed) (Carrique-Mas *et al.*, 2007). Performance was judged by detection of *Salmonella* in eight replicate samples. All fishmeal samples with high initial concentrations of *Salmonella* were still positive 72 hours after organic acid treatment. For medium initial *Salmonella* concentrations at least 50% of samples remained positive after 72 hours and for low initial *Salmonella* concentrations 1-6 of eight samples remained positive after 72 hours. Treatments were more effective at controlling *Salmonella* in meat and bone meal, with 0-2 of eight samples remaining positive at each initial *Salmonella* concentration.

Zinc salts of organic acids appear to be more effective than sodium salts in controlling *Salmonella* (Park *et al.*, 2003). When added to poultry layer mash containing approximately 10^6 CFU/g *S.* Typhimurium at a rate of 1%, log reductions after nine days for zinc acetate and zinc propionate (2.55 and 2.40 $\log_{10}$ CFU/g, respectively) were greater than for untreated layer ration (1.49 $\log_{10}$ CFU/g) (Park *et al.*, 2003). Log reductions for sodium acetate and sodium propionate amended feed (1.71 and 1.66 $\log_{10}$ CFU/g, respectively) were less than for the corresponding zinc salts and less than the corresponding untreated layer ration (1.84 $\log_{10}$ CFU/g).

While acid decontamination may not be as effective as heat decontamination, it does provide a level of residual protection against post-production contamination/recontamination of the

feed (Carrique-Mas *et al.*, 2007; Rouse *et al.*, 1988). There is also evidence that organic acids may create an intestinal environment unfavourable to *Salmonella* colonisation from other sources (Al-Natour and Alshawabkeh, 2005; Humphrey and Lanning, 1988).

Reduction of salmonellae in feed by organic acids may take several days and it is possible that feed may have been consumed and colonisation established before sufficient inactivation has had time to occur (Hinton and Linton, 1988).

The efficacy of organic acids in controlling *Salmonella* contamination is increased at higher rates of inclusion, by application in a liquid form, and use of products with a high proportion of free acid (Panel on Biological Hazards, 2008). Although higher concentrations of organic acids in feed may result in improved *Salmonella* control there are also implications for cost, corrosion of equipment, safety of workers and animal palatability (Panel on Biological Hazards, 2008).

Chemical treatment of feed is not routinely used in New Zealand, but may be employed in feedmills or rendering plants when a persistent *Salmonella* contamination exists or when an avian pathogenic *Salmonella* is isolated (e.g. *S.* Typhimurium DT160). Products reported to be used in broiler feed include organic acid formulations, such as Sal Curb™ [1], or *Salmonella*-binding agents, such as the mannanoligosaccharide product BioMos™ [2], which are added at the mixing stage.

Formaldehyde-based treatments

A number of studies have shown improved decontamination of feed by formaldehyde when compared with organic acids (Carrique-Mas *et al.*, 2007; Duncan and Adams, 1972; Smyser and Snoeyenbos, 1979). However, formaldehyde may be less effective in providing residual protection due to its volatility (Khan *et al.*, 2003).

2.4 Cleaning Practices in the Feedmill Environment

The feedmill environment, including incoming raw materials, is essentially dry, allowing minimal scope for bacterial growth. The exception to this is the conditioner in which a significant amount of moisture in the form of steam is introduced into the feed. However, the introduction of steam during this process would likely inactivate any vegetative bacteria such as *Salmonella*.

Crushing plants, where plant protein products such as soy meal are prepared, are also dry environments allowing minimal scope for bacterial growth.

In contrast, the rendering environment producing meat and bone meal contains both wet and dry environments, with associated wet and dry (physical removal) cleaning regimes. A major focus of rendering plant design is the strict separation of wet and dry areas.

Cleaning of feedmills usually concentrates on the physical removal of loose and adhering material (Lake *et al.*, 2005). Modern systems incorporate cleaning as well as dust control measures. Some operators have reported the use of sanitisers in particular situations (in

[1] http://www.keminonline.com/specifications/salcurb-liquid.pdf
[2] http://www.alltech.com/About/bio-Mos.htm

storage areas used for meat and bone meal, affected areas following *Salmonella* detection). Both feedmills and rendering plants are primarily designed for material handling, with good material handling design sometimes inconsistent with good hygiene design. Some equipment in the rendering process is sealed or closed and is essentially uncleanable (MIRINZ, 1994). Feed or meal can therefore be recontaminated from macro biofilms in which *Salmonella* can grow to high numbers.

While the heating process of the rendering system is sufficient to destroy vegetative cells including *Salmonella* spp., there is a risk of recontamination following heat treatment. An important factor that contributes to widespread *Salmonella* contamination in rendering plants is the build up of moist meal deposits (e.g. edges of casings around augers) and thorough dry cleaning of the rendering plant and removal of these moist meal deposits regularly would reduce the risk of further contamination past the heat treatment step.

2.5 Sampling and Testing for *Salmonella* in Animal Feeds

2.5.1 Sampling

2.5.1.1 Feed and feed ingredients

Sampling for detection of *Salmonella* in animal feed is difficult, as the organism is often present at low concentrations, masked by competing microorganisms which may prevent recovery and is likely to be unevenly distributed within a large volume of feed.

Taking a number of small subsamples is believed to be more effective that taking a single larger sample and a number of approaches to achieving this have been included in published studies.

2.5.1.2 Feed mill environment

It has been reported that dust and fine particles are more likely to be contaminated with *Salmonella* than feed ingredients or finished feed and sampling of dust in filters and other equipment has been shown to be a good indicator of *Salmonella* presence in a feed mill (Davies and Wray, 1997; Panel on Biological Hazards, 2008). Examples of indicator sampling points for dust samples include:

- Intake augur pits;
- Ingredient bins;
- Coolers;
- Crumblers and pellet shakers;
- Finished product bins; and
- Outloading gantry.

2.5.2 Testing

Salmonellae may be detected in feed or feed ingredients by culture, immunological or molecular methods (Panel on Biological Hazards, 2008). The low water activity of most animal feed materials results in dehydration of bacterial cells and suitable isolation methods for *Salmonella* should give injured and stressed cells the opportunity to recover and multiply (Panel on Biological Hazards, 2008).

Given that *Salmonella* are likely to be present in feed at very low concentrations, in a stressed or injured state and amongst competing microflora, current methods require first a non-selective incubation followed by an enrichment step which allows the *Salmonella* to grow while inhibiting the growth of other bacteria (MIRINZ, 2005). Since the *Salmonella* may have been subjected to heat treatment, they may be sublethally injured and this requires the use of gentle initial conditions to allow cell repair prior to selective enrichment. Such an approach is routinely used for dry foods and feed (D'Aoust *et al.*, 1993; Panel on Biological Hazards, 2008).

Once the enrichment has been performed a variety of detection methods are available. The method selected will depend on factors such as cost, the need for rapid results, sensitivity and specificity. Details are included in Appendix 1.

3 EXPOSURE ASSESSMENT

Information from other countries on the prevalence of *Salmonella* in animal feed and animal feed ingredients, serotypes found, concentrations measured and doses causing colonisation in animals is included in Appendix 2.

At present, information on *Salmonella* isolated from different sources is not well coordinated. A study was recently conducted to investigating strategies for a national *Salmonella* surveillance programme (Lake and Sexton, 2009).

The following sections summarise information currently available from a range of information sources.

3.1 Prevalence of *Salmonella* in New Zealand feed

3.1.1 Feed ingredients

Table 3 summarises consolidated New Zealand industry data on the prevalence of *Salmonella* in feed ingredients for the years 2006-2008 (James Fick, New Zealand Feed Manufacturers' Association, personal communication).

Table 3: Prevalence of *Salmonella* in feed ingredients in New Zealand, 2006-2008

Year	Number of samples tested	Number *Salmonella* positive (%)	Types of feed ingredients *Salmonella* positive (number of isolates)[1]
2006	3551	84 (2.4)	MBM (56), palm kernel (13), offal (4), broll (4), copra (2), canola (2)
2007	3213	105 (3.3)	MBM (64), offal (19), broll (7), copra (4), blood (4), palm kernel (3)
2008	3862	147 (3.8)	Soy meal (60), MBM (34), broll (26), copra (10), offal (7), palm kernel (3), ground wheat (3)
2009	2800	118 (4.2)	MBM (61/1034; 5.9%), soy meal (17/422; 4.0%), broll (14/650; 2.2%), copra (11/87; 12.6%), palm kernel (6/272; 2.2%), offal (3/248; 1.2%)

MBM – meat and bone meal

[1] For 2009, numbers of samples of each ingredient type tested were provided. For example, for MBM 1034 samples were tested, with 61 (5.9%) testing positive for *Salmonella*

Unfortunately, no breakdown of the number of samples tested into different ingredient types was possible for 2006-2008, precluding assessment of prevalence of *Salmonella* in individual feed ingredients in those years. Values for the prevalence of *Salmonella* in feed ingredients from the 2009 year are within the range for these product types reported internationally (see Appendix 2, Table 12), although no comparative data are available for some product types (e.g. copra).

While detailed information on individual isolates is not maintained centrally, previously investigations involving traceback of individual isolates have been informative.

3.1.2 Finished feed

Table 4 summarises consolidated New Zealand industry data on the prevalence of *Salmonella* in finished compound animal feed for the years 2006-2008 (James Fick, New Zealand Feed Manufacturers' Association, personal communication).

Table 4: Prevalence of *Salmonella* in animal feed in New Zealand, 2006-2008

Year	Number of samples tested	Number *Salmonella* positive (%)	Types of finished animal feed *Salmonella* positive (number of isolates)
2006	3675	38 (1.0)	Pellets (29), mash (9)
2007	3441	33 (1.0)	Pellets (23), mash (10)
2008	3658	11 (0.3)	Pellets (4), mash (7)

The apparent prevalence of *Salmonella* in finished animal feed in New Zealand is similar to recent prevalence figures reported internationally (see Appendix 2, Table 11). For example, prevalence of *Salmonella* in a range of feed types in the EU in 2006 was in the range 0.6-0.8%. The data in Table 4 suggest an improvement in the situation with respect to *Salmonella* in pelleted feed from the 2007 to the 2008 year. This seems to be associated with a sharp drop in the number of *S.* Tennessee isolates from finished feed (24 in 2006, 12 in 2007 and 1 in 2008; see Table 5). However, these results represent a mixture of routine structured monitoring and problem investigations and may overestimate the prevalence of *Salmonella* contamination in these products.

3.1.3 *Salmonella* serotypes in New Zealand feed

Table 5 summarises information on *Salmonella* serotypes isolated from feed ingredients, finished feeds and the feedmill environment for the years 2006-2008. These serotypes relate to the isolates reported in Table 3 and 4. The information was provided by the New Zealand feed industry (James Fick, New Zealand Feed Manufacturers' Association, personal communication).

Table 5: *Salmonella* serotypes isolated from feed ingredients, finished feed and feedmill environment in New Zealand, 2006-2008

Sample type	Year	Total isolates	Dominant *Salmonella* serotypes (number of isolates)*
Feed ingredients	2006	84	Derby (12), Heidelberg (10), Oranienburg (10), Species (7), Senftenberg (6), Agona (6), Species group B (6), London (4), STM (3), STM 101 (3), Mbandaka (2), Kentucky (2), Anatum 15+ (2), Brandenburg (2)
	2007	105	Senftenberg (31), Heidelberg (12), Tennessee (9), Infantis (8), Agona (7), STM (4), Species 6,7:K (4), Brandenburg (3), Mbandaka (3), Oranienburg (3),
	2008	147	Infantis (16), Senftenberg (15), Tennessee (15), Species (14), STM (9), STM 42 (8), Banana (7), Lexington 15+ (6), STM 160 (5), STM RDNC (5), Mbandaka (4), Derby (3), Livingstone (3)
Finished feed	2006	38	Tennessee (24), Kentucky (3), Anatum 15+ (3), Oranienburg (2)
	2007	33	Tennessee (12), Give 15+ (9), Mbandaka (3), Rissen (2), STM 101 (2)
	2008	11	STM RDNC (5)
Feedmill environment	2006	137	Tennessee (50), Kentucky (34), Species (27), Infantis (5), Senftenberg (4), Species group E (4), Hindmarsh (2)
	2007	66	Tennessee (39), Give 15+ (9), Kentucky (3), Mbandaka (3), Senftenberg (3), Infantis (2), Rissen (2)
	2008	39	Tennessee (16), SE 9a (4), STM (4), Infantis (3), STM RDNC (3), Give 15+ (2), Species (2)

* Serotypes are only listed if more than one isolate was typed for a sample type in a particular year
STM = *Salmonella* Typhimurium
RDNC = not conforming to any recognised definitive type

Salmonella typing information for non-human isolates is also published quarterly by ESR's Enteric Reference Laboratory[1].

Results are published for poultry feed and for meat and bone meal, a common component of animal feed. Table 6 summarises information on major serotypes detected in these products for the years 2004-2007.

[1] http://www.surv.esr.cri.nz/enteric_reference/enteric_reference.php

Table 6: Major *Salmonella* serotypes in New Zealand poultry feed and meat and bone meal, 2004-2007

Serotype	Total	Number of isolates*							
		Poultry Feed				Meat and Bone Meal			
		2004	2005	2006	2007	2004	2005	2006	2007
Agona	11	2	4	5					
Anatum	68	4	1	2	4	21	12	8	16
Anatum 15+	21		6	9	3			2	1
Brandenburg	79	23	14	7	4	19	4	1	7
Cubana	9	9							
Derby	116	11	64	19	4	3	6	4	5
Give	30				30				
Havana	17	3		2	4	2	3	1	2
Heidelberg	16	5	2	9					
Hindmarsh	10	4	5	1					
Infantis	58	4	3		6	7	15	19	4
Kentucky	2	2							
Kiambu	23	6	2	3	1	1	1	6	3
Lille	2						2		
London	6			4	1	1			
Mbandaka	20	2	4	3	9				2
Montevideo	21				2		8	4	7
Oranienburg	4			2	2				
Orion 15+	7			1				4	2
Rissen	4				4				
Senftenberg	37	6	10	5	3	11			2
Tennessee	39	13	9	4	1	1	7		4
STM1	5	5							
STM8	2	2							
STM9	2	2							
STM41	2						2		
STM42	3	3							
STM101	9		2	5	2				
STM154	1								1
STM156	8	7		1					
STM160	31	15	9	7					
STM RDNC	9	5		1	3				
Group B4,12:-:1,2	2		2						
Group C6,7:k:-	4				4				

* Only serotypes for which greater than one isolate was typed in at least one year are included
STM = *Salmonella* Typhimurium
RDNC = not conforming to any recognised definitive type

The patterns of serotypes detected are reasonably consistent from year to year. Serotypes are also reasonably consistent with those detected in feed products in other countries (see Appendix 2, Table 13 and associated commentary). Analysis of serotyping records suggests

that most of the poultry feed isolates were from raw feed ingredients, rather than from finished compound feed. However, the nature of the feed ingredient is only recorded for a small number of isolates. These data do not allow any conclusions to be drawn on routes of introduction of specific serotypes or *Salmonella*, in general, into the feed environment.

3.2 Prevalence of *Salmonella* in Meat from Food-producing Animals in New Zealand

In order to consider the likely contribution of animal feed to *Salmonella* contamination in food-producing animals and the human food chain, it is necessary to consider the total level of *Salmonella* contamination in the meat from food-producing animals in New Zealand. A European assessment concluded that in regions with low *Salmonella* prevalence in food-producing animals, *Salmonella* contaminated feed represents a major source for introduction of *Salmonella* into the food production chain, while in regions with high prevalence the relative importance of contaminated feed is difficult to quantify (Panel on Biological Hazards, 2008).

Baseline surveys of *Salmonella* on bovine (1995-1996) and ovine (1996-1997) carcasses at slaughter (pre-chill) were conducted (Dr Roger Cook, NZFSA, personal communication). Carcass contamination was assessed on three pooled 100 cm^2 swabs per carcass. *Salmonella* was not detected on any of 941 (149 heifers, 272 steers, 268 cows and 252 bulls) bovine carcasses, representing a prevalence of 0.0% (95th percentile confidence interval 0.0-0.4%). *Salmonella* was detected on 2/500 ovine carcasses, representing a prevalence of 0.4% (95th percentile confidence interval 0.05-1.4%).

The National Microbiological Database (NMD) programme involves systematic collection of information on the microbiological status of meat from certain farmed animal species in New Zealand. Table 7 summarises the species currently included in the NMD for *Salmonella* and a summary of results from inception to date. Information is reproduced from the NZFSA's *Salmonella* Risk Management Strategy (NZFSA, 2009).

Table 7: National Microbiological database for *Salmonella* in New Zealand

Meat type	NMD *Salmonella* status	Programme active period	Percentage positives to date*
Sheep and Lamb meat	Stopped	1997-2006	Carcasses post-slaughter 0.19-0.93 Carcasses post-chilling 0.00-0.17 Primal cuts 0.00-0.02 Bulk product 0.03-0.14
Beef meat	Active	1997-	Carcasses post-slaughter 0.01-0.03 Carcasses post-chilling 0.00-0.49 Primal cuts 0.00-0.02 Bulk product 0.00-0.01
Young calf veal	Active	1998-	Carcasses post-slaughter 0.99-4.95 Carcasses post-chilling 0.00-5.52 Primal cuts 0.14-0.72 Bulk product 0.28-1.41
Goat meat	Active	1999-	Carcasses post-slaughter 0.15-0.75 Carcasses post-chilling 0.00-7.87 Primal cuts 0.00-0.30 Bulk product 0.00-0.28
Farmed deer meat	Active	2001-	Carcasses post-slaughter 0.00-0.10
Ostrich/emu meat	Active	2001-	Carcasses post-slaughter 0.00-0.68
Broiler chicken meat	Active	2001-2010	Carcasses post-slaughter 1.2-1.6** Carcasses post-slaughter 0.03-0.5**
Pig meat	Active	Aug 2009 – Mar 2010	Carcasses post-slaughter 0.43-2.19 ***

* 95th percentile confidence interval
** Dr Roger Cook, NZFSA, personal communication
*** 915 carcasses tested to date (Dr Roger Cook, NZFSA, personal communication)

The prevalence of *Salmonella* contamination in the meat of food-producing animals in New Zealand is generally low, as determined by the NMD programme. Further information on sampling sites, sampling frequencies and performance standards for the NMD can be found on the NZFSA website[1].

[1] http://www.nzfsa.govt.nz/animalproducts/legislation/notices/animal-material-product/nmd/schedule-1-technical-procedures-nmd-final.pdf

3.3 Prevalence of *Salmonella* in Foods of Animal Origin in New Zealand

A survey of *Salmonella* in uncooked retail meats also demonstrated low prevalence (Wong *et al.*, 2007). Estimates of the prevalence of *Salmonella* in meat from this survey are consistent with estimates from the NMD programme. A summary of this survey is included in Table 8.

Table 8: Prevalence and serotypes of *Salmonella* in uncooked retail meats in New Zealand, 2003-2005

Meat type	Number of Samples		Prevalence, % (95%CI)	Serotypes detected
	Tested	Positive for *Salmonella*		
Chicken	232	7	3.0 (1.2-6.1)*	*Salmonella* sp. 6,7:k:- Enteritidis PT9a Typhimurium DT1 *Salmonella* sp. 4,12:-:- *Salmonella* sp. 4,5,12:-:- *Salmonella* sp. 4,12:-:- Typhimurium DT160
Lamb/mutton	230	3	1.3 (0.3-3.8)	Brandenburg (x2) *Salmonella* sp. 4:-:2
Unweaned veal	183	1	0.5 (0.0-3.0)	Typhimurium DT1
Beef	232	1	0.4 (0.0-1.6)	Infantis
Pork	231	0	0.0 (0.0-1.6)	

95% CI = 95th percentile confidence interval

* Since 2005, the nationwide broiler prevalence (95th CI) under the NMD programme has decreased from 2.4-3.4% to 0.03-0.5% (Dr Roger Cook, NZFSA, personal communication). A similar decrease in prevalence on uncooked retail meat may have occurred.

None of the serotypes listed in Table 8 have been reported in finished animal feed in New Zealand (see Table 4), although the date ranges for the uncooked meat data and the animal feed data do not overlap. The detection of *S.* Brandenburg in both sheepmeat and animal feed ingredients is more likely to be due to contamination in sheep persisting in feed ingredients of animal origin than contamination in the feed ingredients causing colonisation in sheep, as sheep are generally not fed compound feed.

To examine the prevalence of *Salmonella* in New Zealand pork, a total of 100 New Zealand produced chilled pig carcass samples and 110 imported (Australia, USA, Canada) pork samples were obtained from processors between October 2004 and May 2005 (Wong *et al.*, 2009). The domestic pig carcasses originated from four New Zealand abattoirs. Ninety-five of the carcasses came from the South Island. The pig carcasses were swabbed with a sponge over a 100 cm^2 template. Swabs of pork from Canada and the USA were taken before the meat was cooked under Porcine Reproductive and Respiratory Syndrome (PRRS) requirements. The imported meat was either excised or swabbed as for domestic samples. *Salmonella* was tested using a presence/absence procedure.

Salmonella was not isolated from domestic pig carcasses or from pork imported from Canada and the USA; only samples of imported Australian pork were positive.

3.4 Commentary on Overseas Data

Overseas data indicate temporal trends in the prevalence of *Salmonella* contamination in finished feed and feed ingredients (Appendix 2, Tables 11 and 12). While earlier studies often observed *Salmonella* in greater than 10% of some product types, more recent data (post-2000) rarely include prevalence figures for *Salmonella* in excess of 10%.

While many different *Salmonella* serotypes may be detected in feed and feed ingredients, there is no doubt that there are certain serotypes that are more frequently observed in feed. This is almost certainly due to these serotypes having superior resistance to biotic stresses that may occur during feed processing (heat, desiccation, acid). For example, *S.* Senftenberg is a common serotype in feed, both in New Zealand and overseas. The heat and desiccation resistance of this serotype is well documented (Broennum Pedersen *et al.*, 2008; Liu *et al.*, 1969; Ng *et al.*, 1969). Other serotypes commonly associated with feed overseas and in New Zealand include *S.* Mbandaka, *S.* Livingstone, *S.* Anatum, *S.* Agona, *S.* Rissen, *S.* Tennessee, *S.* Infantis and *S.* Oranienburg.

4 EVALUATION OF ADVERSE HEALTH EFFECTS

Salmonella colonisation in pigs and poultry is typically asymptomatic, while colonisation in ruminant animals is less common, but more likely to result in clinical signs (Panel on Biological Hazards, 2008). Therefore, concerns associated with *Salmonella* colonisation in food-producing animals are usually with the potential for the *Salmonella* to be transmitted to humans and cause salmonellosis. This concern is reflected in the second of two risk questions that this profile seeks to address:

- What is the flow-on effect (from introduction of *Salmonella* into food-producing animals) for human exposure?

The following material summarises human salmonellosis.

4.1 Salmonellosis (non-Typhoidal)

Incubation: 6-48 hours (usually 12-36 hours).

Symptoms: Diarrhoea, abdominal pain, vomiting, nausea and fever lasting 1-7 days. Hospitalisation rate estimated at 22.1%, case fatality rate 0.8% (Mead *et al.*, 1999). Hospitalisation rates in New Zealand tend to be lower than those reported internationally and the rate was reported to be 13.2% in 2007 (ESR, 2008).

Condition: Salmonellosis.

Toxins: Toxins are not produced in foods.

People Affected: The young, old, and immunocompromised are particularly at risk. In addition people of less privileged socioeconomic groups and those living in higher population densities are more at risk.

Long Term Effects: Septicaemia and subsequent non-intestinal infections can occur. Reactive arthritis, including Reiter's syndrome, may occur 2-4 weeks after gastrointestinal symptoms. Approximately 2% of a population exposed to a triggering infection may develop reactive arthritis, which may last for anywhere from a few days up to a year or longer (Smith, 1994). Salmonellosis has also been associated with development of inflammatory bowel disease (IBD) (Helms *et al.*, 2006) and irritable bowel syndrome (IBS) (DuPont, 2008; Smith and Bayles, 2007).

Treatment: The infection is usually self-limiting although fluid replacement may be required. Antibiotic treatment seems to be either ineffective or results in relapse or prolonged faecal shedding. Certain groups, e.g. new born children, may benefit from antibiotic treatment.

Dose-response: Dose-response relationships for *Salmonella* may be influenced by both the serotype and the food in which the salmonellae are ingested. A summary of available information on dose-response characteristics of *Salmonella* spp. is included in Appendix 3.

4.2 Adverse Health Effects in New Zealand

4.2.1 Incidence

The number of cases and rates per 100,000 population of salmonellosis in New Zealand are shown in Table 9. The incidence data are also shown graphically (by year) in Figure 3, while the number of cases (by month) are shown in Figure 4.

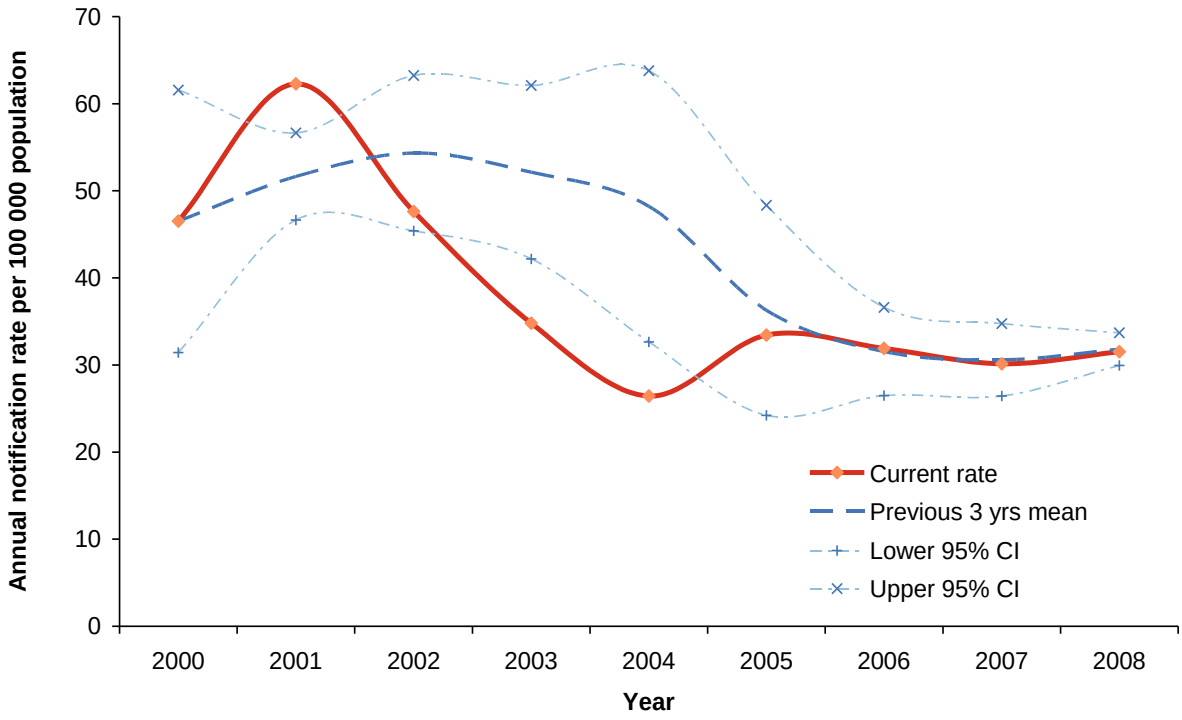
Table 9: Incidence data for salmonellosis in New Zealand, 2000-2008

Year	Number of cases	Incidence (cases/100,000)
2000	1795	48.1
2001	2417	64.7
2002	1880	50.0
2003	1401	37.6
2004	1081	28.9
2005	1382	37.0
2006	1335	31.9
2007	1274	30.1
2008	1346	31.5

Data from Annual Report Concerning Foodborne Disease in New Zealand 2008 (Williman *et al.*, 2009)

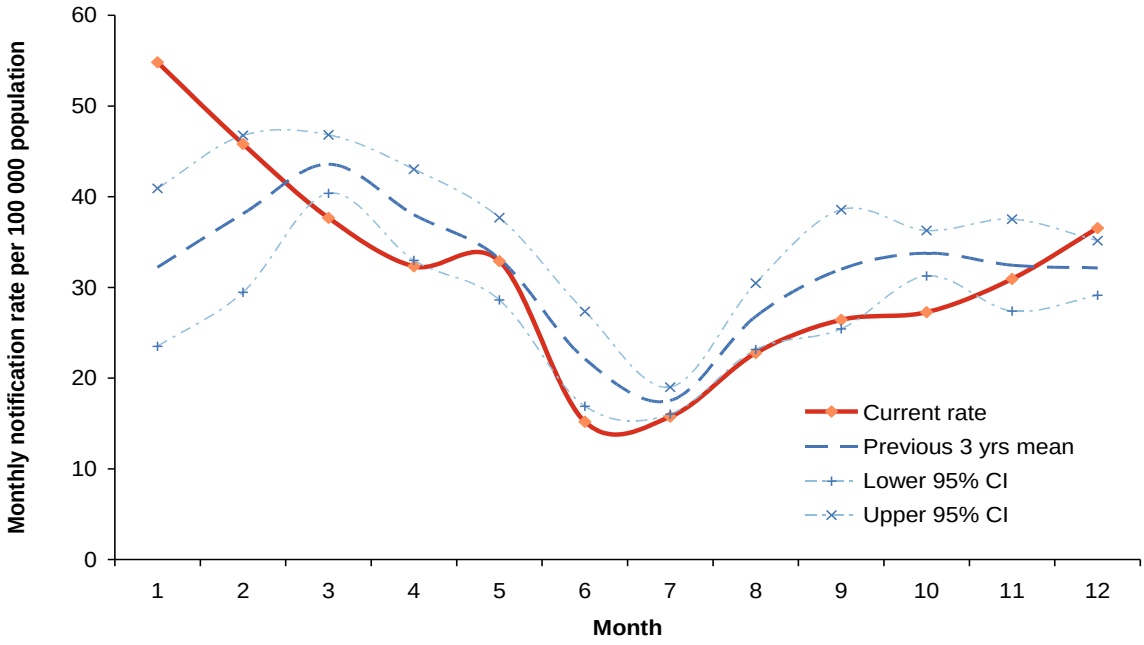
Notifications for salmonellosis have remained reasonably consistent over a long time period. Since 2004 the annual number of notified cases has been particular stable at approximately 1,300 cases per annum.

Figure 3: Salmonellosis notification rate by year 2000 – 2008



Reproduced from (Williman *et al.*, 2009)

Figure 4: Salmonellosis notifications by month January 2008 – December 2008



Reproduced from (Williman *et al.*, 2009)

The frequency of salmonellosis is characterised by a late summer peak and a winter trough. Two changes to this cyclic pattern have occurred since 1998:

- A spring peak occurred in 1998 and each subsequent year, corresponding to the emergence of *S.* Brandenburg as an important cause of human salmonellosis in New Zealand; and
- The winter trough has become less pronounced due to the increasing numbers of STM 160 cases since July 2000 (Anonymous, 2001).

4.2.2 Clinical consequences of *Salmonella* infection

Hospitalisation and fatality rates for notified cases of salmonellosis in New Zealand are given in Table 10. These outcomes are not always reported for each case, so percentages are expressed in terms of the number of cases for which outcomes are known.

Table 10: Outcome data for salmonellosis in New Zealand, 2000-2008

Year	Hospitalised cases	Fatalities	Reference
2000	215/1554 (13.8%)	7/1802 (0.4%)	(Lopez *et al.*, 2001)
2001	279/1934 (14.4%)	2/2417 (0.1%)	(Sneyd *et al.*, 2002)
2002	206/1473 (14.0%)	1/1870 (0.05%)	(Sneyd and Baker, 2003)
2003	167/1118 (14.9%)	0/1401 (0.0%)	(ESR, 2004)
2004	109/871 (12.5%)	0/1081 (0.0%)	(ESR, 2005)
2005	142/1134 (12.5%)	1/1382 (0.07%)	(ESR, 2006)
2006	148/1111 (13.3%)	1/1335 (0.07%)	(ESR, 2007)
2007	110/833 (13.2%)	1/1274 (0.08%)	(ESR, 2008)
2008	123/896 (13.7%)	1/1346 (0.07%)	(ESR, 2009)

Hospitalisation and fatality rates for New Zealand are quite consistent from year to year. It has been noted that disease outcomes may differ with serotype and in a US study hospitalisation rates were reported to range from 14.4% (*S.* Hartford) to 67.0% (*S.* Dublin), while fatality rates ranged from 0.0% (various serotypes) to 3.0% (*S.* Dublin) (Jones *et al.*, 2008). Serotypes commonly observed in animal feed in New Zealand (section 3.1.2) and overseas (Appendix 2) are of low to moderate virulence, as judged by their potential to result in invasive disease and the proportion of cases resulting in hospitalisation and death (Jones *et al.*, 2008).

4.2.3 Animal feed associated outbreaks of human salmonellosis in New Zealand

During January and February of 2003 contamination of broiler poultry feed with *Salmonella* Typhimurium DT1 was detected in the Canterbury region through industry testing (Wong, 2003). The contamination was thought to have originated from wheat used in the feed formulation. Increases in the prevalence of *S.* Typhimurium DT1 on chicken at retail and in the number of notified human cases of salmonellosis, albeit small, were observed during this period (Wong, 2003).

Chicken, whole and portions, from supermarkets, fast food outlets and restaurants was tested for *Salmonella* on eight occasions with sampling dates from 11 February 2003 to 7 March 2003. At the first sampling, 36% of samples (9/25) were *Salmonella* positive with 8/9 positive samples typed as *Salmonella* Typhimurium DT1. At the second sampling (13 February 2003), 17% (4/24) of samples were *Salmonella* positive, with half of the isolates typed as *Salmonella* Typhimurium DT1. One further *Salmonella* Typhimurium DT1 positive sample was found at the third sampling, with no further *Salmonella* positive samples found on the subsequent five sampling occasions.

No human cases of *Salmonella* Typhimurium DT1 were notified in Canterbury during October 2002-January 2003, but seven cases were notified during February 2003.

4.2.4 Serotypes in New Zealand human cases and feed isolates

Of the animal feed types available in New Zealand, *Salmonella* serotype reports produced by ESR's Enteric Reference Laboratory only include serotype data for poultry feed, with separate typing data for meat and bone meal, a major component of animal feed. Previous investigations have revealed that many isolates recorded as animal feed are actually meat and

bone meal (Dr Roger Cook, NZFSA, personal communication). As has been previously noted in international studies (Hald *et al.*, 2006), *Salmonella* serotypes can be grouped into three categories; serotypes found in animal feed, but not associated with human disease, serotypes found in human cases, but not found in animal feed, and serotypes found in both animal feed and human disease cases.

Analysis of *Salmonella* typing data for 2004-2007 identified just three serotypes that were amongst those most commonly detected in both poultry feed or MBM samples and human cases; *S.* Brandenburg, *S.* Infantis and *S.* Typhimurium DT160. However, this should not be viewed as suggesting that human cases are due to the occurrence of these serotypes in animal feed. *S.* Brandenburg is believed to be transmitted directly from colonised animals to humans, with no indication of transmission by food[1]. Therefore, the occurrence of *S.* Brandenburg in animal feed/MBM and human cases is likely to be due to a common source (i.e. colonised animals), rather than due to the organism in feed being transmitted to animals, then food, then humans. Similarly, epidemiological studies on *S.* Typhimurium DT160 implicated contact with wild birds (Thornley *et al.*, 2003) and it is plausible that the presence of this serotype in animal feed/MBM and human cases is due to a common source, rather than a causal connection. Less is known about the epidemiology of *S.* Infantis in New Zealand.

Based on industry data, the predominant *Salmonella* serotype detected in finished feed during 2006 and 2007 was *S.* Tennessee (James Fick, New Zealand Feed Manufacturers' Association, personal communication). In 2006, no human isolates were typed as *S.* Tennessee by ESR's Enteric Reference Laboratory. In 2007, one human case was due to *S.* Tennessee, while in 2008 three human isolates of *S.* Tennessee were identified. This suggests little or no transmission of *S.* Tennessee from finished animal feed through food-producing animals to humans during this period.

[1] http://www.nzfsa.govt.nz/consumers/food-safety-topics/foodborne-illnesses/salmonella-brandenburg/faq.htm

5 EVALUATION OF RISK

International evidence for animal colonisation and human disease resulting from *Salmonella* contamination of animal feed and risk assessments related to animal feed are included in Appendix 3.

5.1 Risk Assessments

5.1.1 New Zealand

No New Zealand risk assessments were found that directly assessed the risk of human salmonellosis due to contaminated animal feed.

A risk model framework for *Salmonella* in the poultry food chain has been established, including a feed component (Lake *et al.*, 2006). The model predicts a low probability of broiler chickens becoming colonised with *Salmonella* if pelletised poultry feed is produced with an appropriate conditioning (steam treatment) step.

An NZFSA report addressing attribution of salmonellosis cases to food sources considered the proportion of cases due to foods of animal origin, but did not address animal feed specifically (NZFSA, 2007). A review of salmonellosis outbreaks similarly did not consider animal feed as a potential primary source of foodborne outbreaks (King and Lake, 2007).

5.1.2 Overseas

A Danish study assessed the potential for *Salmonella* in imported soybean meal, used for animal feed, to result in human salmonellosis (Hald *et al.*, 2006). Of 82 serotypes found in both animal production and human cases, 45 were also found in feed. A semi-quantitative ranking identified *S.* Agona, *S.* Senftenberg, *S.* Kentucky, *S.* Newport, *S.* Tennessee, *S.* Mbandaka, and *S.* Oranienburg as having the highest potential impact on human health from transmission via animal feed. A previously published attribution model (Hald *et al.*, 2004) was used to estimate that, during 1999-2003, 1.7% of human salmonellosis cases and 2.1% of domestically-acquired human salmonellosis cases in Denmark could be attributed to feedborne serotypes from consumption of beef and pork.

The EFSA Panel on Biological Hazards carried out a microbiological risk assessment for feed for food-producing animals (Panel on Biological Hazards, 2008). *Salmonella* was identified as the major hazard for microbial contamination of animal feed, with oilseed meal and animal derived protein the major risk materials for introduction of *Salmonella* into feed mills and compound feed. It was concluded that in regions with low *Salmonella* prevalence in food-producing animals, *Salmonella* contaminated feed represents a major source for introduction of *Salmonella* into the food production chain, while in regions with high prevalence the relative importance of contaminated feed is difficult to quantify.

5.2 Economic Costs and Burden of Disease

The annual health burden of salmonellosis and its sequelae for New Zealand has been estimated in terms of both Disability Adjusted Life Years (DALYs) and Cost of Illness (Lake *et al.*, 2010). These estimates concern foodborne salmonellosis in total, not the proportion due to transmission from animal feed into food-producing animals and subsequently causing infections in humans.

The total burden of foodborne salmonellosis was 111 DALYs (90% CI 68-177). The burden of salmonellosis is mainly due to the primary gastrointestinal disease, although 40% of the total DALYs are due to morbidity associated with sequelae (reactive arthritis, inflammatory bowel disease). Salmonellosis is fourth in the ranking (after foodborne campylobacteriosis norovirus infection and perinatal listeriosis). The proportion of salmonellosis cases considered to be foodborne (on the basis of an expert elicitation) was 61% (minimum 45%, maximum 69%).

The annual cost of foodborne salmonellosis to New Zealand society was estimated to be $2.8 million (95% CI $1.9 – 4.0 million) (Lake *et al.*, 2010).

5.3 Summary of Foodborne Human Health Risk

5.3.1 Risks associated with animal feed

Salmonellosis is a significant disease in New Zealand, with 1,000-2,000 notified cases per annum. It has been estimated that this may equate to approximately 17,000 cases in the community per annum (Cressey and Lake, 2007). The proportion of these cases that are due to contaminated animal feed is unknown.

Increases in human cases of a specific *Salmonella* serotype in New Zealand have been demonstrated subsequent to a known feed contamination incident (Wong, 2003). There is evidence to suggest that *Salmonella* prevalence in food-producing animals in New Zealand is low (NZFSA, 2009; Wong *et al.*, 2007). It has been suggested that in regions with low *Salmonella* prevalence in food-producing animals, *Salmonella* contaminated feed represents a major source for introduction of *Salmonella* into the food production chain (Panel on Biological Hazards, 2008).

A Danish study estimated that approximately 2% of domestically-acquired salmonellosis cases could be due to transmission of *Salmonella* from contaminated imported soybean meal via pigs and cattle. New Zealand also imports significant quantities of soybean meal for use as an animal feed ingredient. Although use patterns for soybean meal are likely to differ between Denmark and New Zealand, it is likely that imported soybean meal used for animal feed will contribute to human salmonellosis in New Zealand. Other feed ingredients (e.g. meat and bone meal) and other transmission routes (e.g. poultry) would potentially also contribute to the salmonellosis burden.

Some recent trends in the animal feed industry in New Zealand have the potential to contribute to an increased risk of transmission of *Salmonella* from feed to food producing animals. These are the increased importation of feed materials that are fed without further heat treatment and the increased feeding of food and industrial by-products, including

material that may have been rejected from the human food chain (Davidson and Pearson, 2009b).

While few data are available (see Appendix 2), it is likely that when *Salmonella* is present in feed ingredients, concentrations will be low (<100 organisms/g). Processing of animal feed into a pelletised form includes a heat-processing step (conditioning) that would be expected to substantially reduce the already low levels of *Salmonella* in feed ingredients. However, these processes will also tend to select for serotypes with good resistance to biotic stresses, which may then become established in the feedmill environment.

The fact that the most common *Salmonella* serotype in finished animal feed in New Zealand (*S.* Tennessee), based on industry data, occurs infrequently amongst human cases argues against animal feed as a significant source of human salmonellosis in New Zealand. However, the available information on the *Salmonella* status of feed and feed ingredients in New Zealand is not sufficiently comprehensive to fully assess animal feed as a source of human salmonellosis cases.

While the potential for introduction of novel *Salmonella* serotypes through imported feed ingredients cannot be discounted, the similarity in serotypes present in feed ingredients in New Zealand and internationally suggests that the risk of this occurring is probably quite low. The serotypes commonly found in feed and feed ingredients are generally of low to moderate severity with respect to hospitalisation and fatality rates of cases (Jones *et al.*, 2008).

5.3.2 Risks associated with other foods and transmission routes

It has been estimated that approximately 60% of salmonellosis cases in New Zealand are due to foodborne transmission (Cressey and Lake, 2005; NZFSA, 2007). Poultry and eggs were estimated to be the main identified food contributors to salmonellosis, but approximately half of the foodborne cases were attributed to 'miscellaneous other' foods (NZFSA, 2007). However, the absence in New Zealand of *S.* Enteritidis types that can penetrate into eggs (PT4 and DT104) means that this food type is likely to be of lower risk here. Overseas travel was estimated to account for 6.5%, direct animal contact a further 10% and person to person contact 5% of salmonellosis cases (NZFSA, 2007).

Analysis of outbreaks in New Zealand most often implicated a food source for the infection, but the wide variety of suspected foods makes attributing risk very difficult (King and Lake, 2007).

5.4 Risk Management Questions

What is the risk of introduction of salmonellae into animals (poultry broilers and layers, red meat) by contamination of feed?

While the ability of animals to become colonised with salmonellae through consumption of contaminated feed has been demonstrated (see Appendix 3 for incident summaries), there is insufficient information on the prevalence and concentration of *Salmonella* contamination of feed in New Zealand and the associated dose-response relationships for various animal species (see Appendix 3) to estimate the risk.

What is the flow-on effect for human exposure?

The potential for human cases of salmonellosis to result from exposure to foods produced from animals colonised with *Salmonella* is well known and the potential for human cases originating from contaminated feed has been demonstrated. Transmission of *Salmonella* from feed to poultry and to humans has been demonstrated in New Zealand (Wong, 2003). However, there is insufficient evidence to determine the proportion of salmonellosis in New Zealand that is ultimately due to contaminated animal feed.

5.5 Risk Assessment Options

It is likely that human salmonellosis due to contaminated animal feed will occur in New Zealand, although there is insufficient information to estimate the proportion of cases due to this transmission route. Quantitative risk assessment offers a means of assessing the public health impact of transmission of *Salmonella* by this route. However, successful completion of a quantitative risk assessment would be critically dependent on the filling of key data gaps.

5.6 Data Gaps

Data gaps identified in this risk profile include:

- Prevalence and concentration of *Salmonella* in feed and feed components in New Zealand;
- Distribution of *Salmonella* serotypes on carcasses and in meat of food producing animals in New Zealand; and
- Dose-response relationships for *Salmonella* colonisation of food-producing animals.

NZFSA have taken initiatives that have the potential to inform the first two of these data gaps. The inclusion of a porcine component in the National Microbiological Database (NMD) programme is beginning to provide improved information on the prevalence of *Salmonella* on pig carcasses, and will allow an estimate of what might be on meat and bones that are raw material for MBM, and provide opportunities to compare serotypes found with those found in animal feed. A recent report completed for NZFSA outlines a strategy for a national *Salmonella* surveillance programme (Lake and Sexton, 2009). Implementation of such a programme would add context to available data, particularly on the specifics of samples submitted for serotyping.

6 AVAILABILITY OF CONTROL MEASURES

Risk management information from countries other than New Zealand is included in Appendix 4.

6.1 Risk Management Strategy

The NZFSA published a *Salmonella* Risk Management Strategy 2009-2012 (NZFSA, 2009). The strategy outlines three strategic goals for *Salmonella* risk management in New Zealand:

- To achieve a 30% reduction in reported annual incidence of foodborne salmonellosis after five years;
- To detect and control exotic genotypes that are known to be more virulent and/or have multiple antibiotic resistance, and that require specific risk management strategies; and
- To support market access.

The strategy also lists five objectives:

- To quantify the proportion of foodborne salmonellosis cases attributable to:
    - Specific foods
    - Animal feeds
    - Domestically produced versus imported foods
    - Multi-resistant and virulent *Salmonella* genotypes associated with foods;
- Identify sources of *Salmonella* contamination of specific foods and animal feeds;
- Determine the relative value of different interventions throughout the food chain in reducing the risk of salmonellosis;
- Make prioritised risk management decisions on appropriate *Salmonella* control measures across the food chain, and according to data availability; and
- Design and implement an effective monitoring and review programme to support strategic goals.

The strategy lists rendered animal products and other animal feed as specific sources of *Salmonella* and identifies initial activities around information gathering and conducting pilot studies and a survey, to confirm the New Zealand situation. Information is also to be gathered from New Zealand renderers, to obtain an understanding of the *Salmonella* prevalence /incidence in rendered products.

6.1.1 Reviews

The NZFSA reviewed the regulation of animal feed, including pet food, for the domestic market (NZFSA, 2006). The review concluded that current frameworks for management of risks are adequate for animal feed containing:

- Only plant material;
- Products from live animals (e.g. dairy products, eggs and honey);
- Primary processed animal material;
- Rendered animal material; and
- Products containing animal material for export.

The review identified significant risks for export trade and domestic animal health from manufacturers involved in secondary processing of animal feed containing animal material

resulting from death of the source animal. The identified risks particularly relate to procurement of source material for secondary processing from unregulated sources.

6.2 Relevant Food Controls

6.2.1 Codes of practice

The New Zealand Feed Manufacturers' Association has a Code of Good Manufacturing Practice[1] and is currently developing a Code of Practice specifically for control of *Salmonella* in animal feed[2].

6.2.2 Legislation

The sourcing, processing and distribution of animal feed in New Zealand is regulated under the Agricultural Compounds and Veterinary Medicines Act 1997 (ACVM Act), the Animal Products Act 1999 (APA) and the Biosecurity Act 1993 (NZFSA, 2006). The ACVM Act and the APA are administered by the New Zealand Food Safety Authority, while the Biosecurity Act is administered by Biosecurity New Zealand.

The ACVM Act regulates importation of all animal feed, including feed of plant origin, feed of animal origin and chemical additives. Regulations covering both imported and domestically produced material include labelling, animal feed being fit for purpose and not resulting in certain effects, and incorporation of therapeutic or pharmacological substance and feed additives in animal feed.

The APA regulates animal feed composed partly or completely of animal material processed in New Zealand. The APA has a major focus on primary processing, but also covers on-farm production, secondary processing and export (NZFSA, 2006).

The Biosecurity Act regulates exclusion, eradication and management of pests and unwanted organisms. Two sets of regulations under this act apply to animal feeding; the Biosecurity (Ruminant Protein) Regulations 1999 and the Biosecurity (Meat and Food Waste for Pigs) Regulations 2005. The former prohibits feeding of ruminant protein to ruminants and is in response to the bovine spongiform encephalopathy (BSE) outbreak in Europe.

6.3 Commentary on Risk Management Options

Control of *Salmonella* contamination in the animal feed industry is complicated by the diversity of products involved, with some receiving heat treatment and some not. Further complication is introduced through the wide diversity of materials that may be used for or in animal feed. Overseas data (Appendix 2) suggests that none of these source materials can be assumed to be free of *Salmonella*.

The New Zealand feed industry has experienced recent trends towards greater use of imported plant protein material (soybean meal, palm kernel meal) and little information is available on *Salmonella* control in the industries from which these products are sourced. Data from the New Zealand feed industry showed most *Salmonella* isolates were from animal

[1] http://www.nzfma.org.nz/Codes/codes_of_practice.php
[2] http://www.nzfma.org.nz/Documents/newsletter_autumn_2009.pdf

protein products (mainly domestically sourced) and plant protein products (mainly imported), while relatively few isolates were from grains, grain by-products or other ingredients. However, the relative sampling rates of the different ingredient categories are unknown. A recent EFSA review of microbial risks in animal feed emphasised that *Salmonella* control in feed needs to include control at the crushing and rendering plants that produce animal and plant protein products respectively (Panel on Biological Hazards, 2008). This was also identified in a New Zealand assessment of *Salmonella* contamination in a feedmill and it was suggested that a price incentive be offered for provision of *Salmonella*-free meat and bone meal for feed production (P.D. Lowry, 1989, MIRINZ Confidential Report).

Application of HACCP principles, including good manufacturing practices and general hygiene procedures are recognised as important measures for *Salmonella* control (Panel on Biological Hazards, 2008). Such measures are either in place or under development in the New Zealand feed industry, although it is uncertain what level of application of these principles is achieved outside the membership of the New Zealand Feed Manufacturers Association. The members of the association are responsible for the production of more than 85% of the animal feed produced in New Zealand. The New Zealand Feed Manufacturers' Association is currently working on a specific Code of Practice for control of *Salmonella*.

The unevenly distributed nature of *Salmonella* contamination of animal feed ingredients means that testing of material is unlikely to be an effective primary control measure. However, application of well-structured testing programmes would provide a measure of the effectiveness of other control measures and allow assessment of any emerging trends. The EFSA review concluded that establishment of microbiological criteria for *Salmonella* in the feed production chain was appropriate, but should be based on one or more hygiene criteria at critical stages of the production chain, rather than be based on end product testing (Panel on Biological Hazards, 2008).

Although suitable application of heat treatment is likely to be the best measure for decontamination of animal feed, the residual protection provided by chemical treatments, such as organic acids and formaldehyde, suggests these products may also contribute to reducing *Salmonella* contamination in feed and reducing *Salmonella* colonisation in food-producing animals. Both heat treatment and chemical additions are used in the compound feed industry in New Zealand, although there is evidence to suggest that neither measure can be considered to be completely effective (delays in application of chemical treatment following positive *Salmonella* detection, continued occasional *Salmonella* positives in finished compound feed).

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APPENDIX 1: HAZARD AND FOOD

The information contained in this Risk Profile is current to the date of publication. Please be aware that new information on the subject may have arisen since the document was finalised.

The following information is taken from a number of different sources but, unless otherwise referenced, is primarily derived from a data sheet prepared by ESR under a contract for the Ministry of Health in 2000-2001. The data sheets are located on the NZFSA website and are intended for use by regional public health units. The datasheets will be updated from time to time, and placed on this website: http://www.nzfsa.govt.nz/science/data-sheets/index.htm

Note that in the following text the term "D" is used. In microbiological terms "D" refers to a 90% (or decimal or 1 log cycle) reduction in the number of organisms.

Salmonella

Growth and survival

The following information in sections 2.1.2, 2.1.3 and 2.1.4 is referenced to ICMSF unless otherwise stated (ICMSF, 1996).

Growth:

Temperature: Minimum 7^{o}C, growth greatly reduced at <15^{o}C. Maximum 49.5^{o}C. Optimum 35-37^{o}C. Some evidence for growth at temperatures <7^{o}C exists, but this is serotype specific, the data are still not universally accepted and doubts surrounding the experimentation have been noted.

pH: Minimum 3.8, optimum, 7.0-7.5, maximum 9.5. The minimum pH is influenced by other factors such as temperature, the acid present and the presence of nitrite, etc.

Atmosphere: Can grow in the presence or absence of air. The growth rate on beef muscle stored at 20^{o}C under nitrogen is only slightly less than that obtained when stored under air. At high concentrations of CO_2 (50-60%), growth is strongly inhibited on crab meat, beef steak and ground beef at a temperature of 10-11^{o}C, but at 20^{o}C there is little inhibition (Jay *et al.*, 2003).

Water activity: Minimum 0.94, optimum 0.99, maximum >0.99.

Survival:

Salmonella is known to survive well in foods and on surfaces.

Temperature: *Salmonella* can survive for long periods under refrigeration. Survival for >10 weeks in butter stored at –23 and +25^{o}C has been noted. Salmonellae can survive for 28 days on the surfaces of vegetables under refrigeration. Some foods, including meat, appear to be protective of *Salmonella* during freezing and frozen storage. Jay *et al*. (2003) report that rapid freezing promotes survival and that lower storage temperatures and less fluctuations in temperature give greater survival. Storage temperatures near the freezing point result in most death or injury. In minced chicken breast (pH 5.8), 60-83% of *Salmonella* cells survived

storage at -20°C for 126 days, whereas at -2°C and -5°C only 1.3% to 5.8% were still viable after 5 days.

pH: *Salmonella* appear to be significantly less tolerant of low pH (pH 2.5; hydrochloric acid) than *Shigella* spp. or *Escherichia coli*. These last two organisms possess additional acid survival systems that are not present in salmonellae (Jay *et al.*, 2003).

Water Activity: Survival in environments with low water activity (a_w) is a characteristic of these organisms. For example, they can survive in bitter chocolate (0.3-0.5) for months. Exposure to low a_w environments can greatly increase the subsequent heat resistance of these organisms.

Inactivation (CCPs and Hurdles)

Temperature: Death is greater during the freezing process than subsequent frozen storage, but those that survive remain viable during frozen storage. Freezing does not ensure the inactivation of salmonellae in foods.

D times: 60°C usually 2-6 min; 70°C usually 1 min or less. Some rare serotypes (e.g. *S.* Senftenberg) are significantly more heat resistant than the others, but this organism is not considered to be important as a food pathogen (Doyle and Mazzotta, 2000).

D times for *Salmonella* can depend on the type of food involved. Long D times have been reported for experiments with *Salmonella* Typhimurium in milk chocolate. Values reported were up to 1050 min at 70°C, 222 min at 80°C and 78 min at 90°C.

pH: Jay *et al*. (2003) report that at low pH values, the nature of the acidulant determines the rate of death. Temperature is also a factor, for example, inactivation of *Salmonella* Enteritidis PT4 is more rapid in commercial mayonnaise of appropriate acetic acid content at 20°C than at 4°C.

Water activity: At a_w levels below those allowing growth, salmonellae die slowly. The rate of death decreases as the a_w is lowered and also decreases as the temperature is reduced (Jay *et al.*, 2003).

Radiation: Lamuka *et al*. reviewed the effect on survival of salmonellae when irradiated (Lamuka *et al.*, 1992). Gamma irradiation of chicken at 2.0 kGy eliminated 99% of the microbial load (Katta *et al.*, 1991) and a dose of irradiation between 2 to 3 kGy effectively destroyed all salmonellae on chicken (Dickerson *et al.*, 1991).

Salmonella **Serotypes in New Zealand**

The non-typhoidal *Salmonella* are divided into approximately 2000 serotypes. Most of these are capable of causing disease in humans, although a few have restricted host ranges for example, *S.* Pullorum and *S.* Gallinarum are highly host-adapted to poultry. Neither of these poultry serotypes have been detected in New Zealand since 1985.

The ESR Enteric Reference Laboratory at the Kenepuru Science Centre provides *Salmonella* typing services for New Zealand. In addition to isolates from human cases sent by clinical laboratories, the laboratory also provides typing for isolates from animals and foods

submitted by various sources. Isolates derived from poultry originate from Animal Health Laboratories (sent to ESR via the Ministry of Agriculture and Forestry (MAF)) as well as directly from poultry producers. Summaries of the isolates submitted by Animal Health Laboratories are published in the MAF Biosecurity Authority journal Surveillance. All isolates are reported by the Enteric Reference Laboratory as annual and quarterly tables on the ESR website (www.esr.cri.nz) under publications.

Serotypes of major concern overseas

Two serotypes of particular human health relevance overseas are *S.* Enteritidis phage types capable of transovarian transmission into eggs (especially phage type 4 (PT4)) and the antibiotic resistant *S.* Typhimurium Definitive phage type 104 (DT104).

S. Enteritidis PT4 became the most prevalent *Salmonella* causing human infection in the United Kingdom during the 1980s and 1990s. This was, in part, due to the fact that chicken eggs can be contaminated with *S.* Enteritidis PT4 internally or externally by the time they are laid, or can subsequently become contaminated after lay (Advisory Committee on the Microbiological Safety of Food, 1993). Similar problems occurred in the USA, but involved a wider range of phage types.

The number of reported cases of salmonellosis in the United Kingdom during the 1990s was relatively constant at around 30,000 cases per year (Institute of Food Science and Technology, 1997). The most common *Salmonella* involved was *S.* Enteritidis, followed by *S.* Typhimurium. The antibiotic resistant DT104 made up an increasing proportion of the *S.* Typhimurium isolates from 1991 to 1996, although its prevalence has been declining since 1998/99. The resistant nature of DT104 has presented difficulties in treatment, and a relatively high mortality rate (3%) occurred amongst cases. Isolates showing a multi-drug resistance remain a concern. The most common food sources were comminuted meats, especially sausages and burgers.

Test Methods for *Salmonella* in Animal Feed

EFSA (Panel on Biological Hazards, 2008) comment that standard cultural methods have not been validated for testing individual feed components, but a more recent paper (Koyuncu and Haggblom, 2009) has looked at this. They used three standard cultural methods and determined that that there was considerable variability in the concentration that needed to be present in the different feeds for reliable detection to occur. For example, all three methods detected *Salmonella* inoculated at 1-10 CFU/25 g in rape seed meal, wheat grain and feed mill scrapings, but 10^2-10^3 CFU/25 g was required when the organism was inoculated into palm kernel meal. Cultural methods can be slow, with several days being needed for a result to be obtained. PCR and immunological systems are faster to perform than culture, but since they both still need the enrichment step, claims by manufacturers with respect to rapidity need to be considered in this context.

Cultural and immunological methods have been reviewed (Maciorowski *et al.*, 2006). Both the FDA and USDA still recommend culture-based methods. In summary, recommended media include pre-enrichment in lactose broth, followed by selective enrichment in Rappaport-Vassiliadis (RV) and tetrathionate broths. Subsequent plating is to bismuth sulphite, xylose lysine desoxycholate and Hektoen enteric agars for isolation. Many other media are available which could be used for the same purpose. There are numerous

commercially-available products for the detection of *Salmonella* that use immunology, specifically antigen-antibody interactions. Accredited rapid tests are listed by the AOAC (http://www.aoac.org/testkits/testedmethods.html#Microbiological). A comparison of three standard cultural methods (Nordic Committee on Food Analysis NMKL71, Modified semisolid RV method, and EN ISO 6579:2002) found them to be equally effective at detecting *Salmonella* in feed components (Koyuncu and Haggblom, 2009).

It must be remembered that a rapid kit relying on an immunological reaction will only indicate the presence of the pathogen. If there is a need to isolate the pathogen for serotyping, for example, there is a need to continue with a cultural approach from the enrichment broth. Enumeration of *Salmonella* may be achieved using most probable number methods which could involve the use of immunological methods for the detection of positive tubes.

The use of the polymerase chain reaction (PCR) for the detection of *Salmonella* has been reviewed (Maciorowski *et al.*, 2005). The review lists several commercial PCR kits for *Salmonella* detection, but none of them appears, from the information presented, to have been tested on animal feeds (an exception is reported below). PCR methods are undoubtedly faster that cultural methods for the purposes of detection and can be more sensitive. However, problems lie in the carry-over of PCR inhibitory compounds from the matrix being tested, and the possibility that cells testing positive by PCR may not be viable.

In a specific study, the probability of detecting feed contaminated at 1 CFU per 25g was 0.81 using enrichment and PCR. The method was also shown to be more sensitive than conventional culture (Löfström *et al.*, 2004). A more recent comparison of the NMKL71 cultural method and a PCR method using 250 samples showed them to be broadly comparable (Löfström *et al.*, 2008). The cultural method showed some inadequacies when testing acidified feed samples and the PCR method performed less well with rape seed samples. The advantage of PCR over culture was the speed of analysis (24 h vs 72 h). Investigations of a commercial PCR system (BAX™, Qualicon, Wilmington, DE, USA) showed some problems and required modifications of the enrichment procedure to improve results (Maciorowski *et al.*, 2000). BAX™ and conventional methods for testing feeds were not in good agreement although presumptive *Salmonella* colonies were not confirmed.

Real-time PCR offers the *potential* to quantify *Salmonella* in feeds (Malorny *et al.*, 2008). Because the quantification of cells is the aim, the sample cannot be enriched as this would lead to an increase in their number. Since the method detects DNA, the DNA would need to be isolated directly from the sample, or the cells extracted followed by DNA isolation. The detection of dead cells then becomes a problem if they still contain amplifiable DNA. Current direct isolation methods also suffer from the inability to detect low concentrations of cells; a problem tackled through the use of short enrichments to give semi-quantitative data. The review concludes that enrichment is necessary for low concentrations of cells and so the advantages of real-time PCR are that it is faster, cheaper and amenable to automation. Because no gel needs to be run to visualise PCR products it is faster than conventional PCR.

APPENDIX 2: EXPOSURE ASSESSMENT

Overseas Data on Prevalence/Concentration of Hazard in Food

Tables 11 and 12 summarise studies on the prevalence of *Salmonella* contamination of finished animal feed and animal feed ingredients. The information in these tables is mainly restricted to more recent studies. An extensive review of studies into the *Salmonella* prevalence in poultry feed prior to 1980 has been published by Williams, but these data have not been included because of questions over the relevance of data of this age (Williams, 1981).

Table 11: Reported Prevalence of *Salmonella* in overseas animal feed

Country	Feed type	Samples tested	Positive for *Salmonella* (%)	Year	Reference
Belgium	Pig feed	332	10.2	1999-2001	(Korsak *et al.*, 2003)
EU	Cattle feed	2763 2919 1630 4158 4141	1.3 0.8 0.4 0.7 0.7	2002 2003 2004 2005 2006	(EFSA, 2007)
EU	Pig feed	3972 4449 6076 6115 6234	0.7 0.5 0.7 0.7 0.6	2002 2003 2004 2005 2006	(EFSA, 2007)
EU	Poultry feed	2186 3309 7070 13655 13819	0.3 0.7 2.0 0.9 0.8	2002 2003 2004 2005 2006	(EFSA, 2007)
Ireland	Poultry – finished feed	5	0.0	??	(Whyte *et al.*, 2003)
Japan	Layer	783 1232 1771 2060 2106 2466	0.0 0.6 1.2 0.2 0.1 0.7	1993 1994 1995 1996 1997 1998	(Shirota *et al.*, 2001)
Netherlands	Broiler, start Broiler, growth Broiler, finish Layer and breeder Turkey Mash Pellet	39 80 43 156 42 145 215	0.0 1.3 0.0 20.5 2.3 21.4 1.4	1990-1991	(Veldman *et al.*, 1995)
Netherlands	Cattle feed	2467 2438	0.5 0.3	2005 2006	(Product Board Animal Feed, 2007)
Netherlands	Pig feed	3301 2917	0.4 0.3	2005 2006	(Product Board Animal Feed, 2007)
Netherlands	Poultry feed, total	9768 8581	0.4 0.3	2005 2006	(Product Board Animal Feed, 2007)
Netherlands	Poultry feed, breeders	693 486	0.1 0.0	2005 2006	(Product Board Animal Feed, 2007)
Netherlands	Poultry feed,	2939	0.2	2005	(Product Board Animal

Country	Feed type	Samples tested	Positive for *Salmonella* (%)	Year	Reference
	broilers	2158	0.1	2006	Feed, 2007)
Netherlands	Poultry feed, layers	3357 3001	0.8 0.7	2005 2006	(Product Board Animal Feed, 2007)
Saudi Arabia	Animal feed	346	12.1	1977-1980	(Nabbut *et al.*, 1982)
UK	Pig and poultry meals	3114 2644	0.6 0.7	2006 2007	(Veterinary Laboratories Agency, 2008)
UK	Pig extrusions	1258 1219	0.2 0.1	2006 2007	(Veterinary Laboratories Agency, 2008)
UK	Poultry extrusions	3640 3411	0.4 0.1	2006 2007	(Veterinary Laboratories Agency, 2008)
USA	Cattle feed	295	9.8	1995	(Krytenburg *et al.*, 1998)
USA	Pig feed -Ground meal -Pelleted -Liquid	 765 334 4	 1.8 5.4 0.0	NS	(Harris *et al.*, 1997)

Table 12: Reported Prevalence of *Salmonella* in overseas animal feed ingredients

Country	Feed ingredient	Samples tested	Positive for *Salmonella* (%)	Year	Reference
Animal Protein					
EU	Fish meal	1824 1249 5280 1362 2414	2.1 1.6 1.1 0.4 1.9	2002 2003 2004 2005 2006	(EFSA, 2007)
	Meat and bone meal	2033 8064 13113 10633 12350	2.9 0.5 1.7 1.3 2.3	2002 2003 2004 2005 2006	
Netherlands	Fish meal Meat and bone meal	130 83	31 4	1990-1991	(Veldman *et al.*, 1995)
Netherland	Blood products Animal meal products Egg products Fish meal Whey products Dairy products	7 166 10 903 1681 158	0.0 12.0 0.0 0.8 0.1 0.0	2005-2006	(Product Board Animal Feed, 2007)
Sweden (imported)	Meat and bone meal Meat meal Feather meal Graves meal Fish meal Bloodmeal Bone meal	6733 6414 3256 960 419 270 242	0.9 2.9 2.5 1.1 0.2 5.2 3.3	1988-1992	(Malmqvist *et al.*, 1995)
UK	Processed animal protein for feedingstuff use	576 1302	2.1 1.5	2006 2007	(Veterinary Laboratories Agency, 2008)
USA	Protein byproducts Beef/bone meal Blood	4 1 6	75 100 50	NS	(McChesney *et al.*, 1995)

Country	Feed ingredient	Samples tested	Positive for *Salmonella* (%)	Year	Reference
	Bone meal Dried plasma Feather meal Feather/blood Fish Meat/bone meal Meat meal Meat/bone/poultry Pork blood Poultry byproduct Poultry	1 1 14 1 4 42 3 1 2 4 17	100 0 36 0 75 64 67 100 0 50 53		
USA	Milk/whey Other protein products (e.g. fishmeal)	14 23	0.0 8.7	NS	(Harris *et al.*, 1997)
USA	Fish meal Meat and bone meal Whey	1 1 1	100 0.0 0.0	NS	(Jones and Richardson, 2004)
Plant protein					
EU	Oilseeds and products	13764 14381 20326 20849 18449	5.3 4.8 5.7 4.3 2.5	2002 2003 2004 2005 2006	(EFSA, 2007)
Netherlands	Tapioca Maize grits	58 15	2 27	1990 – 1991	(Veldman *et al.*, 1995)
Netherlands	Maize gluten feed Rapeseed meal/flakes Soya byproducts Soya beans Soya flakes Soya meal Sunflower seed/flakes Sunflower seed meal	203 8715 1111 4321 60 8166 18 2584	1.5 5.1 1.8 3.4 5.0 2.4 11.1 2.6	2005-2006	(Product Board Animal Feed, 2007)
Switzerland	Soya meal	52	0.0	2002-2003	(Sauli *et al.*, 2005)
UK	Oilseed meals and products for feedingstuffs use Non-oilseed meal vegetable products	9393 8331 7506 7106	1.7 1.0 0.3 0.4	2006 2007 2006 2007	(Veterinary Laboratories Agency, 2008)
USA	Vegetable protein	50	36.0	NS	(McChesney *et al.*, 1995)
USA	Soybean meal	22	0.0	NS	(Harris *et al.*, 1997)
USA	Plant protein	158	0.6	2002	(Myint *et al.*, 2007)
USA	Cottonseed meal Soybean meal	2 10	100 10		(Jones and Richardson, 2004)
Grains					
EU	Cereals	4538 3928 5382 4735 5331	1.2 1.0 0.7 0.5 0.3	2002 2003 2004 2005 2006	(EFSA, 2007)
Netherlands	Barley Oats	384 44	0.8 0.0	2005-2006	(Product Board Animal Feed, 2007)

Country	Feed ingredient	Samples tested	Positive for *Salmonella* (%)	Year	Reference
	Maize Rice and byproducts Rapeseed Rye and byproducts Sorghum and byproducts Wheat Triticale	889 151 43 79 3 1623 176	0.1 0.0 4.7 0.0 0.0 0.1 0.6		
Switzerland	Cereals	80	0.0	2002-2003	(Sauli *et al.*, 2005)
USA	Grain	41	2.4	NS	(Harris *et al.*, 1997)
USA (Colorado)	Dry corn High moisture corn	175 180	4.0 0.6	2001-2002	(Dargatz *et al.*, 2005)
USA	Corn Wheat	19 1	5 0	NS	(Jones and Richardson, 2004)
Grain by-products					
USA	Brewers grains Soybean hulls Wheat middlings	3 5 24	0.0 0.0 4	NS	(Jones and Richardson, 2004)
Other					
USA	Fats/oils	16	0.0	NS	(Harris *et al.*, 1997)
USA (Colorado)	Hay Silage	360 1180	1.7 0.6	2001-2002	(Dargatz *et al.*, 2005)

Serotypes in Feed and Feed Components

Types of all *Salmonella* isolates from animal feed raw materials, dust and scrapings from feedmills and compound feeds in Sweden over two five year periods (1988-1992, 1993-1997) have been reported (Boqvist *et al.*, 2003; Malmqvist *et al.*, 1995). Table 13 summarises the most commonly detected serotypes from each source. It should be noted that far more isolates were typed from raw materials of animal origin (436) and feedmill dust and scrapings (282), than raw materials of vegetable origin (47) or compound feed (15).

Table 13: *Salmonella* serotypes from animal feed raw materials, feedmill dust and scrapings and compound feed in Sweden (1988-1992)

Source	Most common serotypes (in descending order)		Most common sources
	1988-1992	1993-1997	
Raw materials of vegetable origin	*S.* Rissen, *S.* Havana, *S.* Cubana, *S.* Mbandaka, *S.* Senftenberg	*S.* Senftenberg, *S.* Mbandaka, *S.* Agona, *S.* Anatum, *S.* Cubana	Soybean meal, maize meal, rapeseed products, coconut
Raw materials of animal origin	*S.* Montevideo, *S.* Senftenberg, *S.* Anatum, *S.* Lexington, *S.* Tillburg	*S.* Senftenberg, *S.* Montevideo, *S.* Livingstone, *S.* Liverpool	Meat meal, meat and bone meal, feather meal, fish meal, greaves meal, blood meal
Feedmill dust and	*S.* Cubana, *S.* Ohio,	*S.* Livingstone,	

Source	Most common serotypes (in descending order)		Most common sources
	1988-1992	1993-1997	
scrapings	*S.* Livingstone, *S.* Anatum, *S.* Senftenberg	*S.* Senftenberg, *S.* Mbandaka, *S.* Subspecies I, *S.* Cubana	
Compound feed	*S.* Livingstone, *S.* Newport, *S.* Rissen, *S.* Agona, *S.* Anatum, *S.* Cubana	*S.* Infantis, *S.* Agona, *S.* Tennessee, *S.* Subspecies	

While the evidence from this study is largely circumstantial, there are indications that contamination of compound feed may originate from raw ingredients of animal or plant origin or from persistent contamination of the feedmill environment.

A study of *Salmonella* types in Australian animal feed identified similar dominant types, including *S.* Orion (17.7%), *S.* Senftenberg (6.4%), *S.* Havana (5.6%), *S.* Ohio (5.3%), *S.* Singapore (5.0%), *S.* Cerro (4.7%), *S.* Tennessee (4.5%), *S.* Livingstone (4.0%) and *S.* Johannesburg (3.8%) (Murray, 1994). No further analysis of the specificity of types to particular feed components was carried out.

Serotypes of *Salmonella* detected in nine British animal feed mills have been reported (Davies and Wray, 1997). While the publication results do not indicate which types were detected most frequently, types detected in more than one mill included *S.* Tennessee, *S.* Mbandaka, *S.* Indiana, *S,* Schwarzengrund, *S.* Oranienberg, *S.* 4,12:d:-, *S.* Cubana, *S.* Montevideo, *S.* Ohio, *S.* Senftenberg, *S.* Kedougou and *S.* Agona.

In an investigation of *Salmonella* in pig feed and pig feed components in the USA the most commonly detected serotype was *S.* Worthington, followed by *S.* Agona, with single detections of *S.* Anatum, *S.* Derby, *S.* Montevideo, *S.* Senftenberg, *S.* Arkansas, *S.* Infantis, *S.* Orion, *S.* Mbandaka, *S.* Heidelberg, *S.* Kentucky and *S.* Oranienberg (Harris *et al.*, 1997).

In industry monitoring of animal feed (poultry, cattle, pig) in the Netherlands during 2005 and 2006 the most commonly detected serotypes were *S.* Senftenberg, *S.* Lexington, *S.* Mbandaka, *S.* Havana, *S.* Livingstone and *S.* Anatum (Product Board Animal Feed, 2007). The most commonly detected types in selected feed components (soy meal, fishmeal, rape seed meal, sunflower meal) were *S.* Lexington, *S.* Senftenberg, *S.* Rissen, *S.* Agona, *S.* Infantis and *S.* Tennessee.

In contrast, an older study from a quite different geographical region (Saudi Arabia) showed a quite different pattern of dominant serotypes in animal feed, with *S.* Lille, *S.* New-haw, *S.* Livingstone, *S.* Kentucky and *S.* Meleagridis being the serotypes most commonly detected in animal feed (Nabbut *et al.*, 1982).

Concentrations of *Salmonella* in Feed Components and Finished Feed

Salmonella concentrations were determined in poultry feed by the Most Probable Number method (Maciorowski *et al.*, 2000). Concentrations ranged from 5 MPN/10 g to 794 MPN/10 g (0.5-79.4 MPN/g).

Salmonella concentrations in meat and bone meal, a common component of animal feeds, has been determined (Franco, 2005). Over a 12 month period the mean concentration of *Salmonella* in *Salmonella*-positive samples ranged from 0.2 MPN/g to 78.0 MPN/g. The average *Salmonella* concentration over the entire study period (197 samples) was 16.3 MPN/g. The most commonly detected serotypes were *S.* Senftenberg, *S.* Livingstone and *S.* Mbandaka.

APPENDIX 3: EVALUATION OF ADVERSE HEALTH EFFECTS

Salmonella possess systems that enable them to adhere to small intestinal epithelial cells, provided they survive the low pH of the stomach, and other defence mechanisms (Jay *et al.*, 1997). After entering the cell as part of a vesicle (endosome), non-typhoidal salmonellae multiply and release endotoxin. The invading bacterial cells often cause only a limited, localised intestinal event with no systemic involvement, resulting in damage to the mucous membrane of the small intestine and colon. Both invasion and enterotoxin production are required to cause diarrhoea. A small proportion of cases may experience septicaemia or longer-term illness, such as reactive arthritis.

In contrast, *Salmonella* Typhi enter the gastrointestinal tract, invade the local lymphatic tissue and pass via the blood stream to various organs (Jay *et al.*, 1997). The discussion below pertains only to non-typhoidal *Salmonella* infections.

Dose-response

The dose required to cause disease varies with many factors. Low attack rates have been observed in one outbreak where 4-45 cells were consumed, and another where the dose was 6 cells/65g (Anonymous, 1996). Different serotypes may have different dose responses, and doses generally recognised to cause disease at high attack rates are in the range of 10^5 to 10^7 cells. However, these observations simplify a situation whereby there is no threshold dose for infection.

The leading dose-response model has been produced by the joint risk assessments of *Salmonella* in eggs and broiler chickens by FAO/WHO (FAO/WHO, 2002). The model used variously sourced outbreak reports for a dose-response calculation; these reports were screened and a final 20 outbreaks were used in the database; 11 in Japan, 9 in the USA. Several serotypes were associated with the outbreaks and several vehicles of transmission implicated. A beta-Poisson (BP) model was used for the mathematical relationship and a maximum likelihood technique used to generate the curve best fitting the data. While the nature of this distribution and the parameters are not given, the graph shows at the median of the dose response curve that an ingestion of 10^{10} cells results in a probability of around 0.9 of illness, while the ingestion of 10^1 cells results in a probability of around 0.02. This kind of curve explains the low levels causing disease that have been observed, as they represent outbreaks where the food has been widely consumed but only a small proportion of consumers have become ill.

An attempt was made to discern separate dose-response curves for different subpopulations, defined by age and 'susceptibility'. However, comparing attack rates for children less than 5 years old revealed no increased risk, therefore the database may lack the power to reveal true differences. Nonetheless, this new dose-response model derived from outbreak data is considered the best available estimate for the probability of illness upon ingestion of a dose of *Salmonella.*

Dose response models have been developed for individual *Salmonella enterica* serotypes. For example, Holcomb *et al.* have compared models with data for *S.* Typhosa (Holcomb *et al.*, 1999). Teunis *et al.* (1999) have produced a dose-response model for *S.* Meleagridis. The former study indicated that at ingestion of 10^2 or less the probability of disease is very low, a dose of 10^8 gives a probability of around 0.8, and exposures above 10^{11} are needed to obtain

probabilities approaching 1. The Teunis *et al.* (1999) curve is much steeper, with probabilities of disease approaching 0 at doses < 10^4, and approaching 1 at doses exceeding 10^9. These dose response curves are therefore somewhat shifted to a higher dose being required to cause the same probability of disease when compared to the FAO/WHO model based on outbreak data.

A weighted composite dose-response model for human salmonellosis has been reported by Latimer *et al.* (2001). Data from previous human feeding studies were categorised into low/ moderately virulent/pathogenic and highly virulent/pathogenic *Salmonella* strains, with *Shigella dysenteriae* used as a proxy for highly virulent strains. Three single hit dose-response models were applied and based on the goodness-of-fit test, the exponential (E-1pop) and BP were best-fit models for low and moderately virulent strains while the two-subpopulation exponential (E-2pop) and BP models were better for highly virulent strains.

It has been repeatedly reported that the infectious dose is lower when the implicated food has a high fat or protein content. For example, chocolate or peanut butter may protect cells from gastric juices so permitting a lower dose than usual to cause infection. Experimentation has shown this to be the case for high fat foods (minced beef) and high protein foods (egg white). It was concluded that the pH of the microenvironment of the organism is crucial in determining its resistance to stomach acids (Waterman and Small, 1998).

Overseas Data on Prevalence of Adverse Health Effects/outbreaks

Animal colonisation due to contaminated animal feed

While evidence is often circumstantial, investigations have been carried out that link *Salmonella* contamination of animal feed to subsequent colonisations in a number of animal species.

Cattle

The ability of *Salmonella* contaminated feed to cause colonisation in cattle was demonstrated with two dairy cows fed meat and bone meal artificially contaminated with *Salmonella* spp. (Montevideo, Anatum, Cerro, Meunster and Agona) at approximately 1000 organisms/g of feed (Bender *et al.*, 1997). All serovars were intermittently detected in rumen, faecal or necropsy samples from one or both animals, but not from milk samples. No clinical illness was observed in either animal.

A case-control study of an outbreak of *S.* Menhaden in eight dairy herds in California was carried out (Anderson *et al.*, 1997). Use of one particular feed mill and the feeding of animal fat were identified as significant risk factors. *S.* Menhaden causes clinical disease in cattle.

S. Mbandaka was isolated from rectal swabs of cattle from three English dairy farms receiving compound feed from a single feed mill (Jones *et al.*, 1982). *S.* Mbandaka was also isolated from milk filters from two farms, but not from workers in contact with the cattle or wildlife from the surrounding area. Analysis of feed components found *S.* Mbandaka in unopened bags of vegetable fat supplement (palm oil, with palm kernel and ground straw as a carrier base). Levels of *S.* Mbandaka in two bags of vegetable fat were 240 MPN/100 g. *S.* Mbandaka appeared to be non-pathogenic in cattle and colonisation was only detected in a relatively small number of animals and did not persist beyond one month.

Cattle feed contaminated with *S.* Infantis was distributed to Finnish cattle farms during May 1995 (Lindqvist *et al.*, 1999). Analysis of feed and cattle samples before and after this incident, using pulsed-field gel electrophoresis (PFGE) following restriction enzyme digest, allowed discrimination of the feed-associated *S.* Infantis from strains endemic in Finnish cattle. Of 800 farms that purchased feed from the affected mill, 57 farms were culture positive for *S.* Infantis, with the feed-related strain detected in 50 of these farms. Most farms were cleared of the colonisation within 4-6 months. The contamination was not traced to any specific feed component.

Comparison of *Salmonella* from feed components and from cattle faecal isolates from the same farm demonstrated identical PFGE types (Davis *et al.*, 2003). Serotypes recovered from feed included *S.* Braenderup, *S.* Cerro, *S.* Mbandaka, *S.* Meleagridis and *S.* Typhimurium. However, in some cases the serotype was found on the specific farm before detection in feed, while in other cases *Salmonella* was detected in feed, but was not detected in cattle at any time.

Pigs

Logistic regression analysis was used to estimate an odds ratio of 1.6 for the risk of colonisation of pigs with *Salmonella* due to consumption of contaminated or recontaminated feed (Berends *et al.*, 1996). It was further estimated that 15-30% of *Salmonella* colonisations in the finishing period may be attributed to feed.

Bacteriological and serological information was used to examine risk factors for *Salmonella* occurrence in Danish sow herds and related weaners and finishing herds (Kranker *et al.*, 2001). Ready-mixed pelleted feed was a risk factor for *Salmonella* colonisation in sows (OR = 2.44) and finishers (OR = 2.86) compared to home-mixed meal.

A closed pig production system in Belgium was monitored for *Salmonella* over the course of two years (Korsak *et al.*, 2003). While some types detected in feed were also detected in breeding and fattening pigs and in abattoir carcasses, in general the types most commonly seen in pigs were uncommon (*S.* Typhimurium, *S.* Derby, *S.* Anatum) or not seen (*S.* Brandenburg, *S.* Infantis, *S.* Goldcoast) in feed, while a number of types detected in feed were not subsequently detected in pigs (*S.* Bochum, *S.* Hithergreen, *S.* Lexington, *S.* Mbandaka, *S.* Moers, *S.* Odozi, *S.* Plymouth, *S.* Rubislaw, *S.* Schwarzengrund, *S.* Solt, *S.* Utah and *S.* Wien). The authors concluded that the prevalence of *Salmonella* colonisation of pigs from feedstuffs was very low.

In 2003 a detection of *S.* Cubana in Swedish pigs was traced back to contamination of the swine feed production line of a Swedish feed mill (Osterberg *et al.*, 2006). *S.* Cubana was detected on 49 of 77 farms that received potentially contaminated feed. No clinical symptoms due to colonisation were observed.

Poultry

The ability of poultry feed artificially contaminated with *Salmonella* to cause colonisation in chicks has been demonstrated (Gordon and Tucker, 1965; Hinton, 1988; Schleifer *et al.*, 1984). Concentrations of *Salmonella* of less than one organism per gram of feed were sufficient to establish *Salmonella* colonisation in 1-7 day old chicks (Hinton, 1988; Schleifer *et al.*, 1984), while contamination levels of 100-300 organisms per gram were sufficient to result in colonisation of nearly all birds (Hinton, 1988).

A retrospective longitudinal study on *Salmonella* colonisation in the Danish broiler flock was carried out, using multivariable logistic regression to examine the impact of 14 variables (Angen *et al.*, 1996). The largest feedmill, supplying feed to 19.2% of flocks, had the highest odds ratio for *Salmonella* colonisation (OR = 2.3). A similar study carried out in France identified the feeding of meal from day one as a risk factor for *Salmonella* colonisation (OR = 12.2) compared to feeding of small pellets (OR = 1.0) (Rose *et al.*, 1999). Pelleted feed is subjected to heat treatment.

An Australian study observed a significant correlation between *Salmonella* types detected in raw feed components and types detected in finished broiler carcasses (MacKenzie and Bains, 1976). Some serotypes previously not seen in the organisation under study were observed in feed and were subsequently detected in live birds and finished carcasses. The most commonly contaminated feed components were feathermeal (78%) and meat and bone meal (72%). Serotypes commonly observed in both feed components and finished carcasses were *S.* Singapore, *S.* Anatum, *S.* Havana, *S.* Agona, *S.* Derby and *S.* Newington. Several types were common in feed components, but not in broilers (*S.* Cerro, *S.* Eimsbuettel, *S.* Lille, *S.* Senftenberg, *S.* Tennessee).

A Canadian study looking at risk factors for *Salmonella* colonisation of broiler flocks found that *Salmonella* types isolated from broiler feed (both pelletised and mash) were often subsequently found in the used litter from the same raising unit (Hacking *et al.*, 1978). Comparison of the frequency of isolation from feed trucks and the feed system suggested that these observations were not due to contamination of feed from the poultry shed environment. The most commonly isolated serovars were *S.* Montevideo, *S.* Cambridge, *S.* Bareilly and *S.* Cubana.

A study looking at vertical transmission of *Salmonella* from breeder to broiler flocks observed that *Salmonella* types isolated from breeder feed and broiler feed components were also isolated from the caeca of broilers at slaughter (Humphrey and Lanning, 1988). Imported fishmeal and broiler meal were the feed components most often contaminated with *Salmonella*.

A Japanese study isolated identical *Salmonella* serovars from layer feed and eggs produced from the same farm at the same time (Shirota *et al.*, 2001). PFGE typing confirmed that types from feed and eggs were genetically related. The types most commonly isolated from feed and eggs were *S.* Enteritidis, *S.* Infantis, *S.* Bareilly, *S.* Orion and *S.* Derby.

A British study determined *Salmonella* types in feedmills, hatcheries, farms and abattoirs for two different companies (Corry *et al.*, 2002). Persistent feedmills serotypes were the most common *Salmonella* types detected on farms and in abattoirs. Types found in feed, on farms and in abattoirs included *S.* 4,12:d:-, *S.* Enteritidis PT4, *S.* Kedougou, *S.* Montevideo, *S.* Ohio,

S. Binza and *S.* Typhimurium DT104. *S.* Agona, *S.* Mbandaka, *S.* Agama and *S.* Senftenberg were detected in feed and at abattoirs, but not on associated farms, while *S.* Havana, *S.* Indiana, *S.* Kottbus, *S.* Newport, *S.* Derby, *S.* Stourbridge, *S.* Braenderup, *S.* Hadar and *S.* Ajiobo were detected in feed, but were not subsequently detected on broiler farms or associated abattoirs.

Concentrations of *Salmonella* in feed causing colonisation in food-producing animals

Little information was found on concentrations of *Salmonella* in feed that were able to establish colonisation in food-producing animals.

Poultry

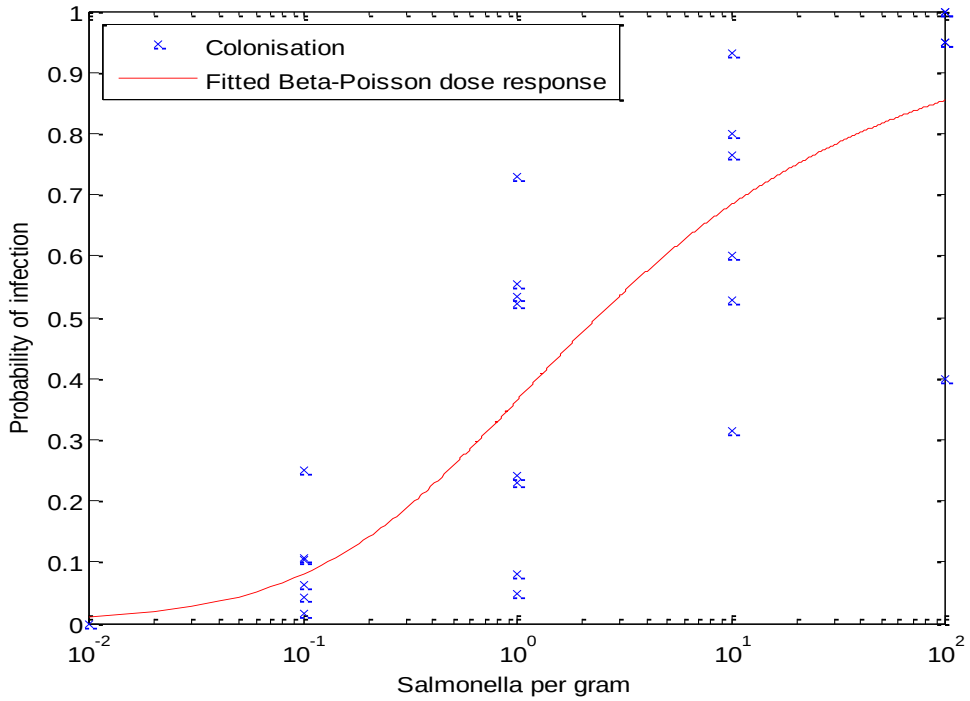
Hinton (1988) fed artificially contaminated feed (concentrations 0.01 – 100 CFU/g) to groups of chicks for 2-3 weeks. No colonisation was found in birds receiving the lowest concentration of *Salmonella*. Colonisation at the highest concentration appeared to be serotype dependent, with *S.* Kedougou resulting in greater than 90% of birds being colonised, while *S.* Livingstone resulted in 35-45% of birds being colonised.

Schleifer *et al*. (1984) demonstrated colonisation of chicks consuming feed artificially contaminated with *S.* Montevideo at feed *Salmonella* concentrations as low as 0.04 CFU/g, although results were variable amongst trials and in another trial no colonisation was found at *Salmonella* concentrations below 5 CFU/g.

Gordon and Tucker (1965) reported 1.9% carriage in chicks consuming feed contaminated with 0.5 CFU/g of *S.* Menston, increasing to approximately 30% at a concentration of 233 CFU/g.

Lake *et al*. (2006) combined the data of Hinton (1988) and Gordon and Tucker (1965) and fitted a Beta-Poisson dose-response curve to the resultant data set. The curve is shown in Figure 5.

Figure 5: Fitted Beta-Poisson dose response to the data in Gordon and Tucker, 1965 and Hinton 1988



Cattle

Two dairy cows (Jersey) consumed feed artificially contaminated with a mixture of *Salmonella* serotypes (Montevideo, Anatum, Cerro, Muenster, Agona) for 55-57 days (Bender *et al.*, 1997). The salmonellae were inoculated into meat and bone meal at a concentration of 1000 CFU/g and the meal was fed at a rate of 600 g/day. No signs of clinical disease were noted in either cow. Rumen samples were *Salmonella* positive on 46 or 30% of tested days for the two cows. Only one faecal sample from one cow cultured positive for *Salmonella* and no milk samples from either cow.

The source of *S.* Mbandaka colonisation in a cattle herd was found to be animal fat, a component of the feed mixture (Jones *et al.*, 1982). The animal fat was found to contain 240 MPN/100 g of *S.* Mbandaka. It was not stated what proportion of the final feed the animal fat constituted.

Human salmonellosis due to contaminated animal feed

A limited number of outbreaks due to transmission of *Salmonella* from feed through animals to humans have been reported. However, the circumstances of these outbreaks suggest that many other outbreaks may go undetected, particularly if the serotypes are already common in the human population. It should be noted that in all cases the evidence linking human cases of salmonellosis to the presence of particular serotypes in animal feed is largely circumstantial, but when viewed in aggregation is highly suggestive.

***S.* Heidelberg in milk (Knox *et al.*, 1963).** During November-December 1961 56 incidents of *S.* Heidelberg infection were reported in and around Cirencester, England, including 77

cases and 46 asymptomatic excreters. The incidents were traced to unpasteurised milk from a cow with asymptomatic *Salmonella* mastitis. *S.* Heidelberg was isolated from meat and bone meal at the farm that supplied cattle feed to the farm. Although the cattle feed did not contain meat and bone meal, both the cattle feed and the meat and bone meal were processed on the same equipment. No other source of *S.* Heidelberg was identified on the farm or environs.

***S.* Virchow in chicken (Pennington *et al.*, 1968; Semple *et al.*, 1968)**. During July 1968 an outbreak involving at least 50 people occurred in Liverpool, England caused by *S.* Virchow infection. This previously rare serotype was isolated from a number of cases during subsequent weeks. The outbreak was traced to dressed chicken from a packing station in Cheshire and associated rearing units. It was hypothesised that the contamination was introduced into chicken breeding units through contaminated feed. Although the breeding flocks were found to be negative for *S.* Virchow at the time of the investigation, this was explained by the greater resistance of adult birds to *Salmonella* colonisation. *S.* Virchow had previously been isolated from poultry feed components (protein supplements, meat and bone meal, offal meal).

***S.* Agona in chicken (Clark *et al.*, 1973)**. Prior to 1970 *S.* Agona was a rare serotype in humans. However, by 1972 it accounted for over 500 cases in the USA and over 700 cases in the UK. Investigation of an outbreak of salmonellosis in Paragould, Arkansas during May 1972 traced the source of infection to a Mississippi poultry farm. While *S.* Agona was not isolated from feed samples taken from the poultry farm, their feed formulation contained 8% Peruvian fishmeal. Ongoing monitoring of imported feed components identified that Peruvian fishmeal was frequently contaminated with *S.* Agona. During 1969-1970 *S.* Agona also emerged a significant public health issue in UK, Israel and the Netherlands. In all three countries the emergence of this serotype was preceded by detection of the serotype in imported Peruvian fishmeal.

***S.* Hadar in turkeys (Rowe *et al.*, 1980; Watson and Kirby, 1985)**. Human isolations of *S.* Hadar were very rare in the UK prior to 1971, but by 1978 accounted for over 14% of all human salmonellosis cases. Consumption of turkey meat was identified as a factor in approximately 46% of cases. While the ultimate source was not conclusively identified, *S.* Hadar was found in the UK in poultry offal meal imported from Israel in 1969 and had become endemic in turkey breeder flocks by the mid 1970s.

APPENDIX 4: RISK MANAGEMENT OVERSEAS

Europe

Legislation

EC Regulation 183/2005 outlines requirements for feed hygiene:
http://eur-lex.europa.eu/LexUriServ/site/en/oj/2005/l_035/l_03520050208en00010022.pdf
There is a legal requirement for European feed manufacturers to be registered and to implement, maintain and document procedures based on HACCP principles.

Reviews

Strategies to control *Salmonella* in the feed production chain have been reviewed by EFSA's Panel on Biological Hazards. There is a legal requirement for European feed manufacturers to implement, maintain and document procedures based on HACCP principles. Risks and potential control measures are discussed relating to *Salmonella* contamination during:

- Primary production of feed, mainly related to the spreading of contaminated fertilisers. Controls include storage, composting, ploughing in, increasing time between spreading and grazing or planting, heat or chemical (lime) treatment of fertilisers.
- Processing of feed ingredients, due to residual contamination of premises, equipment or staff. Controls include cleaning and disinfection, implementation of good hygiene controls, heat treatment and control of moisture of co-products, maintaining good ventilation in silos, controlling vermin and carrying out routine bacteriological testing. Careful selection and monitoring of supplier is also an important control.
- Transport and storage of ingredients. Controls include use of hygienic vehicles and implementation of suitable cleaning and disinfection, protection of feed material from the environment, avoidance of carryover from previous shipments, control of vermin and control of access of wild birds.
- Feedmilling, including handling and storage of ingredients, design of the feedmill, processing, conditioning and pelleting, decontamination, foot traffic, wild birds, insects and rodents. Controls include avoiding moisture build up, strict separation between ingredients and finished feeds (including via ventilation systems or foot traffic), equipment designed to prevent material build up and allow inspection and cleaning, control of temperatures and times during conditioning and pelleting and avoidance of steam leaks that may result in local moisture build up, attention to cooler air microbial quality, thorough cleaning and disinfection and control of animal ingress.
- Transport and storage on the farm. Controls include preventing moisture increase in feed, proofing storage against wild birds and vermin, limiting visitors, use of dedicated clothing and equipment and regular cleaning procedures.
- Distribution in feeding system. Control measures include cleaning and disinfection, maintenance of trough hygiene, control of vermin and visitors.

Codes of practice and HACCP programmes

A large number of governmental and industry Codes of Practice and HACCP programmes have been developed in Europe for control of *Salmonella* in animal feed, including:
DEFRA Codes of Practice for the control of *Salmonella* (UK):
http://www.defra.gov.uk/animalh/diseases/zoonoses/salmonella-cop.htm

Agricultural Industries Confederation (AIC) Universal Feed Assurance Scheme (UFAS):
http://www.agindustries.org.uk/content.output/93/93/Trade%20Assurance/Trade%20Assurance%20Schemes/UFAS.mspx

European Feed Manufacturers Guide:
http://www.fefac.org/code.aspx?EntryID=265

Product Board Animal Feed GMP+ (Netherlands):
http://www.pdv.nl/english/kwaliteit/

International

International Feed Safety Alliance (IFSA) Feed Ingredients Standard:
http://www.ifsa-info.net/lmbinaries/ifis.pdf

USA

Codes of practice and HACCP programmes

Jones (2002) conducted an analysis of sources of pathogen contamination in the feedmill environment and identified critical control points as the basis for a HACCP programme. These included procedures to:

- Exclude contamination from the feed;
- Prevent multiplication of the organism in the feed; and
- Kill organisms within the feed and prevent recontamination.