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Assessment of the risk from *Salmonella* occurring in feedingstuffs and the feed production process

Norwegian Scientific Committee for Food Safety

Panel on Biological Hazards

&

Panel on Animal Health and Animal Welfare

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Content

1. Summary

Globally, *Salmonella* is one of the most important foodborne pathogens. The situation in Norway with respect to *Salmonella* is good, as these bacteria are virtually absent from food-producing animals and domestically-produced food. Consequently, Norway also has a relatively low frequency of domestically-acquired salmonellosis amongst the human population.

Salmonella may, however, occasionally be found in animal feed and the ingredients of feed. The Norwegian Food Safety Authority (Mattilsynet) commissioned the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety (Vitenskapskomitéen for mattrygghet), to assess the risk of *Salmonella* occurring in feed and feed ingredients, and the possible animal and human health implications. In response, an *ad hoc* Working Group of experts was appointed with the mandate to draft a risk assessment.

Experiments have shown that under current conditions for farmed Atlantic salmon in Norway, and with the low occurrences of *Salmonella* observed in the feed, the risk of *Salmonella* in fish feed being transmitted via fish to humans is negligible. Epidemiological data from the Norwegian surveillance systems support this opinion. The occurrence of *Salmonella* in feed for production animals other than fish, is a well-recognized problem worldwide and is a source of disease in animals and for further transmission to humans via the food chain. *Salmonella*-contaminated feed for pets has regularly been detected. Due to the close contact between pets and humans, this potential route of transfer of *Salmonella* should not be overlooked.
Importation of raw materials for the production of animal feed may constitute a possible “port of entry” for *Salmonella* into Norway, which may ultimately infect animals and humans.

Whereas *Salmonella*-contaminated feedingstuffs do not affect the health of fish in Norway, such feedingstuffs represent a health risk for animals other than fish, as is well documented in the scientific literature.

There is little information on the risk of *Salmonella* cross-contamination from feed and its ingredients, and the feed production factories, to other sectors of society and the external environment, especially wildlife. *S.* Typhimurium is occasionally carried by some Norwegian wildlife. As this serovar is relatively infrequent in feedingstuffs, there seems to be no significant transfer of *Salmonella* between these compartments.

There is only limited information on the health significance of possible exposure of factory workers, or others handling contaminated material, to *Salmonella*-contaminated feedingstuffs.

Resistance to antimicrobial agents is currently not widespread among *Salmonella* isolates from feedingstuffs in Norway.

1. Samandrag

På verdsbasis representerer *Salmonella* eit av dei viktigaste smittestoffa som kan gi matvareborne sjukdomar. Som ei fylgje av låg førekomst av *Salmonella* hos husdyr og i matvarer, har Noreg ein svært låg førekomst av innanlandssmitte med denne med denne bakterien hos menneske. Det har likevel synt seg at salmonellabakteriar regelmessig kan påvisast i dyrefôr eller i ingrediensar til slikt fôr. Med dette som bakgrunn tinga Mattilsynet ei risikovurdering frå Komiteen for hygiene og smittestoff i Vitenskapskomiteen for mattrygghet. Til å utføre denne vurderinga vart det nedsett ei *ad hoc* arbeidsgruppe som fekk i oppdrag å lage eit utkast til risikovurdering.

Nyleg utførde eksperiment har vist at under vanlege oppdrettstilhøve for norsk laks og med låge konsentrasjonar av *Salmonella* i fôret, er det lite sannsynleg at konsumenten skal kunna bli smitta via fisk eller fiskeprodukt. Fara for dette vert rekna som neglisjerbar. Dette synet vert og støtta av epidemiologiske data for førekomst av humane infeksjonar med *Salmonella*. Det har lenge vore kjent at fôr til landlevande husdyr kan vera forureina med *Salmonella*, og at desse kan verta overførde til menneske via matvarer. Tidvis har det vorte påvist salmonellabakteriar i fôr til kjæledyr. Med tanke på den nære kontakten det er mellom kjæledyr og menneske, kan smitte denne vegen ikkje utelukkast. Når det gjeld import av ingrediensar til dyrefôr, kan dette representera ein mogeleg innfallsport for *Salmonella* som seinare kan tenjast å infisere dyr eller menneske.

Medan fôrvarer som er infiserte med *Salmonella* ikkje representerer eit dyrehelseproblem for fisk i Noreg, kan slikt fôr utgjere eit helseproblem for andre dyr enn fisk. Tidlegare vitskapeleg dokumentasjon har vist dette.

Mykje er framleis uklårt når det gjeld *Salmonella* og dyrefôr. Mellom anna veit ein lite om mogeleg smitte mellom dyrefôr, ingrediensar til slikt fôr eller produksjonsanlegg og andre delar av samfunnet eller det ytre miljøet, inkludert viltlevande dyr. Delar av villfaunaen i Noreg er tidvis infiserte med *S.* Typhimurium. Sidan dette ikkje er ein vanleg førekomande serovariant i fôrvarer, ser det ikkje ut til at det er nokon utbreidd kryssforureining med *Salmonella* mellom desse områda.

Ein veit også lite om kva mogelege negative helseverknadar arbeidarar ved fôrfabrikkar kan verta utsette for ved kontakt med *Salmonella*-infiserte fôrvarer.

Resistens mot antimikrobielle midlar hos *Salmonella* isolert frå fôrvarer er så langt ikkje noko utbreidd problem i Noreg.

2. Background

The situation in Norway with respect to *Salmonella* is good, as these bacteria are virtually absent from food-producing animals and domestically-produced food. Large resources are invested to maintain the very low occurrence of *Salmonella* in the domestic animal population and the domestic food chain. *Salmonella* may occasionally be found in feed and ingredients for such feed. The occurrence of *Salmonella* in feed for animals, other than fish, is a well-recognized problem worldwide and is a source for further transmission to humans via the food chain (Shirota *et al.* 2000;Veldman *et al.* 1995).
Because food-producing animals are the primary source of pathogenic *Salmonella* infections in humans, it follows that bacterial contamination of animal feed contributes to the burden of foodborne illness. The impact of reducing *Salmonella* contamination of animal feeds on the risk of human foodborne salmonellosis is difficult to assess.

3. Terms of reference

The Norwegian Food Safety Authority commissioned the Norwegian Scientific Committee for Food Safety to undertake a risk assessment on the occurrence of *Salmonella* in feedingstuffs, mainly for food producing terrestrial animals (including horses), as well as for fish and pets (with principal focus on dogs and cats). The Norwegian Food Safety Authority expressed a particular interest in the following topics[1]:

A. To what extent might *Salmonella*-contaminated feed materials constitute a risk to human and animal health?

1. Identify the potential pathways of infection and the probability of them occurring.
2. Will the risk to human or animal health posed by *Salmonella*-contaminated feedingstuffs be influenced by whether it is of vegetable or animal origin?

B. Assess the use of *Salmonella*-contaminated feed material in a production process with a heat treatment (extruding/pelleting with a core temperature of at least 81°C) in relation to the survival of the *Salmonella*.

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[1] **Oppdrag**
Mattilsynet vil be VKM om en risikovurdering i forhold til salmonellaproblematikk knyttet til produksjon av fôrblandinger til både landdyr og fisk. Mattilsynet ønsker spesielt følgende aspekter belyst:

A. Vurdere i hvilken grad et salmonellainfisert fôrmiddel kan utgjøre et smittepress i forhold til folkehelse og dyrehelse
   1. Vi ber om identifisering av smitteveier og sannsynliggjøring for at de oppstår.
   2. Vil risikoen i forhold til folkehelse/dyrehelse være avhengig av om det er animalske eller vegetabilske fôrmidler som er infisert?

B. Vurdere eventuell bruk av et salmonellainfisert fôrmiddel i en produksjonsprosess med varmebehandling (ekstrudering/pelletering med kjernetemperatur på minst 81°C) i forhold til overlevelse av salmonellabakterier.
   1. Vil overlevelsen av salmonellabakterier avhenge av om det er et vegetabilsk eller et animalsk fôrmiddel som er infisert?
   2. Vil overlevelsen av salmonellabakterier avhenge av sammensetningen til fôrblandingen?
   3. Vil overlevelsen av salmonellabakterier avhenge av produksjonsprosessen (ekstrudering/pelletering)?
   4. Vil overlevelsen av salmonellabakterier avhenge av anlegg eller utstyrs utforming?

C. Vurdere faren for å innføre antibiotikaresistente salmonellastammer ved innførsel av salmonellainfiserte fôrvarer.

D. Vurdere hvilken risiko fôrvarer til selskapsdyr utgjør i forhold til smittepress for dyrehelse og folkehelse.

1. Would the survival of *Salmonella* depend on whether the feed material is of vegetable or animal origin?
2. Would the survival of *Salmonella* depend on the composition of the feed material?
3. Would the survival of *Salmonella* depend on the production process (extruding/pelleting)?
4. Would the survival of *Salmonella* depend on the arrangement and construction of the processing plant or the design of the production equipment?

C. Assess the risk posed by antimicrobial resistant *Salmonella* strains imported with contaminated feed materials.

D. Assess the risk to animal and human health posed by contaminated feed materials intended for pets.

4. Definitions

Salmonella: unless otherwise specified, the term *Salmonella* in this report refers to *Salmonella enterica* and serovars thereof.
Fish: the term "fish" in this report refers to farmed marine fish.
Pets: the term "pets" in this report refers to dogs and cats kept as pets.
Terrestrial animals are defined as non-aquatic animals normally living on land.
Feed materials: defined as product of vegetable and animal origin, organic or inorganic substance intended for feeding of animals directly or in blends.
Compound feedingstuffs: defined as any ready-to-use feedingstuff that is sufficient to cover the nutritional needs of an animal.
Feedingstuffs: defined as any product intended for the feeding of animals

5. Legislation

According to the Norwegian regulations for production of feedingstuffs, an internal process control programme must be implemented in feed mills. This includes a sampling scheme for *Salmonella,* in which a minimum of 3 samples are analysed every 14 days. Samples include feed materials and scrapings from control points. Less frequent sampling is required in establishments that produce small amounts of feed, based on heat-treated protein concentrates and Norwegian crops. Through an official surveillance programme (sampling according to Council Directive 76/371/EEC) random samples of feedingstuffs for terrestrial animals are collected and analysed for *Salmonella*.

Companies producing fish meal or fish oil are required to establish and maintain an internal process control based on the HACCP-system, according to the regulation for fish meal and fish oil. This control includes analyses for *Salmonella* on a minimum of one sample per 50 metric tons produced. The national production of meat and bone meal is subject to a continuous process control that includes analyses for *Salmonella*.
Imported feed material is subject to both official controls and internal checks. Official control regimes depend on whether the consignment comes from third countries or EEA (European Economic Area) countries (intra community trade) and whether the consignment is of vegetable or animal origin. Together with Sweden and Finland,

Norway has national legislation on import control (including intra community trade) for *Salmonella* in feed materials of vegetable origin.

According to national legislation, import of feed materials of vegetable origin must be notified to The Norwegian Food Safety Authority that may perform random sampling for *Salmonella* testing before distribution or use. The number of samples depends upon the size of the load and whether the feedingstuffs are classified as high-risk (soya beans, maize, cotton seed, etc.) or low-risk materials (e.g. wheat, barley).

Feed materials, including fish meal, imported from third countries must be subjected to control for *Salmonella* according to a specified plan before distribution or use. A minimum of one sample per 50 metric tons must be tested for the presence of *Salmonella*.
All *Salmonella* found in production animals, irrespective of whether they are detected in the course of Norwegian *Salmonella* Control Programmes, in connection with clinical problems, or associated with surveys or other investigations, are included in the resistance monitoring (only one isolate per herd) (The Norwegian Zoonosis Centre 2004).

Official control
Import of feed materials of vegetable origin (both from third states and EAA countries) must, according to national legislation, be notified to The Food Safety Authority, which may perform random sampling. Feed materials of animal origin (excluding fish meal) from third countries can only be imported to the EAA through approved border inspection posts. Before import, notification must be given (using a web based system called "traces"). The consignment is subject to document control, identity control and physical control. For feed materials of animal origin, the physical control includes tests for *Salmonella*. *Salmonella*-contaminated feedingstuffs of animal origin are not released for trade, but returned, or decontaminated.

Own check
According to national legislation, establishments importing feed materials both from third countries and EEA countries are also required to sample and analyse for *Salmonella,* according to a specific plan, before distributing or using the consignment.
Norwegian establishments producing compound feedingstuffs for fish are required to establish and maintain an internal control system based upon HACCP-principles. A minimum of four samples per 14 days must be examined for *Salmonella* and if *Salmonella* is detected, The Norwegian Food Safety Authority must be notified immediately. Through an official surveillance programme run by the National Institute of Nutrition and Seafood Research (NIFES) on behalf of The Norwegian Food Safety Authority, random samples of Norwegian compound feed for fish are collected at the establishments and analysed for *Salmonella*.
According to the Norwegian regulations for production of feedingstuffs, an internal (process) control programme must be implemented in feed mills.

6. Feed production process

6.1. Fish feed production process

Around 700 000 metric tons of fish feed is produced annually in Norway (Statistisk sentralbyrå, http://www.ssb.no). Since the feed conversion factor for coldwater fish is approximately 1, this amount is sufficient for the production of around 700 000 metric tons of farmed fish. The feed factories are spread along the Norwegian coast from Rogaland to Troms. There are three significant feed companies operating in Norway. These are Ewos, Biomar and Nutreco.
Fish feed is largely based on the marine ingredients fish oil and fish meal. In addition, about 15 % of the diet is wheat or other starch-rich ingredients. A recent development, to reduce feed cost and improve fish farming sustainability, is the partial replacement of marine ingredients with vegetable ingredients. Thus, fish feeds may contain up to 30 % vegetable proteinaceous ingredients, such as wheat gluten, soybean meal or rapeseed meal. The major component in fish feed is however, still fish meal and fish oil, constituting between 60 and 80 % of the diet. A typical salmon diet contains between 30 and 40 % oil and between 30 and 40 % proteins. In addition, it may contain up to 10 % starch. Water level is usually below 10 %. According to the Norwegian Seafood Federation (Fiskeri- og Havbruksnæringens Landsforening, March 2006), fish feed can consist of 30-45% fish meal, 15-25% fish oil, 5-15% vegetable oil, and 25-30% vegetable proteins, including wheat proteins. In Norway, approximately 1.1 million metric tons of industrial fish is used for fish meal and fish oil production, giving a total production of around 400 000 metric tons. Of this approximately 100 000 metric tons is exported. In addition around 200 000 metric tons is imported. Thus, it can be estimated that the majority of the fish meal and fish oil produced in Norway is used in the fish feed industry. Furthermore, the main part of fish farmed in Norway is exported.

The different feed ingredients, except the oil fraction, are ground in a hammer mill through a 1 mm sieve, and are then mixed. After mixing, the feed mash is subjected to a process termed conditioning. During conditioning, steam and hot water are added to increase the temperature of the mash to around 95 °C as it leaves the conditioner (Strahm 2000). This addition of steam and water increases the water content to around 25 to 30 %. Retention time in the conditioner usually varies from 30 to 180 seconds. The conditioner may sometimes be pressurized. Immediately following the conditioning process, the feed mix is transported to an extruder. In the extruder, the feed material is forced towards a die by one or two screws. Due to the small opening of the die, the internal pressure increases, and the friction and the heat applied should theoretically cause the temperature to increase to between 115 to 130°C (Sørensen 2002). In reality, the temperatures at this stage are 98-110 °C for 0.3-1.8s (Bjørn-Arne Næss, Mattilsynet, personal communication). The retention time in the extruder is usually between 15 and 20 seconds. A knife cuts the feed into pieces as it passes the die. Due to the sudden drop in pressure, which causes the water to boil, the pellet expands. Upon drying and cooling, the pellet obtains its hard and porous structure. Drying is performed using air with a temperature not exceeding 80-90°C, but with a target temperature close to that temperature. The oil in the diet is added to the pellets after extrusion, at a temperature not exceeding 70 °C. In this process, the fat is sprayed onto the pellet under vacuum, followed by slow release of air into the vacuum coater so that the fat is absorbed into the interior of the pellet.

There are also some factories in Norway that do not use high temperatures during the production of fish feed.

6.2. Pet feed production process

Feed for pets is available in a large variety of forms, with extruded feeds constituting a major product form. A total of 44 900 metric tons of feed for dogs and cats is sold in Norway annually, of which 12 % is produced domestically. The majority of countries of origin for imported feeds are EU members, while between 500 and 700 metric tons originates from other countries. Dry food for dogs and cats is produced by an extrusion process very similar to the one used for fish feed production. Canned wet feed for dogs and cats is produced by a boiling process. Other types of feed for pet animals include dried pig ears and chewing bones, and 900 metric tons of such feed is sold annually. The production of pet feeds includes some kind of heating process.

6.3. Production of feed for terrestrial, food-producing animals

Total feed production for terrestrial, food-producing animals in Norway is approximately 1.5 to1.7 million metric tons. This feed is produced in feed mills of varying sizes distributed throughout the country. The main ingredients used are cereals grown in Norway, amounting to around 1 million metric tons annually. Other ingredients of domestic origin include animal fat, fish meal and fish ensilage, shell meal and wheat bran. Imported ingredients include soybean meal, soy oil, rapeseed meal, maize, maize gluten, sorghum, wheat and various minor ingredients such as minerals and vitamins. A typical pelleted diet contains 45 to 50 % starch, 16 to 22 % protein, 10 to 20 % dietary fibre and 4 to 10 % fat. Water content is approximately 12 %.

Usually the ingredients are weighed out, followed by grinding in a hammer mill fitted with a sieve with a diameter of 3 to 4.5 mm. The feed then enters a conditioner where steam is added such that the temperature rises to around 75 °C and water content rises from 13 % to approximately 16 %. Retention time is 30 to 60 seconds. Thereafter, the majority of the feed enters a pellet press, where friction causes the temperature to rise to 81-90 °C. For some ruminant feeds, an expansion process is included after the conditioner and prior to the pelleting process. In the expander, some water may be added, and the temperature increases to around 90 to 110 °C due to friction and pressure caused by a screw that presses the feed through a constrained outlet area. After the conditioning/expansion process, the feed is pressed through cylindrical holes with a diameter of between 2.5 and 7 mm and thus pellets are formed. Retention time in the pellet press is 30 seconds maximum. The pellets are thereafter cooled in a cooler. The usual working principle of the cooler is by air, at an ambient temperature, being blown through the pellets in a counter flow direction. Cooling time is usually between 10 and 20 minutes. The cooled pellets are ready for storage or for delivery to the farm.
Animal feed is also produced at the farm level, but information on the extent of such production is unavailable.

7. Hazard identification

The potential mechanisms for feed contamination are as follows:

- Feed materials might already have been contaminated with *Salmonella*,

- *Salmonella* might survive the heat treatment during the feed production process. The extent of survival will depend upon the heat resistance properties of the *Salmonella* strain, as well as the fat content and water activity of the feed materials (Grau 1989),
- Compound feedingstuff may be re-contaminated after heat-treatment,
- All possible cross-contamination pathways, including the environment.

Salmonella isolates in feed may be resistant against antimicrobial agents of clinical relevance, and such isolates may harbour antimicrobial resistance genes, representing an additional animal and public health risk.

8. Hazard characterization

Salmonella are Gram-negative, flagellated, facultative, anaerobe bacteria. The taxonomy of *Salmonella* is rather complicated, and has been a subject of scientific debate for many years (Bell 2002). Detailed phylogenetic analyses by multilocus enzyme electrophoresis and DNA sequencing have demonstrated that the genus *Salmonella* includes the two species *Salmonella bongori* and *Salmonella enterica. S. enterica* is divided into seven distinct groups or subspecies, i.e. *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *indica* (VI), and the diphasic groups IV and VII (Boyd *et al.* 1996). Furthermore, differences in lipo-polysaccharide and flagellar structure generate the antigenic variation that is reflected in the more than 2500 known serovars.
This risk assessment focuses on *S. enterica.*

8.1. Salmonellosis in the human population

Salmonellosis is one of the most common food borne bacterial infections in Norway, and it is reported that more than 1500 human cases occur annually (www.msis.no). However, only approximately 15% of reported cases were infected within Norway, and, among these, *S*. Typhimurium was the dominant serovar.
Due to underreporting, the actual number of infections with *Salmonella* is probably much higher. A recent American population survey estimated that there were almost 70 unregistered cases of human salmonellosis for each culture-confirmed case involving non-bloody diarrhoea (Voetsch *et al.* 2004).

The many *Salmonella* serovars may give infections of highly varying severity, ranging from a lack of symptoms or a mild and self-limiting infection, to a fatal outcome. Depending on the serovars in question, the properties of the contaminated food, and the person-to-person variations in susceptibility, the dose of infection may vary from 10^1 to 10^6 *Salmonella* cells. The incubation time is typically one to three days, but may also be as short as 12 hours or more than a week. The majority of *Salmonella* serovars result in a self-limiting intestinal infection, lasting typically up to one week. The symptoms include diarrhoea, stomach pain, and fever, and occasionally vomiting. In some cases, further accompanying symptoms (sequelae) occur, and may include pains, meningitis, urinary tract infections or infections in the heart linings (Bell 2002). Depending on the form, some sequelae may last for months after the disappearance of the initial symptoms. One additional complicating food safety factor for salmonellosis is the carrier condition that some recovered patients acquire. Even though the number of *Salmonella* in faeces usually declines after recovery, some persons may shed bacteria for a considerable time, and therefore represent a source of contamination, especially if involved in food production or preparation.

Although different *Salmonella* serovars may vary in their degree of virulence, we must presume that all are pathogenic. Human volunteer feeding studies of putative "nonvirulent" serovars established that all the serovars tested caused diseases if given in sufficient doses (McCullough and Eisele 1951).
The most important *Salmonella* serovars in terms of number of infected persons throughout the world are *S.* Typhimurium and *S.* Enteritidis, which account for approximately 70% of reported cases. These serovars are frequently involved in large outbreaks, which sometimes reach many thousand cases (Stan Bailey and Maurer 2001). *S.* Typhimurium has established itself among the wild bird and hedgehog populations of Norway.
According to the data from the Norwegian Surveillance System for Communicable Disease (MSIS) (www.msis.no), the most commonly isolated *Salmonella* isolates from humans in Norway in 2005 were: *S.* Enteritidis, *S.* Typhimurium, *S.* Typhimurium DT104, *S.* Stanley, *S.* Virchow, *S.* Saintpaul, *S.* Hadar, *S.* Newport, *S.* Java, *S.* Branderup, and *S.* Infantis

8.2. *Salmonella* infection and salmonellosis in animal populations

There are many different types of serovars associated with salmonellosis in animals. Some serovars attach to the intestinal tract causing severe diarrhoea and potentially life-threatening dehydration and electrolyte imbalances, while others tend to target joints. Some serovars of *Salmonella* have the potential to cause abortions in animals. In addition to infection of the gastrointestinal tract, *Salmonella* may occur in the mesenteric and hepatic lymph nodes, and sometimes in the gall bladder, liver and spleen (Kampelmacher 1963). Young animals are more susceptible than older animals to infection with *Salmonella.* In young animals and in adults whose resistance has been lowered, spread of *Salmonella* beyond the mesenteric lymph nodes may occur and the infection may establish in the reticuloendothelial cells of the liver, from where it may invade the blood stream. Once a systemic infection has been established, salmonellosis as a disease can develop with manifestations such as septicaemia, enteritis, abortion, and can be localized in various tissues as a result of bacteraemia (Radostis *et al.* 2000).

Generally, animals develop salmonellosis when their immune systems are compromised. Some animals are carriers of *Salmonella* and, frequently, carrier animals cannot be cured with antimicrobials or other drugs. When infected, pigs usually become asymptomatic carriers for variable periods. Cattle can become both asymptomatic carriers for variable periods, or diseased animals. The latter situation is often the case when cattle are infected with *S.* Dublin, which is a serovar well adapted to cattle (Ekperigin and Nagaraja 1998). *Salmonella* types that spread systemically can also be localised in the spleen or liver. *S*. Abortus-ovis, *S*. *diarizonae* 61:k:1,5,(7) and *S*. Montevideo have all been isolated in association with abortion in sheep (Alvseike 2001;Radostis *et al.* 2000).

Salmonella has the potential to spread easily from animal to animal. Carrier animals spread the bacteria in their manure and other discharges. Contaminated footwear, clothing, vehicle tyres, feed and water containers and other equipment are all capable of spreading *Salmonella*.

A study from Washington state, USA provided evidence for a role of cattle feed in transmission of *S. enterica* (Davies *et al.* 2004).

From a global perspective, the following *Salmonella* variants are most often isolated from production animals:

- *S.* Enteritidis from eggs, and *S.* Pullorum and *S.* Gallinarum from birds,
- *S.* Typhimurium, *S.* Dublin and *S.* Newport from cattle,
- *S.* Typhimurium and *S.* Dublin from sheep and goats,
- *S.* Choleraesuis from pigs.

Serovars of *Salmonella* like *S.* Dublin are not recognised as food borne pathogens, but invasive serovars like *S.* Enteritidis and *S.* Typhimurium are by far the commonest and may be associated with subclinical infections in farm livestock. *S.* Enteritidis is also a very common cause of infection transferred by many foods, and has, amongst others, been shown to be able to infect eggs if the parent bird is infected.

Avian-adapted *S.* Gallinarum causes fowl typhoid and pullorum disease in chickens, resulting in high morbidity and mortality among poultry flocks (Bullis 1977). However, this agent is probably no longer of significance in modern industrialised poultry production, and retrospective analysis of epidemiological data suggests that the emergence of *S.* Enteritidis as an egg-associated pathogen was triggered by the elimination of *S.* Gallinarum from poultry populations in the United States and Europe in the 1970s (Baumler *et al.* 2000;Rabsch *et al.* 2000). During the 1990s *S.* Enteritidis became the most frequent *Salmonella* variant detected in poultry populations in many parts of the world.

8.3. Fish as vectors of *Salmonella*

Salmonella have not generally been regarded as fish pathogens, with the possible exception of *Salmonella arizonae* (Austin and Mclnotsh 1991). Experiments have shown that even after administration of very high doses of *Salmonella*, Atlantic salmon did not exhibit any signs of disease (Nesse *et al.*, 2005). Nevertheless fish may be exposed to *Salmonella* spp. through consumption of contaminated feed or by residing in contaminated water.

Relatively little has been reported about the persistence, or possible dissemination, of *Salmonella* in fish exposed to these bacteria via feed. A few experiments in fresh water fish, e.g. Rainbow trout (*Onchorhynchus mykiss*), Israeli mirror carp (*Cyprinus carpio*) and Tilapia (*Tilapia aurea*), have shown that high doses of orally-administered *Salmonella* could result in persistence of *Salmonella* in the gastrointestinal tract, and sometimes also the entry of *Salmonella* into the internal organs and muscle tissue (Baker and Smitherman 1983;Buras *et al.* 1985;Hagen 1966;Heuschmann-Brunner 1974).

Only one publication describes the fate of orally-administered *Salmonella* in marine fish (Nesse *et al.* 2005a). In this study, farmed Norwegian Atlantic salmon were experimentally fed *Salmonella*-contaminated feed. Two *Salmonella* serovars were used, both strains originating from Norwegian fish feed factories. The results showed

that both the persistence and the dissemination of the bacteria in the fish were highly dependent on the dose administered. No clinical signs were observed.

8. 4. Possible *Salmonella* cross-contamination between feed factories and wild life

Wild birds, rodents and insects may carry *Salmonella* (Davies and Wray 1997;Olsen 1998;Roth 1994), and resident rodents and insects in *Salmonella*-positive feed factories have been shown to carry the same *Salmonella* types as those found in the factories (Davies and Wray 1997); Nesse, unpublished data). Furthermore, gulls have been considered as indicators of environmental microbial contamination (Girdwood *et al.* 1985), and have also been suggested to be transmitters of *Salmonella* from one site to another, mainly from abattoirs, refuse tips and sewage, to other sites (Murray 1991). Gulls have been shown to be capable of carrying *Salmonella* over long distances, through their migratory behaviour (Coulson *et al.* 1983). During the breeding season, parental gulls may travel up to 60 km from the colonies, while non-breeding individuals may travel even further (Cramp 1985). The results from a recent Norwegian study indicate possible risks of *Salmonella* cross-contamination between fish meal and feed factories and wild-living gulls (Nesse *et al.* 2005b). In this study, samples with the same *Salmonella* pulsed field gel electrophoresis (PFGE)-profiles as those found in the factories were collected from gulls as far as 100 km away from the factories. Transmission of *Salmonella* from gulls to domestic animals has also been suggested, related to contamination of drinking water by large numbers of birds (Coulson *et al.* 1983;Johnston *et al.* 1979;Sharp *et al.* 1983;Williams *et al.* 1977). Conversely, other reports have suggested gulls present a minimal health risk to humans as carriers of *Salmonella* (Fenlon 1981;Girdwood *et al.* 1985;Refsum *et al.* 2002;Schubert *et al.* 1999;Sørensen 2002), although gulls roosting on drinking water supplies may present a risk (Benton *et al.* 1983;Fennel *et al.* 1974;Refsum 2003) as illustrated by the outbreak at Herøy in Norway (Refsum *et al.* 2002).

8.4.1. The wildlife reservoir of *S.* Typhimurium in Norway

In 1987, a nationwide outbreak of *Salmonella* Typhimurium O:4-12 that could be traced to chocolate bars, occurred in Norway. This epidemic strain had also been regularly encountered as the aetiologic agent of fatal salmonellosis among wild passerine birds, suggesting an epidemiological link between avian and human cases. A case-control study of sporadic cases caused by this variant ((Kapperud *et al.* 1998) identified the following risk factors: drinking untreated water, having direct contact with wild birds or their droppings, and eating snow, sand or soil. The conclusions from molecular epidemiology of isolates from humans and avian wildlife support earlier results that wild passerines constitute an important source of infection to humans in Norway, whereas based on PFGE analysis, it was suggested that gulls and pigeons only represent a minor source of human serovar Typhimurium infections (Refsum *et al.* 2002). Wild passerines, gulls, and pigeons may also constitute a source of infection for domestic animals and feed plants, or vice versa.

S. Typhimurium-infected hedgehog populations most probably constituted the primary source of infection during two human disease outbreaks in Bergen and Moss (Eurosurveillance, 2000, volume 4). Bacteriological investigations during the outbreaks demonstrated that the hedgehog populations in the areas concerned were

colonised with two distinct *Salmonella* strains, each occurring in geographically separate areas.

Although some Norwegian wildlife may be occasional carriers of *S.* Typhimurium, this serovar is relatively infrequent in feedingstuffs. Thus there seems to be no significant transfer of *S.* Typhimurium between these two compartments.

8.5. Risk factors in feed production

Normally, the temperatures used during the feed production are sufficient to eliminate *Salmonella*. However, some factors, like high concentrations of *Salmonella* in feed ingredients, may result in the production of *Salmonella*-contaminated feed.
Assuming that the heating processes are sufficient for eliminating *Salmonella* contamination, there are two major risk factors in the feed production process. One is insufficient heating of the first batch of feed that goes through the processing system following a halt in production. It is necessary to process a certain amount of feed before the temperature increases and is stabilized at the desired level. This feed is usually returned and is processed again once the process has been stabilized. The risk in such a situation is that the process line posterior to the part with a high temperature may become contaminated with *Salmonella* from the feed. This risk is associated with the other major risk factor in the feed production process, namely the risk of recontamination of the feed after heat treatment. In addition to these contamination pathways, there is also the risk for cross-contamination between the section of the feed production plant handling raw, non-heat-treated materials and the section of the plant handling heat-treated feed. In order to avoid this, it is necessary to enforce systems to prevent such cross-contamination occurring.

8.6. Growth and survival of *Salmonella*

Bacteria in the genus *Salmonella* are typical intestinal organisms, being able to colonize the gastrointestinal tract of animals and humans. However, the ability of *Salmonella* spp. to survive and multiply in environments other than the intestine, e.g. food and feed, is well documented (D`Aoust. 1997).
Salmonella may grow in the temperature range between 5 and 46°C. Under suboptimal conditions, e.g. at temperatures below 30°C, *Salmonella* tend to form biofilms on both inert and organic surfaces. In this state, bacteria are better protected against environmental stresses (Costerton *et al.* 1999;Donlan and Costerton 2002)
The bacterium may grow in a pH range from 3.8 to 9.5 and at water activities (a_w) above 0.94 (Bell 2002).
As is the case for heat resistance, the survival of *Salmonella* spp. in stored food and feeds is enhanced by a lowered a_w (Grau 1989). It is known that the ability of *Salmonella* strains to survive air drying varies considerably (Humphrey *et al.* 1995;Jorgensen *et al.* 2000), but in frozen or dried products, *Salmonella* are reported to survive for months or even years (D'Aoust *et al.* 1993;D'Aoust 1994a;D'Aoust *et al.* 1995;D`Aoust. 1997;Grau 1989). Outside the pH range allowing growth, *Salmonella* are reported to die during storage (Grau 1989).

8.7. Heat resistance of *Salmonella*

Salmonella spp. in liquids and food or feed with a high water activity ($a_w > 0.97$) are readily destroyed by heat. However, the heat resistance of *Salmonella* is strongly

determined by strain, physiological state, and the matrix in which the bacterium is found (Doyle and Mazzotta 2000).
There are considerable variations among *Salmonella* strains regarding the sensitivity to heat, and one of the most resistant is reported to be *S.* Senftenberg. It is interesting to note that this serovar is one of those most frequently found in animal feed (Grau 1989).
The physiological state of the cells is also of great importance for heat resistance. A higher heat resistance is found among *Salmonella* cells grown in nutritionally rich media, as compared to minimal media, among cells derived from stationary rather than logarithmic phase, and among *Salmonella* cells previously stored in dry environments or grown at high temperatures (D`Aoust. 1997). The ability of *Salmonella* spp. to acquire higher heat resistance after repeated exposure to sub-lethal temperatures should be especially noted. This phenomenon could be explained by a change in the fatty acid composition of the cell membrane of heat-stressed *Salmonella*. This heat adaptation is important for the possible development of specific heat-adapted “house strains” in feed production facilities.
There is a general tendency for heat resistance to increase with decreasing water activity (a_w) (Bell 2002;Grau 1989). As an example, the decimal reduction time (D-value) at 68°C for *Salmonella* spp. in milk (which has a high a_w) varies from 0.28 to 10 seconds depending on the serovar, whereas the D-value at 71°C for *Salmonella* spp. in chocolate (which has a low a_w) varies from 4.5 to 6.6 hours depending on the serovar (Bell 2002). In addition, the solutes used to alter the a_w plays an important role, and studies have shown that, at the same a_w, sucrose gives a better heat protection than glycerol (D'Aoust *et al.* 1994;D'Aoust 1994b).
It is not possible to predict the heat resistance of *Salmonella* in products with a low a_w (Grau 1989). For such products, the kinetics of heat destruction must be determined in the product itself. We are not aware of any specific studies regarding survival of *Salmonella* in different stages of the feed production process.

8.8. Antimicrobial resistance

During the years 1999-2002, 32 *Salmonella* isolates from Norwegian fish feed factories were tested for antimicrobial susceptibility. The isolates were susceptible to all antimicrobials tested, except for one isolate, which was resistant to streptomycin (NORM-NORM-VET 2000;NORM-NORM-VET 2001;NORM-NORM-VET 2002).
In general, available data, although very limited, indicate that antimicrobial resistance is not widespread amongst those *Salmonella* that are isolated from Norwegian animals (NORM-NORM-VET 2000;NORM-NORM-VET 2001;NORM-NORM-VET 2002;NORM-NORM-VET 2003;NORM-NORMVET 2004). The possible presence of multi-drug resistant *Salmonella* in imported feed and feedingstuffs should not be excluded. However, we are unaware of data regarding antimicrobial resistance profiles from *Salmonella* isolated from imported feed and feedingstuffs.
Worldwide, the prevalence of antimicrobial resistance among *Salmonella* from human infections has increased during recent decades. This increase has been linked to the selection pressure exerted by antimicrobials used for food-producing animals (Angulo *et al.* 2000;Cohen and Tauxe 1986;Glynn *et al.* 1998;van den Bogaard and Stobberingh 2000). *Salmonella* may acquire antimicrobial resistance genes, probably through the acquisition and exchange of mobile genetic elements with other *Enterobacteriaceae,* within the intestinal lumen. Of special concern is a multi-drug resistant variety, *S.* Typhimurium DT104 which has received much

research attention in recent years. This multi-drug resistance pattern has also been spread to other serovars, e.g. Agona.

9. Exposure assessment

9.1. *Salmonella* in feed materials

Internationally, fish meal, meat and bone meal, maize and soy products have been shown to have a relatively high prevalence of *Salmonella* (Jones and Richardson 2004;Veldman *et al.* 1995). In general, heat-treated products appear to have a higher probability of *Salmonella* contamination than non-heat-treated products.
According to the annual report on trends and sources of zoonoses and zoonotic agents in Norway (www.zoonose.no), approximately 53 000 samples from feed materials were tested in Norway in the five-year period from 2000 to 2004 and *Salmonella* was detected in 0.2% of these samples (Appendix I). These samples were tested as a part of compulsory surveillance programmes, as well as voluntary surveillance programmes, by the Norwegian feed material industry. In addition, some samples of feed material have been tested by the compound feedingstuff factories. These results are not readily available and are therefore not included here. Imported fish meal had the highest prevalence of *Salmonella* (1.33%).

Fifteen different serovars of *Salmonella* were detected in feed material samples. In Norwegian fish meal, the dominant serovars were Senftenberg and Montevideo. These serovars have been known to be resident “house strains” in some factories over a number of years. This may also be the case for the serovar Mbandaka, which was detected in Norwegian meat and bone meal over two succeeding years (2002 - 2003).

9.2. Fish

9.2.1. *Salmonella* in compound feedingstuffs for fish

Salmonella is believed to be introduced into fish feed factories via feed ingredients. In addition, contamination by birds, rodents and insects has been suggested, but not proven. Once introduced into a feed factory, a *Salmonella* strain may persist in the factory for years as a so-called “house strain” (Nesse *et al.* 2003).
During the five-year period 2000-2004, approximately 8800 samples from compound fish feed were tested (Appendix I) in Norway. Of these, 0.33% was positive for *Salmonella*. The serovars identified were Agona, Senftenberg, Montevideo, Kentuckey and Livingstone. The first three are known to be “house strains” in several Norwegian fish feed factories via the factories internal controls. This may also be the case for *S.* Kentuckey and *S.* Livingstone.

9.2.2. Possible links between *Salmonella* in feed and *Salmonella* in farmed fish

Only one publication describes the fate of orally-administered *Salmonella* in marine fish (Nesse *et al.* 2005a).These results show that both the persistence and the dissemination of *Salmonella* in the fish were highly dependent on the dose administered. In Norwegian fish farming, food is withheld from the salmon for the last one to two weeks before slaughter. This is to minimize the number of bacteria that would normally be found in the digestive tract, as these bacteria would contribute to the spoilage of the salmon meat and deterioration of product quality. From the results of the study it was demonstrated that, for *Salmonella* to persist in the fish longer than

one week, the dose had to be more than 1 x 10^6 times higher than that previously found in naturally contaminated feed. We therefore conclude that, given the low concentrations of *Salmonella* in fish feed, under the usual conditions for farmed Atlantic salmon in Norway, the risk of *Salmonella* in fish feed being passed on to the consumer via the fish should be considered negligible.

9.3. Animals other than fish

9.3.1. *Salmonella* in compound feedingstuffs for poultry, cattle and pigs

As for fish feed factories*, Salmonella* is believed to be introduced into feed factories for terrestrial animals via the feed ingredients. In addition, contamination may be introduced or maintained by birds, rodents or insects. Some *Salmonella* clones seem to be able to persist in the factories for long periods, probably as sessile communities in biofilms, where they are better protected against environmental stresses, including disinfection.

In general, complete feedingstuffs and protein concentrates (supplementary feedingstuffs) intended for poultry, pigs, and cattle must be subject to heat treatment until a core temperature of at least 81°C is reached. The entire batch must be heat-treated, and the production has to be performed in a line where all the other feedingstuffs are subject to heat treatment.

In the five-year period 2000-2004, 520 samples from compound feedingstuffs were tested and one sample was positive. The serovar identified was *S.* Agona, which is a known ”house-strain” in at least one factory.
S. Typhimurium is found occasionally in Norwegian feed factories. However, observations indicate that this serovar does not readily become established as a “house-strain” in comparison to some of the other serovars. The serovar has not been isolated from compound feedingstuffs.

9.4. *Salmonella* in feedstuffs for pet animals

Salmonella contamination in feed for pets has been found regularly. Surveys have revealed a high prevalence of *Salmonella* spp. in dog treats made from dried hide, such as chewing bones (NORM-NORM-VET 2000). Dog treats made from hides that are imported from third countries must be accompanied by a certificate that documents that the lot has been controlled for *Salmonella* spp.
In the period from 2000 to 2004, a total of 1700 samples of pet feed were examined for *Salmonella*. Of these, 1.71 % were positive, with Senftenberg, Typhimurium and Havana as the most frequently found *Salmonella* serovars (Appendix I) (www.zoonose.no).

9.5. Possible links between *Salmonella* in feed and *Salmonella* in animals

It has long been known that *Salmonella* in feedingstuffs may infect animals. There are numerous examples of outbreaks of *Salmonella* infections in animals that can be traced back to contaminated feeds (Anderson *et al.* 1997;Glickman *et al.* 1981;Gordon and Tucker 1965;Gray 1958;Gray *et al.* 1958;Newell *et al.* 1959;Zecha *et al.* 1977). In the summer of 2003, some 50 farms in the south of Sweden were contaminated by *Salmonella* Cubana. The source of the contamination was demonstrated to be feedingstuffs, and the feed plant that delivered the contaminated

product was closed for decontamination for quite some time (Swedish Board and Agriculture, www.sjv.se/home/infocus/outbreakofsalmonellainsweden.4.7502f61001ea08a0c7fff131521.html). Experimental studies confirm that animals given feed contaminated with different *Salmonella* serovars may develop colonization and infection with that organism (Gordon and Tucker 1965).

In Norway, any finding of *Salmonella* in animals, humans or feed is notifiable. All *Salmonella* isolated from feed, live animals, food, and humans are referred to the National Reference Laboratory at the Norwegian Institute of Public Health for confirmation where serotyping is performed to identify the serovar. Phage typing is performed for all *S.* Typhi and *S.* Paratyphi, for multiresistant *S.* Typhimurium, and for outbreak isolates of *S.* Typhimurium and *S.* Enteritidis. All *Salmonella* isolates are tested for antimicrobial susceptibility.
Nationwide official control programmes for *Salmonella* in Norway were launched in 1995, and these constitute the basis for the additional guarantees agreed upon in the EEA agreement. These programmes cover both live animals (cattle, swine, and poultry) and meat (cattle, swine, sheep, and poultry). The aim of the programmes is to provide reliable documentation on the prevalence of *Salmonella* in animal food production and to detect any increased occurrence of infections with *Salmonella* among food production animals in Norway. The programme for live animals includes analyses of faeces, meconium, or organs from poultry (breeders, layers, and poultry for meat production) and faeces from breeder pigs, as well as analyses of lymph nodes from randomly selected slaughtered cattle and pigs. The necessary total number of samples required to detect *Salmonella* at an animal prevalence level of 0.1% (with 95% confidence levels) is collected annually from the cattle and swine population at slaughter (sampling of lymph nodes) and from poultry before slaughter (faecal samples). In addition, all elite breeding pig herds and all poultry flocks exceeding 250 animals are surveyed at herd level.
In the five-year period 2000-2004, *Salmonella* was isolated 7 times from poultry, 17 times from cattle, 21 times from pigs, 3 times from horses and 8 times from dogs and cats (Appendix I). *S.* Typhimurium was the most frequent serovar isolated from cattle, pigs and poultry.
In total, *S. diarizonae* is the *Salmonella* most frequently detected in Norwegian domestic animals, and is mainly isolated from sheep. However, restrictions are not imposed on such flocks since the pathogenicity of this bacterium for humans is considered very low. However, carcasses found positive for *S. diarizonae* are not used for human consumption.

The two known factory ”house-strain” serovars *S.* Agona and *S.* Senftenberg were isolated from poultry and cattle (see Appendix I). In addition, the known “house-strain” serovar, *S.* Montevideo was isolated from poultry in 2005. Contaminated feedingstuffs were probable sources. In addition, the serovar *S.* Javiana was isolated once from a factory sample and in four samples from cattle. It is unknown whether or not this infection was acquired from feed. Nine isolates displayed serovars that had not been found either in feed material, compound feed or feed factories. The sources of these infections are unknown.

9.6. Possible links between *Salmonella* in feed and *Salmonella* isolated from humans

Appendix I gives an overview of the *Salmonella* isolates of different serovars and from different sources (feed, feedingstuffs, feed factories, animals and humans) in Norway during the five-year period between 2000-2004 (Source: Zoonosis reports 2000-2004 www.zoonosis.no and MSIS; www.msis.no).

9.6.1. Occupational risks

The contamination levels of today suggest a low risk to the production workers and other handlers of fish feed, but this could be expected to increase proportionally with the concentration of *Salmonella*. Increased contamination levels will also increase the risk of cross-contamination with the production environment.

9.6.2. *Salmonella* from fish feed

Given the low concentrations of *Salmonella* in feed, the risk of *Salmonella* in fish feed being transmitted on to the consumer via the fish, under the usual conditions for farmed Atlantic salmon in Norway, is considered negligible. In order for *Salmonella* in fish feed to represent a health hazard for the fish consumer, the concentrations must be considerably higher than those commonly found in natural feed samples.
In the five-year period between 2000-2004, a relatively high number of people in Norway were infected with *S.* Livingstone, a serovar which was also found in Norwegian fish feed. However, nearly all the isolates found in humans originated from an imported food product. We are not aware of any association between the isolates recovered from the feed and the human cases.

9.6.3. *Salmonella* from feed for poultry, domestic ruminants and pigs

It has been well established that *Salmonella* from colonized food animals can be transmitted to humans through the food chain (Crump *et al.* 2002). In addition, several incidents in other countries have been reported in which human illness was traced back to contaminated feed. Probably the best known incident is one from the 1970s in which Peruvian fish meal contaminated with *S.* Agona was found to be the source of a number of human infections in USA, Great Britain, Israel and The Netherlands (Clark *et al.* 1973).
S. Typhimurium is by far the most frequent serovar isolated both from humans infected in Norway, and from Norwegian cattle and pigs (Appendix I). It is estimated that almost half the domestically-acquired human cases can be explained by infections from either wild birds or hedgehogs (Heir *et al.* 2002).
The most common “house-strains” in Norwegian feed factories and feed material factories, *S*. Agona, *S*. Montevideo and *S*. Senftenberg, have also been isolated from a number of human cases. Without more information on the source of infection, we cannot exclude the possibility that some of the bacteria might have originated from the feed, either through ingestion of products from feed-infected animals, or through direct or indirect contact with contaminated feed or feed material. During a five-year period, the incidence rates of these serovars per 100 000 inhabitants were approximately 0.25%, 0.20% and 0.10%, respectively. This indicates that the risk of being infected with *Salmonella* originating from feed produced in Norway under the current conditions is very low, i.e. less than 1 per
100 000 inhabitants per 5 years. The risk will increase if the level of contamination in feed material, feed factories and feedingstuffs increases. Also the rare serovar *S.*

Javiana was isolated in a feed factory, from cattle and from humans, but we have no information on any epidemiological relationship between these isolates.

10. Risk characterization

10.1. Answers to the questions:

The following specific questions were raised in the risk assessment request from The Norwegian Food Safety Authority:

A. To what extent might *Salmonella*-contaminated feed materials constitute a risk to human and animal health?

1. Identify the potential pathways of infection and the probability of them occurring.

In general terms, this risk depends on the prevalence and concentration of *Salmonella* in feed materials, the efficacy of heat-treatment during processing, and the possibilities of cross-contamination of the end product. The following pathways represent risks for infection of fish, production animals and humans from feed:

- The pathway from process/process environment to process workers in the factory represents an occupational risk. Data from environmental samples collected inside factories support the view that *Salmonella* infections of humans may occur via this pathway.

- The pathway through the feed-chain represents the most important route for transfer to animals. Although *Salmonella* infection in production animals is rare in Norway, feed is the most important infection source.

- The pathway through the feed-chain/food-chain from feed materials through production animals and meat, probably represents one of the most important pathways for transmission from feed to humans. However, this route seems to be an unusual cause of *Salmonella* infections in humans in Norway.

- The pathways through fish feed and fish are probably of lesser importance for infection in humans, since fish appear to have a high threshold for the establishment of *Salmonella* infections and *Salmonella* is not a hazard to the fish itself.

- The pathways through dogs (in particular) and cats to humans through direct contact or through the home environment are documented as possible pathways.

- The pathway from feed materials or from the feed processing environment via the external environment (water and/or wildlife) to production animals or humans must also be considered. *Salmonella* from factories has been shown to have infected gulls, rodents and insects. Additionally, drinking water contaminated with *Salmonella* by a gull has caused an outbreak among

humans in Norway (Refsum, 2003). The *Salmonella* variants carried by birds such as passerines, gulls and pigeons are often specific variants of *S.* Typhimurium not often found in feed materials. However, these birds might contaminate feed materials during processing, or feedingstuffs, with *Salmonella*.

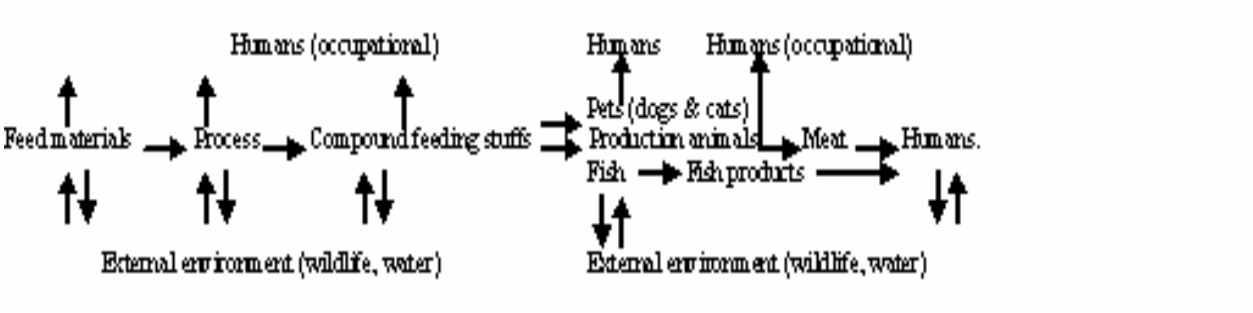

Figure 1. Pathways presenting risks for transfer of, and infection with, *Salmonella* in fish, terrestrial production animals, pets and humans.

2. Will the risk to human or animal health posed by *Salmonella*-contaminated feedingstuffs be influenced by its source, i.e. whether it is of vegetable or animal origin?

According to the available information reviewed, the risk posed by *Salmonella* contamination of feedingstuffs will not be influenced by the source of origin (whether vegetable or animal). There are differences in the prevalence and distribution of serovars among different raw materials, but the human and animal health significance of these differences is unknown.

Furthermore, there may be differences in the ability of different raw materials (vegetable or animal) to sustain the growth of *Salmonella*, but information on such differences is unavailable.

B. Assess the possible use of *Salmonella* infected feed material in a production process with a heat treatment (extruding/pelleting with a core temperature of at least 81°C) in relation to the survival of the *Salmonella*.

1. Would the survival of *Salmonella* depend on whether the feed material is of vegetable or animal origin?

According to the available information reviewed, the risk posed by *Salmonella* contamination of feedingstuffs will not be directly influenced by the source of origin (whether vegetable or animal). The survival will be directly linked to the properties of the feed material itself, such as fat content, water activity, pH, and possibly protein and carbohydrate composition. To the extent that there are fundamental physical and biochemical differences between feed materials of vegetable and animal origin, these may play a role in survival of *Salmonella*.

2. Would the survival of *Salmonella* depend on the composition of the feed material?

The survival of *Salmonella* in contaminated compound feedingstuff is significantly influenced by its composition. Several factors are of relevance, most importantly water activity and fat content. Lowered water content and higher fat content protects bacteria during production and storage, resulting in prolonged survival.

3. Would the survival of *Salmonella* depend on the production process (extruding/pelleting)?

The production process will significantly affect the survival of *Salmonella*. During extruding and pelleting the number of bacteria will be reduced, but the efficacy of heat treatment is dependent on several factors, of which the initial number, the fat content and the water activity are most important. In addition, the evenness of water and fat in the raw materials is crucial. The concentration of *Salmonella* initially present in contaminated products will affect the survival of bacteria due to the kinetics of bacterial death during processing. Equipment involved in heat treatments may also possess dead-ends, where the temperature during processing may be inadequate to kill *Salmonella*.

4. Would the survival of *Salmonella* bacteria depend on the arrangement and construction of the processing plant or the design of the production equipment?

The survival and contamination potential of *Salmonella* will depend significantly on the arrangement and construction of the production facility, as well as on the design of the production equipment. Insufficiently high temperatures for *Salmonella* inactivation may occur for the first batches during initiation of production. If inadequate insulation or ventilation is found in hot sections of the production, water condensation may be an additional problem as it provides possibilities for bacterial growth.
Factors of special relevance in this context are the possible lack of physical divisions between clean and unclean areas in the facility, unsatisfactory sanitation with respect to rodents, birds and insects in the production area, and the lack of adequate cleaning and disinfection of premises. One factor often encountered is the poor hygienic design of equipment or parts of equipment, making proper cleaning and disinfection difficult.

C. Assess the risk posed by antimicrobial-resistant *Salmonella* strains imported with contaminated feed materials.

The data from the Norwegian monitoring programme for antimicrobial resistance in bacteria from feed, food and animals (NORM-VET), although limited, indicate that antimicrobial resistance is not widespread among the *Salmonella* isolates that are sometimes isolated from feed or animals in Norway. The presence of antimicrobial resistant and multi-drug resistant *Salmonella* isolates in imported feedingstuffs and feed should not be discounted. S*almonella*-contaminated feedingstuffs and feed containing such isolates can be disseminated in farms / households.

The occurrence of antimicrobial resistant *Salmonella* currently appears to be low, however, the situation may change, depending on from which countries the feed is imported.

D. Assess the risk to animal and human health posed by contaminated feed materials intended for pets.
The risk to human health posed by the handling and feeding of compound pet feed is negligible, but the risk associated with pet treats is significant, but remains unquantified in Norway. The risk is associated with direct contact with the pet treat and the indirect spread of *Salmonella* from pet treats to humans through the pets themselves. There is a lack of information regarding the occurrence of *Salmonella* infections in pets in relation to feed. Factors contributing to the lack of the information include the ability of the dogs and cats to shed *Salmonella* without exhibiting clinical signs of illness.

11. Major data gaps

The following data have been identified as lacking during the course of the preparation of this document:

- Data on concentration of different *Salmonella* serovars in contaminated feedingstuffs intended for food-producing animals
- Characterization and quantification of the impact of the feed matrix effects, host-pathogen interactions and virulence factors and their effect on the probability of infection and/or illness
- Knowledge on the expression of virulence factors in humans by the predominant *Salmonella* serovars in feedingstuffs
- Epidemiological data concerning cross-contamination between feedingstuffs factories and other sectors of the environment
- Data on the prevalence and concentrations of different *Salmonella* serovars in contaminated feed material intended for pet animals
- Prevalence of *Salmonella* infections among workers at feed processing factories
- Data on possible differences in growth rates of *Salmonella* serovars on substrates of vegetable or animal origin.

12. Conclusions

- With the low prevalences and concentrations of *Salmonella* observed in fish feed under the current conditions for farmed Atlantic salmon in Norway, the risk of *Salmonella* in feed being passed on to the consumer via the fish product is considered negligible. Epidemiological data from the Norwegian surveillance systems support this opinion.
- Fish feed contaminated with *Salmonella* does not constitute a hazard to fish health under current Norwegian conditions.
- The occurrence of *Salmonella* in feed for production animals other than fish is a well-recognized hazard worldwide. It is a source for salmonellosis in animals and a potential source for further transmission to humans via the food chain.
- *Salmonella* have regularly been detected in pet treats. Considering the close contact between pets and humans, transfer of *Salmonella* from pet treats

directly to humans, or indirectly through the pets, should not be overlooked. Infected pet animals might also be source of infection to production animals.

- There is limited information on the risk of *Salmonella* cross-contamination from the feed, the ingredients and the factories to other parts of the environment, including wildlife. However, the risk is not negligible.
- There is only limited information on the health significance of possible exposure of factory workers to *Salmonella*-contaminated feed or its ingredients.
- Some Norwegian wildlife are occasional carriers of *S.* Typhimurium. As this serovar is relatively infrequent in feedingstuffs, there seems to be no widespread transfer between these compartments.
- Import of raw materials for the production of feed may constitute a possible "port of entry" for new *Salmonella* serovars into Norway. Some of these strains may present resistance profiles that are currently rare in Norway.
- The occurrence of antimicrobial resistance among *Salmonella* isolated from feedingstuffs in Norway is currently low. Thus, feed does not seem to present an important source for the spread of antimicrobial resistant *Salmonella* in Norway.

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Appendix I.

Number of positive *Salmonella* isolates of different serovars and from different sources during the five year period 2000-2004 (Source: Zoonosis reports 2000-2004 and MSIS).

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