



Assessment of the human-health impact of Salmonella in animal feed

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Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Hald, T., Wingstrand, A., Pires, S. M., Vieira, A., Coutinho Calado Domingues, A. R., Lundsby, K. L., Dalhoff Andersen, V., & Thrane, C. (2012). *Assessment of the human-health impact of Salmonella in animal feed*. Danmarks Tekniske Universitet (DTU).

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Assessment of the human-health impact of *Salmonella* in animal feed

By

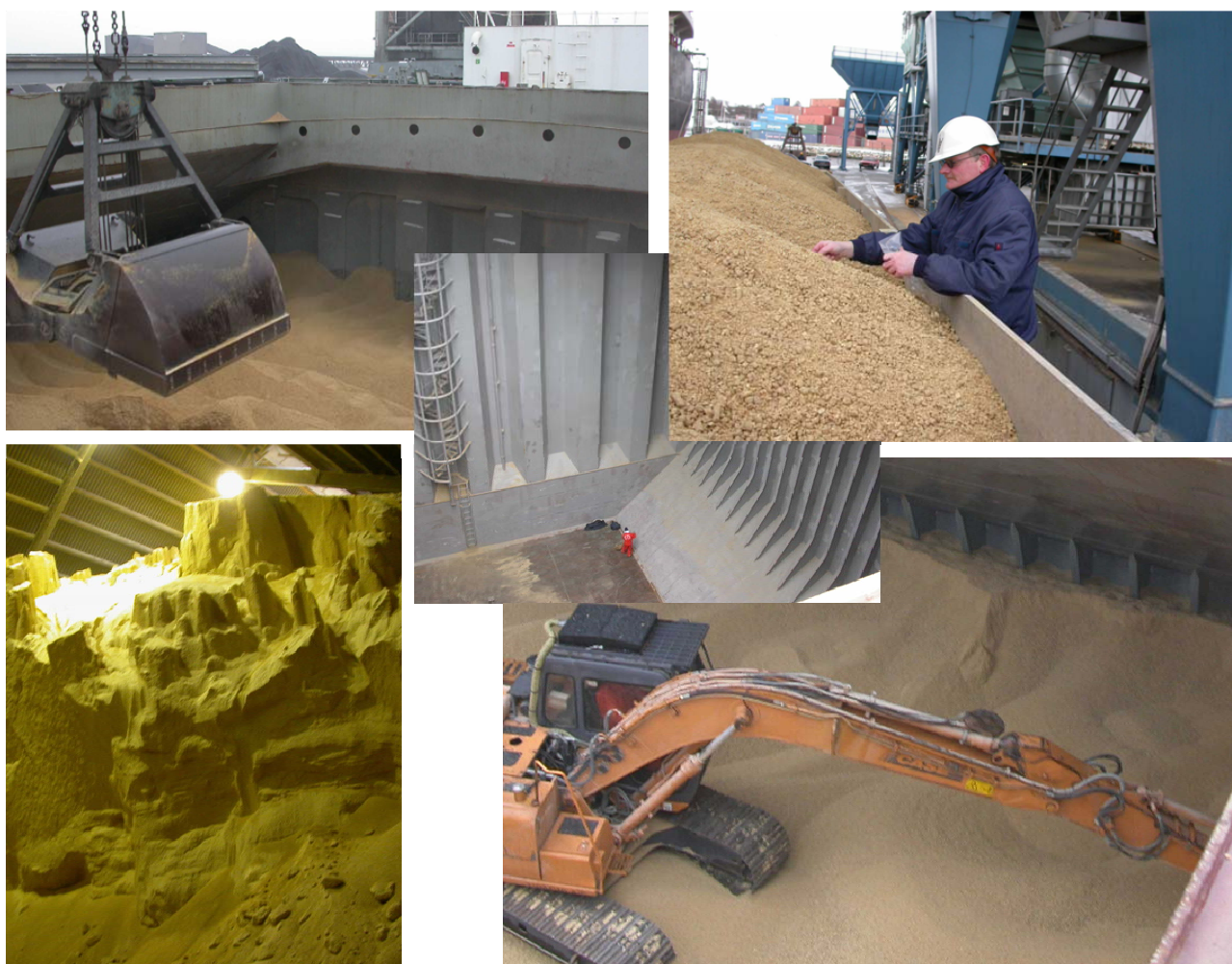
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April 2012



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Background

Several studies have proved that animal feed is frequently contaminated with foodborne bacterial pathogens. This accounts for single feedstuffs but also for heat-treated final feed. Several studies conclude that most samples positive for *Salmonella* originate from the raw materials section of feed mills (Jones et al. 2004). Most final feed from feed mills is heat-treated and theoretically the occurrence of *Salmonella* in final feed should be non-existing. This of course is only the case if the heat-treatment is sufficiently effective and if no recontamination occurs. A Danish study conducted by Israelsen et al. (1996) revealed different critical points in the pelleting process in Danish feed mills and demonstrated that heat-treated feed is easily recontaminated at different points in the production line. This study is supported by a recent study by T. B. Pedersen et al. (unpublished data 2007) where the effect of the heat-treatment of poultry feed on bacterial reduction was studied. The findings in this study indicate the possibility of low-level *Salmonella* contamination in final (heat-treated) feed. It has been demonstrated that as few as one *Salmonella* organism in feed consumed by young chicks can lead to infection (Milner et al. 1952), therefore, though feedstuffs might not in general be heavily contaminated, feed as the source of infection in animals should not be overlooked.

Several incidents have been reported in which human illness was traced back to contaminated animal feed. A semi-quantitative risk assessment of human health impact of *Salmonella* contamination of soybean feed products has been conducted by Hald et al. (2006). In this study it was estimated that up to 1.7% of the total number of registered human cases and up to 2.1% of the domestically acquired infections in the period 1999-2003 could be attributed to feed-borne serovars acquired through the consumption of Danish pork and beef. This study indicates that there is a link between contamination of animal feed and human salmonellosis. The total contribution of contaminated animal feed to human salmonellosis in Denmark is unknown. There is a need to collect all relevant data in order to assess the magnitude of the problem and to assess the methods currently available to prevent and control the problem. Therefore we performed a systematic review with the overall aim to assess the human-health impact of *Salmonella* in animal feed including an evaluation of existing strategies for prevention, control and reduction of *Salmonella* in animal feed.

In the review, we focused on assessing the animal feed as the primary source of introduction of *Salmonella* into the “farm to fork” chain. *Salmonella* introduced into the production systems through other transmissions routes, such as by rodents or birds and poor hygiene management were not considered. The study questions were defined, discussed and agreed upon by a project advisory group. The systematic review was conducted using a programme called SRS 4.0, which is a web-based programme using TrialStat’s electronic systematic review (ESR) methodology. Another aim of the study was also to evaluate the systematic review process as a tool to address a broad and complex issue as the one at hand. The more detailed objectives and methodologies of the study are described in the following section.

1. The systematic review process

A systematic review (SR) consists in a formal process of literature review that can be used to identify, assess and summarize the results of otherwise unmanageable quantities of research, providing a reliable basis for the decision makers (Sargeant et al. 2005).

Systematic reviews follow a clearly defined research protocol to reduce sources of bias and chance-effects at all stages of the review (Sargeant et al. 2006, Higgins et al. 2006).

The steps involved in a systematic review process are (Sargeant et al. 2005, Higgins et al. 2006):

1. Assemble review team
2. Question formulation
3. Literature search
4. Relevance screening of title (in case of an excessive amounts of references)
5. Relevance screening of abstract
6. Quality assessment
7. Data extraction and synthesis
8. Reporting

By following a systematic and transparent approach and assessing the methodological quality of the studies, it is possible to focus the review in the studies of higher quality and, by summarizing the results from multiple studies, increase the power on the conclusions (Sargeant et al. 2005).

An important decision on a systematic review process is to assemble the reviewers. For this project a group of four people with documented expertise in *Salmonella* and/or animal feed, as well as methodological expertise for critical review of epidemiological methods was assembled.

To perform the systematic review, a web-based SR management software called SRS 4.0 from TrialStat (TrialStat Corporation. 2006) was used. Besides managing the references and its access for the reviewers, it allows for the creation of answering forms, handles differences between reviewers, and provides an easy and complete data extraction.

1.1. Study questions

The identification of the study questions took into account the following considerations:

- The questions should be relevant to decision-makers and clearly defined *a priori*;
- The questions should be structured in terms of population(s) (e.g. feed type and animal species), intervention(s), and outcome(s);
- The questions should be sufficiently broad to allow examination of variation in the study factor and across relevant populations.

The reviewers, in collaboration with the advisory board¹ for the project, developed three study questions associated with the main objectives of the study.

Table 1 displays for the main objectives of this study, the correspondent developed study question and also the expected type of study designs able to answer the study questions.

Table 1: Description of the objective, corresponding study questions, and possible study designs.

Objective	Study question	Study designs
1: Assessment of the association between <i>Salmonella</i> in animal feed and <i>Salmonella</i> infection of Danish broilers, table-egg layers, cattle, farmed fish, slaughter pigs and humans.	1: On a qualitative or semi-quantitative scale, what is the association between <i>Salmonella</i> in animal feed and <i>Salmonella</i> infections in broilers, table-egg layers, cattle, farmed fish, slaughter pigs and humans?	<ul style="list-style-type: none"> • Outbreak studies • Risk factor studies • Experimental studies
2: Identification of factors, associated with animal feed (pH, structure etc.), that determine whether exposure to <i>Salmonella</i> lead to infection in broilers, table-egg layers, cattle, farmed fish and slaughter pigs.	2: Which factors, associated with animal feed, determine whether exposure to <i>Salmonella</i> leads to infection in production animals (i.e. pH, structure etc)?	<ul style="list-style-type: none"> • Intervention studies • Risk factor studies • Experimental studies
3: Assessment of available preventive measures, control methods and methods to reduce <i>Salmonella</i> in animal feed.	3: Which interventions can be used to prevent, control and reduce the presence of <i>Salmonella</i> in animal feed?	<ul style="list-style-type: none"> • Intervention studies • Risk factor studies • Experimental studies

1.2. Search of literature

The purpose of the literature search was to identify all primary research that could potentially address the study questions.

Search terms were constructed using the key components of the review questions like feed type(s), populations, interventions, outcomes.

The main database search was performed first and foremost in English. But to the extent needed, other languages such as Danish, German, Norwegian, Swedish, Portuguese and Spanish were included, especially when searching scientific publications or when searching for country specific surveillance data. Furthermore, no publication year limit was imposed in the search.

The following electronic databases² were used in the search for literature: *Food Science and Technology Abstracts (FSTA)*, *BioSIS*, *CAB international*, *Science Direct*, *PubMed*, *ISI Web of*

¹ 1 person from the Pig Research Centre, 1 person from the Danish Poultry Council, 1 person from DAKOFO, 2 persons from the Danish Plant Directorate, 1 person from the Danish Dairy Board and 1 person from the Danish Food and Veterinary Administration.

² For details about the search terms used, please consult Appendix 1.

Knowledge and *AGRICOLA* database. In addition to these databases, the websites from the *Dansk Svineproduktion* and from the *Faculty of Agricultural Sciences, University of Aarhus* were also used to search relevant reports and studies.

A search on conference proceedings was performed for the following conferences: *International Society for Veterinary Epidemiology and Economics (ISVEE)*, *International Pig Veterinary Society (IPVS) Congress Proceedings*, *I3S International Salmonella and Salmonellosis*, *International Symposium on the Epidemiology and Control of Salmonella in Pork (Salinpork/Safepork)* and the *International Symposium on Ecology of Salmonella in Pork Production*.

Also, the *Current Research Information System (CRIS)* was used to search for literature not published in international peer-reviewed journals.

Finally, references cited by studies identified as relevant were checked for relevance. If a reference title appeared relevant, the reference database was checked to determine if this reference had been captured by the search already. If not, the abstract was included in the review.

The literature search was performed in the last trimester of 2007. Following completion of the literature search, the relevant references were uploaded to the SRS system and any duplicates were removed, yielding a total amount of 4,199 references.

1.3. Title and abstract relevance screening³

Title screening

An initial title screening of the 4,199 references was conducted before the relevance screening. In this step, the reviewers had to quickly assess the relevance of a reference by reading only its title and assessing if the study focused on human salmonellosis, *Salmonella* and production animals/animal products, or *Salmonella* in animal feed.

Each title was reviewed by two reviewers and a conservative approach was used, meaning that only the references where the two reviewers concurred on the non-relevance of the title were excluded.

In the title screening, 2,066 references were excluded and 2,133 references passed to the abstract screening (Figure 1).

Abstract screening

In the relevance screening, the reviewers assessed the relevance of the studies by reviewing the abstracts. Studies that passed this screening were taken to the quality assessment.

The relevance of the studies was assessed on the basis of three specific criteria: (1) reference focusing on *Salmonella* or other *enterobacteriaceae* in animal feed; (2) reference describing factors associated with feed or feed additives that determines whether exposure to *Salmonella* leads to infection in production animals; and (3) reference describing interventions that can be used to prevent, control or reduce the **presence** of *Salmonella* in animal feed.

³ To see the complete question forms used by the reviewers, please consult Appendix 2.

Due to the large number of references in this step, each reference was reviewed only by one reviewer. However, a conservative approach was used, meaning that if one of either criteria (1) or (3) above was indicated in the abstract, the reference passed to the next step. 1,807 references were excluded and 326 passed to the quality assessment step (Figure 1).

1.4. Quality assessment⁴

In the quality assessment step, the methodological soundness of the studies was evaluated by reading the full articles through a check-list of quality criteria.

The quality assessment criteria referred to the study characteristics, study setting, study population, outcome measurements, statistical analysis and presentation of the results. Some quality criteria were specific to certain study designs (for example randomization is not an issue with observational studies or risk assessment studies involving models).

Out 326 references assessed, 32 studies did not comply with the quality assessment criteria and were discarded (Figure 1). The 295 references⁵ passing the quality assessment step described different study designs: 20 controlled-trial studies (5 randomized, 4 non-randomized and 11 with no indication of randomization), 35 cross-sectional studies, 2 case-control studies, 8 cohort studies, 4 observational case-based studies, 50 prevalence studies i.e. studies primarily based on surveillance and monitoring data, 2 outbreak studies, 6 risk assessment studies and 16 review studies.

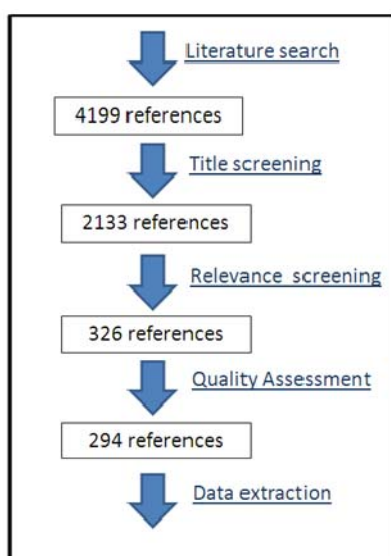


Figure 1: Steps in the systematic review and number of references passing through each step.

1.5. Data extraction

After filling in the quality assessment form and assuring that the quality criteria were acceptable, the reviewers were asked to fill in an Excel spreadsheet that was previously prepared and which covered the main chapters of this report: hazard identification and characterization, *Salmonella* contamination from feed to feeding (exposure assessment), public health relevance of *Salmonella* in feedstuffs, strategies to control *Salmonella* in the feed-chain, monitoring and control of *Salmonella* in feed, and background information on feed production and consumption in Denmark.

⁴ To see the complete question forms used by the reviewers, please consult Appendix 2.

⁵ To see the complete list of references to pass the quality assessment, please consult Appendix 3.

1.6. Synthesizing the information

The information extracted from the systematic review is synthesized in this report. Additional relevant literature was used as appropriate.

2. Hazard identification and characterization

Salmonella is one of the most important foodborne zoonotic pathogens, with significant health and economic impact in both humans and animals (Voetsch et al. 2004). Nontyphoidal *Salmonella* is a worldwide leading cause of foodborne illness and it is estimated that every year about 93.8 million cases of gastroenteritis occur globally due to *Salmonella*, with 155,000 deaths. An estimated 80.3 million of these cases are foodborne (Majowicz et al. 2010). *Salmonella* is often a contaminant of feeding stuffs at the farm level or at the feed mills and may consequently lead to infections in food-producing animals and human.

2.1. *Salmonella* in humans in Denmark and EU

Foods of animal origin are the most important sources of foodborne salmonellosis in humans.

According to Danish *Salmonella* source attribution estimates, the main sources of *Salmonella* infections in Denmark in 2009 were table eggs (12.3%), pork (7.6%), imported beef (3.1%), imported pork (2.0%) and imported turkey (2.0%), with the remaining sources contributing to a minor proportion of human cases (Anonymous. 2010).

According to surveillance data, a total of 131,468 confirmed cases of human salmonellosis were reported by 27 European countries to the European Food Safety Authority (EFSA) in 2008. Overall, the notification rate of reported confirmed cases of human salmonellosis in EU showed a decrease between 2004 and 2008. During these years, 10 European countries showed significant decreasing trends, whereas 7 countries showed significant increase. In Denmark, the incidence of human salmonellosis was 38.5 cases per 100,000 inhabitants in 2009. In 2008, this incidence was 66.8 cases per 100,000 inhabitants. The higher incidence in 2008 was mainly due to a large *Salmonella* outbreak (Ethelberg et al. 2008), and about 43% of all *Salmonella* cases were outbreak related in that year (Anonymous 2010).

When calculating *Salmonella* incidences, there is loss of data at several points along the surveillance chain from patient until official statistics. It is accepted that the reported number of cases do not represent the true burden of salmonellosis in the countries, and some studies have tried to assess the real burden of salmonellosis based on the reported incidences and on estimated multipliers (Wheeler et al. 1999, de Jong et al. 2006, van Kreijl et al. 2006, Simonsen et al. 2008).

A Swedish study investigated underreporting of human salmonellosis in EU countries using travel registers. The study showed higher risk of disease among Swedish residents travellers returning from East Africa (471/100,000 travellers; 95% Confidence Interval (CI) 294-755), or the Indian subcontinent (474/100,000; 95% CI 330-681). In absolute numbers, most cases occurred among

travellers to Southern Europe and Eastern Mediterranean (Ekdahl et al. 2005). Using returning tourists as sentinel population and Norway as reference country, salmonellosis incidence in EU countries were estimated and an “under-reporting factor” was calculated for each country, in comparison to Norway (de Jong et al. 2006). According to this study, the highest burden of salmonellosis was estimated for Bulgaria with 2,741/100,000, followed by Turkey with 2,344/100,000 and Malta with 2,141/100,000.

2.2. *Salmonella* in animals in Denmark and EU

Food animals serve as important reservoirs of *Salmonella* (von Altrock et al. 2000, Davies et al. 1997b, Boqvist et al. 2003). *Salmonella* infections in pigs and poultry are widespread in EU countries, but are often asymptomatic. Ruminants, although less frequently infected, appear to show clinical signs more often (EFSA 2008a). There are several sources of data on *Salmonella* occurrence in food animals and this data vary largely by country, animal species, and efficiency of the different surveillance programs implemented. National surveillance programs for *Salmonella* in food animals are in place in many EU countries.

In Denmark, *Salmonella* surveillance programs for poultry, pigs and cattle are in place and data are annually reported and analyzed (Anonymous 2010). In the EU, a total of 26 countries reported *Salmonella* prevalences in food animals in 2008. This data originated from national surveillance programs on *Salmonella* surveillance in food animals and are published in the EU Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents and Foodborne Outbreaks in the European Union (EFSA. 2010a). Mandatory *Salmonella* surveillance programs in breeding flocks, laying hens, broilers and turkeys are currently in place for all European countries. According to data provided by these programs, 2.8% of the broiler flocks in the EU were positive to *Salmonella* (1.2% in Denmark), while 5.9% of the laying hen flocks (0.6% in Denmark) and 1.8% (0.6% in Denmark) of the breeding flocks were positive. Similarly, 6.5% of the turkey flocks were positive to *Salmonella* in the EU (1.4% in Denmark). Reported data on *Salmonella* prevalences in pigs and cattle are still not sufficient to allow for estimation of prevalences at the EU level.

During the past years EFSA has performed several EU-wide baseline studies assessing the *Salmonella* prevalence in different food animals. These studies are harmonized with regard to sampling design and testing methodologies and the differences between the prevalences found in EU countries could be observed and compared. Analysis of the data provided by these studies showed that the prevalence of *Salmonella* in EU holdings with more than 1,000 laying hens was 30.7% (EFSA 2007b). Similarly, another baseline study showed that the prevalence of *Salmonella* in commercial flocks of broilers with at least 5,000 birds was 23.7%, with a large variation between countries (between 0% and 68.2%) (EFSA 2007a). The overall EU prevalence of *Salmonella* positive holdings with breeding pigs was 31.8% (EFSA 2009). Estimated prevalence of *Salmonella* positive slaughter pigs was 10.3% (EFSA 2008d). Among fattening turkey flocks, approximately one third of the flocks raised over the one year period of the baseline survey were *Salmonella* positive (EFSA 2008e). In all these baseline studies, prevalences varied widely among EU Member States.

2.3. *Salmonella* serovars of Public Health significance

More than 2,500 *Salmonella* serovars have been identified, with prevalence distributions varying between different parts of the world (Popoff 2001). Most have been described as potential causes of human infections. *S. Typhimurium* and *S. Enteritidis* have been reported to be the most common causes of human salmonellosis, but the relative public health importance of other *Salmonella* serovars may be higher in some regions (WHO-GFN). Knowledge of the occurrence and distribution of different serovars in different geographic regions and sources assists in the understanding of *Salmonella* epidemiology (Bangtrakulnonth et al. 2004).

2.3.1. *Salmonella* serovars in humans

The incidence of human salmonellosis fluctuates over time and varies between European countries. Such changes are often accompanied by changes in the incidence of infections caused by different *Salmonella* serovars and phage types. This suggests that the major sources of human salmonellosis change over time, and/or that the predominant *Salmonella* subtypes in the specific animal sources vary over the years (EFSA 2010a). *S. Typhimurium* and *S. Enteritidis* are the most frequent *Salmonella* serovars in humans worldwide, ranking first or second in most countries reporting data to the WHO Global Infection Network *Salmonella* Databank (WHO-GFN). In the EU, *S. Enteritidis* and *S. Typhimurium* are the most commonly reported serovars associated to human illness, accounting for 58% and 21.9% of all reported serovars in human confirmed cases in 2008. Other frequently isolated serovars in humans in the EU include *S. Infantis*, *S. Virchow*, *S. Newport*, *S. Agona*, *S. Derby*, *S. Stanley*, *S. Bovismorbificans* and *S. Kentucky*. However, the prevalence of these serovars is much lower (below 1%) than the prevalence of *S. Enteritidis* and *S. Typhimurium* (EFSA 2010a). Other studies investigating the distribution of *Salmonella* serovars in European countries have been conducted and achieved different estimates, depending on the country and detection method applied.

In Denmark, *S. Typhimurium* and *S. Enteritidis* are also the most commonly isolated serovars in humans, accounting for nearly 65% of the isolates in 2009. Other common serovars include *S. Dublin*, *S. Newport*, *S. Virchow* and *S. Agona*, each of these showing prevalences below 2.2%.

2.3.2. *Salmonella* serovars in food-producing animals

A review of the *Salmonella* serovar distribution reported by European countries to EFSA shows that the dominant serovars varied between animal reservoirs, but it was clear that *S. Enteritidis*, *S. Typhimurium* and *S. Infantis* were the most frequently observed and widely distributed serovars among food-producing animals. Other serovars appeared as important for specific animal sources, such as *S. Derby* in pigs and cattle, *S. Dublin* in cattle, *S. Hadar* in broilers and *S. Saintpaul*, *S. Kottbus* and *S. Bredeney* in turkeys (EFSA 2010a).

Occurrence of species-adaptation and association with animal clinical disease is observed in some serovars, with relevant examples being *S. Gallinarum* and *S. Pullorum* (causing fowl typhoid and pullorum disease respectively in poultry), *S. Choleraesuis* (causing enteritis and septicaemia in pigs), *S. Abortusovis* (causing abortion in sheep) and *S. Dublin*, associated with abortion, enteritis and septicaemia in cattle (EFSA 2008c).

According to the baseline studies performed by EFSA, the observed flock prevalence of *S. Enteritidis* and *S. Typhimurium* in broiler flocks varied greatly, from 0% to 39.3%. The five most frequently isolated *Salmonella* serovars from broiler flocks in the EU were, in decreasing order, *S. Enteritidis*, *S. Infantis*, *S. Mbandaka*, *S. Typhimurium* and *S. Hadar* (EFSA 2008a). *Salmonella* Derby and *Salmonella* Typhimurium predominated in both pig breeding holdings and pig production holdings (EFSA 2009). Among slaughter pigs, *S. Typhimurium* and *S. Derby* were widespread and dominant in the EU countries, with *S. Enteritidis* being relatively prevalent in some eastern EU Member States (EFSA 2008d). Similar *Salmonella* serovars distributions are observed in Denmark, with *S. Typhimurium* being associated with pig herds, *S. Enteritidis* to layer flocks, and *S. Typhimurium* and *S. Infantis* in broiler flocks (Anonymous 2010).

2.3.3. Ability of different *Salmonella* serovars to spread and cause disease in humans and animals

Differences in the distribution of serovars from human salmonellosis may be a consequence of differences in serovar distribution and prevalence of *Salmonella* in food animals, differences in animal production, food processing, food preparation and hygiene and or different food consumption patterns (EFSA 2008a). Additionally, even though all serovars of *Salmonella* are potentially pathogenic for humans, the degree of host adaptation varies, which may affect the pathogenicity. Non-typhoid and ubiquitous serovars, such as *S. Typhimurium* and *S. Infantis*, affect both humans and a wide range of animals, and are those with principal zoonotic significance (Mølbak et al. 2006b) and the ability of these to infect animals and eventually infect humans via food seems to vary (Hald et al. 2006, Pires et al. 2010). Although these serovars are in principle non-host-adapted, strong associations between certain serovars or phage types within a serovar and a given animal reservoir may occur e.g. *S. Enteritidis* in laying hens (Hald et al. 2004).

In humans, while *S. Enteritidis* are often associated with the consumption of contaminated eggs and poultry meat, *S. Typhimurium* are more commonly associated with the consumption of pig, poultry and bovine meat (EFSA 2010a). Eggs from laying hens are considered the most important source of *S. Enteritidis* infections and consequently the most important source of human salmonellosis in EU, but a certain proportion of human *S. Enteritidis* infections are also assessed to be attributable to broilers. *S. Enteritidis*, *S. Infantis*, *S. Mbandaka*, *S. Typhimurium* and *S. Hadar* were other frequent isolated serovars in broilers and all these serovars, with the exception of, *S. Mbandaka* are frequent causes of *Salmonella* infections in humans. *S. Infantis* are widely distributed among different animal reservoirs (EFSA 2010a). A spatial cluster analysis on the data obtained from the baseline studies revealed a cluster of *S. Infantis* in pigs in Northern Europe and in broilers in Eastern Europe, indicating that pork and broiler meat may be important sources of these infections in humans. However, *S. Infantis* was also commonly observed in laying hens and turkeys, so a proportion of the infections from these sources cannot be ruled out. Similar analyses on the data obtained from the baseline studies showed pigs are a main source of *S. Typhimurium* infections in humans in Western Europe, whereas in Eastern Europe, the disease burden may be more evenly shared between broilers and pigs. Other important serovars in humans included *S. Hadar*, *S. Virchow* and *S. Derby*. Whereas the two first were primarily found to be associated with the poultry reservoir and particular broilers, *S. Derby* was particularly observed in pigs, but also in turkeys (EFSA 2007a, EFSA 2009, EFSA 2008d, EFSA 2008e).

An increase in the occurrence of antimicrobial resistance among *Salmonella* isolated from animals and humans has been observed in several countries. It is estimated that between 20% and 40% of all *Salmonella* infections are caused by antimicrobial resistant strains (McDermott 2006). Infections caused by antimicrobial resistant *Salmonella*, particularly quinolone-resistant *Salmonella*, are associated with higher fatality rate, occurrence of hospitalization and hospitalization for longer time periods than patients with infections caused by susceptible strains (Mølbak 2006a, Helms et al. 2002).

In Denmark in 2009, 53.6% of the *Salmonella* Typhimurium attributed to domestic food products were caused by types susceptible to all antimicrobials, whereas 36.3% were caused by types resistant to up to three antimicrobials and 3.7% caused by types resistant to four or more antimicrobials. About 48% of the *S. Typhimurium* infections attributed to imported products were caused by resistant types, with 29% of these cases being caused by types resistant to four or more antimicrobials (Anonymous 2010).

2.4. Prevalence of *Salmonella* in feed materials

Animal feed ingredients of both animal and plant origin may be contaminated with *Salmonella*, and large differences regarding the estimated *Salmonella* prevalences were reported by different studies assessing contamination of feed ingredients (EFSA 2008c)

Animal protein and by-product ingredients originating from animals have always been considered a major source of *Salmonella* in feeds and several studies have provided varied data on the prevalence of *Salmonella* in these feed ingredients. The 1993 US Food and Drug Administration (FDA) survey of feed ingredients detected levels as high as 56.4% of the animal protein samples being positive for *Salmonella* (McChesney et al. 1995). In the EU, monitoring has shown contamination of that animal meat and bone meal can be contaminated with *Salmonella*. These data derived from different national surveillance programs in Europe and the levels of *Salmonella* contamination in meat and bone meal varied between 1% and 2.3% between 2006 and 2008. Fish meal presented even higher contamination between these years in the EU (1.9% to 2.9%) (EFSA 2010a). However, these types of products has since 2001 been banned as a source of protein for production animals in the European Union, due to concerns related to the spread of Bovine spongiform encephalopathy (BSE).

Salmonella incidence from feed ingredients of plant origins including the major cereal grains used in feed production have become recognized as sources of *Salmonella* contamination. Early studies were already able to isolate a wide variety of serovars from several seeds and cereal grains including peanut meal, sunflower meal, bran meal, barley, corn sorghum, SBM, and wheat (MacKenzie et al. 1976). In the Netherlands, 5.1% of various vegetable protein ingredients tested between 1999 and 2000 were positive for *Salmonella* (Anonymous 2002b). An investigation of the prevalence of *Salmonella* contamination of soya meals and cereals (mostly oats, corn, wheat, rice), using a cross-sectional survey, could not detect *Salmonella* in any of the samples of soya meals (0/52) and cereals (0/80) (Sauli et al. 2005). In another study, assessing contamination based on results from the *Salmonella* surveillance of feed ingredients before introduction to feed mill, *Salmonella* was isolated from 14.6% of soybean meal consignments and in 10% of rapeseed meal

samples (Wierup et al. 2010). In Denmark, out of 1,061 samples of feed materials from feed business operators own sampling of *Salmonella*, predominantly soybean meal and rapeseed cake, 85 were positive. Routine surveillance of *Salmonella* in feed material detected only 4 *Salmonella* positive samples out of 186 samples analyzed in 2009 (Anonymous 2010). Grains are rarely contaminated with *Salmonella* and forage feed is generally not associated with a risk of contamination with *Salmonella* (EFSA 2008c). In the EU, *Salmonella* contamination in cereals was estimated to be between 0.2% and 0.4%, a much lower level than those estimated for oil seeds and oil products (between 1.8% and 2.5%) (EFSA 2010a).

2.5. Prevalence of *Salmonella* in compound feed

Contamination of compound feed by *Salmonella* has been well documented by a series of studies and reports. Surveillance performed in compound feeding stuffs in the EU in 2008 showed that the proportion of *Salmonella* positive findings ranged from 0% to 3.6% in cattle and pig feed, and up to 8.3% in poultry feed. The average prevalences in EU were estimated as 0.5% for cattle feed, 0.6% for pig feed and 0.9% for poultry feed. These levels were fairly stable between 2006 and 2008, with small differences between yearly estimates. In Denmark, none of the 1,339 analyzed samples of compound feed were positive to *Salmonella* in 2009 (Anonymous 2010, EFSA 2010a).

Pelleted and mash poultry feeds have long been recognized as vectors for *Salmonella* contamination of commercial poultry production systems. A study conducted in the southern United States found that 8.8% of mash feed samples and 4.2% of pelleted feed samples were contaminated with *Salmonella* (Threlfall et al. 2003). Contaminated feed is also an important source of *Salmonella* exposure for pigs and for cattle. *Salmonella* organisms were isolated from 36 of 1,264 (2.8%) feed samples in 30 swine farms in the US (Blaser et al. 1982). In contrast, a 2-year field survey sampling multiple ecological compartments within swine production systems did not detect *Salmonella* in 221 feed samples (Barber et al. 2002). In cattle feeds, 29 (9.8%) out of 295 feed samples examined, collected from six US farms were found to contain *Salmonella* (Krytenburg et al. 1998). Contamination rates varied widely in other studies assessing contamination in cattle feeds. Overall, much lower prevalences of *Salmonella* are detected for swine feeds than for cattle feeds in the US.

2.6. *Salmonella* serovars in feedstuffs

A wide variety of *Salmonella* serovars have been identified in feeding stuffs. Serovars commonly isolated from animal feed are *S. Typhimurium*, *S. Montevideo*, *S. Hadar* and *S. Tennessee*. Surveillance performed by European Member States shows that the occurrence of *S. Typhimurium* and *S. Enteritidis* in feeding stuffs was low and mainly other *Salmonella* serovars were detected. More specifically, *S. Enteritidis* accounted for 6.2% and *S. Typhimurium* accounted only for 4.1% of the *Salmonella* isolated from compound feed for poultry in the EU. *S. Senftenberg* (11.6%) was detected more frequently from poultry feed than these two serovars together. In compound feed for pigs, *S. Agona*, *S. Livingstone*, *S. Senftenberg* and *S. Anatum* were the most commonly detected serovars. Among compound feed for cattle, *S. Senftenberg*, *S. Agona*, *S. Surrey* and *S. Ohio* were the predominant serovars (EFSA 2010a).

In a five year period (2000 to 2004), analysis of surveillance revealed that common serovars in fish feed ingredients, fish feed and fish feed factories accounted for approximately 2% of clinical *Salmonella* isolates from domestically acquired cases in Norway. The predominant serovars found in fish meal were *S. Senftenberg* and *S. Montevideo*. The authors of this review concluded that *Salmonella* serovars in feed ingredients, compound feed or processing environments would pose a negligible public health threat (Lunestad et al. 2007).

A Dutch study identified 28 different serovars in poultry feed samples, where *S. Agona*, *S. Livingstone* and *S. Mbandaka* were the most frequent isolates. No *S. Enteritidis* was found, despite the occurrence of an epidemic in poultry in the Netherlands caused by this serovar at that time (Veldman et al. 1995). Other studies did isolate *S. Enteritidis* and *S. Typhimurium* from feeding stuffs and 20 strains of *S. Enteritidis* were recovered from Japanese commercial layer feeds (Shirota et al. 2001). *S. Worthington* was the most frequently isolated serovar in feeds sampled from multiple swine farms across several states in the United States and *S. California* was the predominant serovar in plant-based feed from Spanish feed mills (Harris et al. 1997, Alvarez et al. 2003). *S. Montevideo* was the most commonly isolated serovar recovered from meat meal in a Canadian study assessing *Salmonella* prevalences in feed mills. The same study showed *S. Agona* and *S. Schwarzengrund* as the most common serovars in feather meal (Hacking et al. 1978).

3. *Salmonella* contamination from feed to feeding

3.1. Primary production of feed

Contamination of feed with *Salmonella* can originate from different sources and a variety of routes. Various feeding stuffs, production facilities and systems, and conservation conditions may be used, offering different opportunities for contamination of feed with zoonotic pathogens. Main sources of contamination are fertilizers on the pasture/fields, ingredients, co-products, dust, wild animals (e.g. birds, rodents) and contaminated equipment.

Ingredients

A large variety of ingredients of vegetable or animal origin are used for production of feed for production animals. Ingredients of animal origin have been shown to have the highest frequencies of contamination, but *Salmonella* can also be isolated regularly from vegetal feed ingredients for farm animals. *Salmonella* spp. has been isolated from varied vegetable feed matrices, including grain and oilseed based products (Sauli et al. 2005, Harris et al. 1997, Williams 1981, Jones et al. 1982, Davies 1992, Köhler 1992). *Salmonella* spp. has been isolated from varied vegetable feed matrices, including grain and oilseed based products. Bacterial contamination of feed ingredients can occur before the ingredients arrive on a farm, either at a feed mill, from transportation vehicles, or during feed storage. Feed ingredients such as canola meal and whole cottonseed are often waste products of other processing operations. Thus, contamination of these feed ingredients may have occurred before or during the recycling process. In oil meal plants, the mechanism of the invasion and survival of *Salmonella* has been identified by Morita et al. (2003, 2004): *Salmonella* is brought into the plant with oilseed through the receiving area and is present in high concentrations with long-term survival in areas with high oil concentrations. The contamination of vegetables may take place either from contact with infected or carrier wildlife or production animals or from the use of manure or sludge as fertilizers. The Danish legislation set rules for the use of manure and sludge as fertilisers in order to prevent or reduce the risk of contamination of the crops, see chapter 6.

In response to the BSE episode, a total ban of the use of processed animal protein in feeds for any animal farmed for the production of food came into force January 1st 2001. Some exceptions have been opened at a later stage, such as the use of fish meal and certain blood products and dicalcium phosphate (by-products e.g. from the production of gelatin) as feed for non-ruminants (Reg. EC 1292/2005). When allowed as ingredients of animal feed, mammalian meat and bone meal (MBM) and poultry offal meal were found to be frequently contaminated by *Salmonella*, a consequence of the risk from the rendering of animals infected with *Salmonella*, some of which could be clinical cases (EFSA 2008c). The risk for the *Salmonella* contamination can also be due to in-house contamination in the rendering plants and recontamination following the heat treatment process. There is also potential risk for the spread of *Salmonella* by feeding animals by some dairy byproducts (in particular raw milk, non-pasteurized white water and whey from raw/unpasteurized milk cheese processing).

Fish meal also has the potential for the spread of *Salmonella*, although it seems to be less contaminated than other animal derived protein feed according to the EFSA zoonoses report from 2005. *Salmonella* contaminated fish meal was the source to the most well known example of feedborne transmission of *Salmonella*, when *S. Agona* emerged as a public health problem in several countries due to contaminated imported fish meal. In the United States a rapid increase of

human infections with *S. Agona* occurred from 1968 to 1972 (Clark et al. 1973), and *S. Agona* human cases occurred simultaneously in European countries.

Salmonella can survive for long periods in the dry products used for the production of feed, as well as in the dry finished feed. This has been shown in studies on non-acidified dry feeds (Stege et al. 1997), and on other dry products (Burnett et al. 2000, Jung et al. 1999) and highlights the importance of keeping storage and manufacturing conditions dry.

Environment

Various sources of environmental contamination can result in the presence of *Salmonella* in animal feed, including rodents and other wild animals, dust (Jones et al. 1994), contaminated equipment (Maciorowski et al. 2004) or leaking cooker seals.

Henzler et al. (1992) reported that the carrier rate in rodents was proportional to the level of contamination in the environment from which they were captured. Moreover, excrement of rodents is a factor that would further amplify environmental contamination. Also, animals that do not directly feed on grain stocks and spilled stocks, but on human garbage and faeces may also contribute to the ubiquity of *Salmonella* spp. in the environment. *Salmonella* has been isolated from wild birds such as crows and gulls, which may feed in refuse dumps containing contaminated food (Nielsen et al. 1975, Kapperud et al. 1983, Murray 1991). Predators such as foxes and domesticated cats may consume both contaminated insects and rodents and in turn become carriers themselves (Singer et al. 1992). Evidence also exists that *Salmonella* may survive in the intestinal tracts of insects (Devi et al. 1991, Khalil et al. 1994, Letellier et al. 1999). Additionally, soil may be a contamination route for *Salmonella*, since the pathogen may resist acidic soils for an extended length of time due to an acid tolerance response, and pH does not appear to affect the adhesion of *Salmonella* to soil particles (Foster 1995).

Salmonella has been detected for long periods in spillage and dust from milling equipment (Davies et al. 1997c).

Co-products

Animal waste, e.g. cattle manure or poultry litter, may be used as feed (Haapapuro et al. 1997). Potential high contaminations of waste with *Salmonella* may result in transmission of the pathogen to animals from feed. Many producers capitalize on the ability of cattle to utilize feedstuffs that may otherwise be wasted, such as straw, vegetables, cotton ginning lint, and poultry litter. Dried poultry waste is derived from undiluted poultry excreta usually collected from cage layer flocks, and dried poultry litter is a combination of excreta and litter (wood shavings or rice hulls) that is used to bed the floor of commercial poultry houses. These products are heat processed to generate a pathogen-free product. In case of processing failure, they may represent a source of *Salmonella* to production animals (Jeffrey et al. 1998).

3.2. Transport and storage

Contamination of feed ingredients during storage and transportation can occur through wild animals (rodents, birds) or pets (e.g. dogs), or be a consequence of cross-contamination from previous

batches of ingredients, e.g. due to insufficient disinfection or inadequate drying of storage rooms or vehicles after cleaning.

3.3. Feed mills

3.3.1. Handling and storage

Salmonella contamination of feed ingredients can occur before these arrive at the farm, either at a feed mill, from transportation vehicles, or during feed storage (Kidd et al. 2002) . Additionally, *Salmonella* present in feed ingredients may multiply during storage. Data obtained from the questionnaires compiled by Sauli et al. (2005) suggested that storage time of soya meals and cereals may range from 1 week to 3 months. It has been observed that *Salmonella* in low numbers may multiply rapidly in moistened feed: in a study by Israelsen et al. (Israelsen et al. 1996), each *Salmonella* originally present in the dry feed multiplied to about 1 million during 48 hour after moistening. This was also shown by other authors, for comparable products (Abushelaibi et al. 2003).

3.3.2. Processing

Feed processing may contribute to *Salmonella* in feed through cross-contamination. A survey by Davies and Wray (1997c) detected *Salmonella* in 16% of poultry feed samples from a feed mill in England, with 24, 13, and 12% of samples from intake pits, ingredient bins, and mixers, respectively. Jones and Ricke (1994) also suggest that feed may be cross-contaminated at the feed mill, and suggest cleaning intake pits with a “neutral” feed, such as corn, containing organic acids. In addition, the source of the air for the pellet is critical and should never originate from sites such as the ingredient receiving and loading areas (Jones et al. 1994). Air ducts should be maintained in sites that are protected from dust and other types of contamination (Jones et al. 1994).

3.3.3. Conditioning and pelleting

Israelsen et al. (1996) studied *Salmonella* contamination in Danish feed manufacturing facilities and found that most feed contamination occurred as a result of growth within the manufacturing system. This study suggested that such growth was correlated with moisture condensation and that the pellet cooler was the primary site where condensation occurs. In addition, the study concluded that contamination rates could be expected to be higher during cool seasons than during warm seasons, because temperature differentials enhanced the chances for condensation. Davies and Wray (1997c) studied the distribution of *Salmonella* in 10 feed manufacturing facilities in Great Britain and found that contamination rates from all samples tested ranged from 1.1 to 41.7%, depending upon the facility. The highest *Salmonella* contamination rates documented by this study were in the pellet cooler, where isolation rates were as high as 85.7% in some facilities.

3.3.4. Decontamination

Since all raw feed components must be considered as a potential source of *Salmonella*, process control and decontamination steps are essential to avoid spread of contaminated feed to production

animals (Sauli et al. 2005). Diverse process steps aimed at reducing or eliminating a contamination with *Salmonella* in feed are available, namely: implementation of heat treatment; use of organic acids; other chemical preservatives (Sauli et al. 2005). Most European countries run some kind of routine testing of raw materials, during the production process or in the final product, and some countries, including Denmark and Norway, has implemented a mandatory program for the control of *Salmonella* in pig feed production. In these two countries, feed for pigs must be heat-treated (Anonymous 2002b, Nielsen et al. 1997). Moist heat can effectively decontaminate feed materials, as well as compound feed as long as sufficiently high temperatures and treatment times are used. Where Good Hygiene Practices/Good Management Practices (GHP/GMP) are in place, the risk of recontamination is minimized. Comparative studies suggest that heat treatment processes used to successfully control *Salmonella* contamination will also be effective for other non spore forming food-borne pathogens (EFSA 2008c). Although heat treatment is generally recognized as the most effective decontamination method, in some circumstances (e.g. pelleted feed for layers) this may not be appropriate. In such cases, chemical treatment of feed may offer an alternative means of protection. Treatment of feed ingredients or compound feed with blends of organic acids, or with formaldehyde products at suitable concentrations, can be effective in reducing contamination by *Salmonella* spp. and other organisms. Furthermore, chemical treatment has a residual protective effect in feed, which helps reduce recontamination and also helps reduce contamination of milling and feeding equipment and the general environment.

Contamination of the treated feed may occur in a later stage of the processing chain. In the feed mill, thorough physical cleaning followed by efficient chemical disinfection is the most important factor to eliminate persistent *Salmonella* contamination (EFSA 2008c). Dry cleaning using vacuum cleaners are used in most feed mills and the accumulated dust must be discarded. Water should be avoided in the cleaning process because residual water may enter processing equipment or containers and accentuate the *Salmonella* problems. Ineffective disinfection may aggravate the *Salmonella* situation and only the recommended concentration of the disinfectant should be used (EFSA 2008c).

3.3.5. Bio-security

Bio-security refers to those measures taken to prevent or control the introduction and spread of infectious agents to a herd/flock. Control measures typically focus on wild animals, birds and insects, and on foot traffic. In a recent study of 121 Danish conventional slaughter pig herds, birds, cats and dogs had access to stored feed in 25%, 20% and 7% of the herds respectively. Birds, cats and dogs had access to stored straw in 41%, 40% and 13% of the farms. In the alternative organic farms and free range farms the access for pets and birds to stored feed was only slightly higher, but their access to stored straw was doubled compared to conventional herds. The occurrence of rodents in the farms was reported to be “low” in 79% of the conventional farms and “some” in 15% of the herds. In 6% of the farms the occurrence of rodents was reported to be “high” or “very high”. The occurrence of rodents in alternative slaughter pig productions was generally reported to be higher than in conventional farms (Sørensen et al. 2011).

It is acknowledge that rodents and wildlife can contaminate feed with pathogens, which makes rodent control and biosecurity in general essential when considering prevention of pathogenic

transfer from feed to animals. Among less documented means to reduce contamination is adding capsaicin (the burning substance in chilli peppers) to poultry feed as a rodent repellent and an alternative to traditional use of rodenticides. A study indicates that adding capsaicin to feed may reduce the amount of feed consumed by rats and mice, and eventually the contamination of feed from urine and faeces from rodent pests (Jensen et al. 2003). Capsaicin in poultry feed was reported not to affect growth of chicken or feed efficiency and was reported not to change the flavour of the meat (Jensen et al. 2003).

3.4. Transport and storage on the farms

Similar to potential contamination of feed ingredients, contamination of feed during storage and transportation on the farms can occur through animals (rodents, wild birds, pets), or be a consequence of cross-contamination from previous batches if storage rooms or vehicles were inadequately cleaned, disinfected or dried after cleaning.

3.5. Feeding systems

Contamination of the feed during its distribution to animals is also possible, via equipment, environment (water, soil, and animal faeces), wild animals or pets or staff. Insufficient cleaning or disinfection of the building or equipments increases the risk of contamination.

4. Public Health relevance of *Salmonella* in feedstuffs

4.1. Assessment of the feedstuffs as a source for *Salmonella* infections in animals

Animals can be infected either via direct contact between animals or from faecal contamination from the surroundings due to a persistent infection of the environment. For the latter part, contaminated feed is a likely source. Many studies have documented or provided circumstantial evidence for feeding stuffs constituting a source of *Salmonella* infection in animals (Table 2). Still, considering the prevalence of *Salmonella* in feedstuffs (Section 2.4-2.5) and the amount of feedstuff consumed (Appendix 4), it is assessed that contaminated feed on most occasions does not give rise to infections in food-producing animals. Which particular factors or combination of factors that determine whether an introduction of *Salmonella* via feed become established in a farm is not predictable at this time (Hald et al. 2006, Veldman et al. 1995), but factors such as storage conditions of feed, the prevalence and concentration of *Salmonella* in the feed and feeding strategies (e.g. dry pelleted vs. wet non-pelleted) are considered to contribute.

The impact of a *Salmonella* infection due to contaminated feedstuffs is different among the production types. Infection at the top of the breeding pyramid has a much larger overall impact since these farms supply breeding stocks by trade, and thereby acts as a continuous source of *Salmonella* infection, whereas infections in commercial herds, where an all-in/all-out production is common, the risk is merely short term unless cleaning and disinfection is not effective. In food production where the animals have a short lifespan as in broiler production, feedstuff contamination can introduce new serovars of *Salmonella* to the flocks and these types will shortly after be present at slaughter on the carcasses (MacKenzie et al. 1976). The long-term consequences related to animal productions, where the level of *Salmonella* in the herd and the effort put in to reducing the level has to be considered, contaminated feedstuff can be the source of endemic *Salmonella* strains that become established in the farm both in animals as asymptomatic carriers and as a persistent contamination of the environment.

Feedstuffs contaminated with *Salmonella* may cause a transient animal infection, as well as cause of animal infections for an extended period, due to a persistent establishment in the farm environment. Ahead of an estimate of a dose of *Salmonella* which may cause infection, a number of influencing factors should be taken into consideration. *Salmonella* bacteria in feed may be protected from the gastric defense mechanisms by a high fat content, thereby enabling infection with only a few numbers of bacteria (Jones et al. 1982). Furthermore, in stressed, immature and young animals as well as already infected animals the infectious dose may be especially low, less than 1 cfu/g (Schleifer et al. 1984, Hinton 1988). Finally, an initial low number of *Salmonella* in the feed may be multiplied because of a warm moist environment where the feedstuffs are stored either at the feed mill or at the farm.

It has also been discussed that the ability of a feed contamination to become established as a farm infection may depend on the serovar, as some serovars appear to be frequently present in feed without giving rise to problems in animals. However, attempts to identify such serovars for possible regulatory purposes have not been successful, since the vast majority of serovars found in feed also have been isolated from animals and humans (Hald et al. 2006).

Table 2: Studies documenting or providing supporting evidence for feedstuffs as a source of *Salmonella* in different animal species and the link via food to humans.

Refid. ⁶	Feed	Animal species	Food	Human	<i>Salmonella</i> serovar
21	+	Poultry	+	(+)	na
31 (rev.)	+	Food animals	+	+	Na
43	+	Pigs			Na
63	+	Cattle			<i>S. Infantis</i>
135	+	Poultry			Na
141	+	Pigs	+	+	<i>S. Cubana</i>
171	+	Poultry		(+)	<i>S. Typhimurium</i>
211	+	Fish	+		Na
215 (rev.)	+	Pork	+		Na
206	+	Dogs			Na
		Cats			
215	+	Pigs			Na
316	+	Food animals			Comparison of serovar distributions
381	+	Pigs			<i>S. Typhimurium</i> <i>S. Derby</i>
382	+	Poultry			Na
383	+	Cattle			Na
417	+	Cattle			Na
433	+	Turkeys			Na
517	+	Poultry			<i>S. 4, 12:b:-</i>
5727	+	Pigs			Na
5742	+	Poultry	+	+	<i>S. Agona</i>
		Cattle			<i>S. Hadar</i> <i>S. Heidelberg</i> <i>S. Virchow</i>
5458	+	Cattle			Na
635	+	Cattle			Na
1648	+	Fish			Na
1132	+	Cattle			<i>S. Mbandaka</i>
3208	+	Cattle			Na
93	+	Pigs	+	+	Comparison of serovar distribution
		Cattle			
371	+	Cattle	+	+	Na
612	+	Cattle			<i>S. Infantis</i>
EFSA 2011	+	Pigs	+		<i>S. Tennessee</i>
		Laying hens			

na = not applicable or many serovars mentioned

In areas, where *Salmonella* occurs endemically in the food-animal population, preventing feed contamination probably plays only a lesser role for controlling *Salmonella* than other factors. For instance persistent environmental contamination has been identified as an important risk factor for infection in laying hens (van de Giessen et al. 1994, Davies et al. 2003, Gradel et al. 2004), and several studies has identified trade with infected animals, poor biosecurity measures and different management strategies as important factors in pig production (Wong et al. 2002). Still contaminated feedstuffs could contribute to the problem by being a source of infection in farms otherwise free from *Salmonella*. Berends et al. (1996) estimated that as much as 15-30% of all *Salmonella* infections in the finishing period of pigs may be attributed to contaminated feed.

⁶ To see the list of references to pass the quality assessment, please consult Appendix 3.

In countries or regions where the endemic infection level in food-producing animals is well-controlled or absent, contamination of the farm environment by *Salmonella* from feedstuffs is considered particularly important and may have serious consequences (Shapcott 1985, Hansen et al. 1990). Sweden is such an example, as feed is found to be the main source when *Salmonella* is found in the swine and poultry meat production (Wierup 2006). The same situation is true for Finland from where several feedborne outbreaks in livestock have been described (Lindqvist and Pelkonen 2007, EFSA 2011). In 2009, a feed-borne outbreak in pigs and laying hens caused by contamination of a feed-mill production line with *S. Tennessee* was reported. *S. Tennessee* was isolated from samples of faeces, farm environment and/or feed samples at 50 pig holdings and 40 laying hen holdings (EFSA 2011). In Denmark, the current *Salmonella* prevalence in laying hens and broilers is also low and it must therefore be expected that distribution of contaminated feed can have similar consequences as seen from the case stories from Finland and Sweden.

The differences between the importance of feed contamination in high (endemic) and low prevalence regions was also demonstrated in a quantitative microbial risk assessment (QMRA) of *Salmonella* in pigs and pork at the EU level. The QMRA considered the effect of different (theoretical) mitigations strategies for preventing *Salmonella* infections in finishing pig herds. The results indicated that (a) by ensuring that breeder pigs are *Salmonella*-free a reduction of 70-80% in high prevalence MSs and 10-20% in low prevalence MSs can be foreseen; (b) by feeding only *Salmonella*-free feedstuffs, a reduction of 10-20% in high prevalence MSs and 60-70% in low prevalence MSs can be foreseen; and (c) by preventing infection from external sources of *Salmonella* (i.e. rodents and birds) a reduction of 10-20% in slaughter pig lymph node prevalence can be foreseen in both high and low prevalence MSs (Snary et al. 2010).

4.2. Assessment of the feedstuffs as a contributing factor for *Salmonella* contamination of food of animal origin

Food-producing animals are the main reservoirs for non-typhoidal *Salmonella* and food of animal origin are the most important sources of human infections (Mølbak et al 2006b, Crump et al. 2002). In a recent study, eggs were estimated to be the most important source for human salmonellosis in EU followed by pork, whereas broiler and turkey meat contributed less (Pires et al. 2011). A similar pattern was seen when analyzing data from foodborne outbreaks occurring in EU (Pires et al. 2010), where eggs through several years have been the most frequently implicated source, although their importance appear to be decreasing as an effect of the implemented harmonized EU monitoring and control (EFSA 2012). In Denmark, the *Salmonella* source account for 2010 estimated pork to be the most important food source of human domestic and sporadic infections (Anonymous 2011).

As presented in section 4.1., there exists sufficient evidence to establish a relationship between feedstuff contamination with *Salmonella* and infections in animals. This will consequently affect the level of *Salmonella* in animals brought for slaughter and thereby affect the contamination of the carcasses (Campbell et al. 1982). From this it may be inferred that contamination of feedstuffs influences the level of meat contamination, although only few studies provide direct evidence for such a link. A study from Canada applying different phenotypic and genotypic methods found an association between different *Salmonella* strains isolated from pelleted broiler feed and *Salmonella* strains found in raw chicken nuggets and strips (Bucher et al. 2007).

For milk and eggs the transmission pathway appears straighter forward as these food products are less exposed to contamination from other sources than the animals themselves. The vast majority of *Salmonella* infections in laying hens with public health significance is caused by *S. Enteritidis* (Pires et al. 2011), which is only rarely found in feed. However, in Japan, Shirota et al. (2001) have demonstrated an association between *S. Enteritidis* strains found in feed and those isolated from eggs indicating that if laying hens are infected through the feed, the infection passes on to the egg and hence to humans, but that this will be highly dependent on the serovar, where *S. Enteritidis* is the serovar most consistently associated with vertical transfer of *Salmonella*.

In general, contaminated milk must be expected to pose an insignificant risk to consumers, because it is pasteurized before consumption. Exceptions are persons exposed to raw milk. Dairy products made of unpasteurized milk such as raw milk cheeses may also constitute a higher risk, although the fermentation process under normal circumstances will reduce the contamination to an insignificant level. Still, cattle feedstuffs have been implicated in a milkborne outbreak caused by *S. Heidelberg* as described by Knox et al. (1963).

4.3. Assessment of the feedstuffs as a contributing factor for *Salmonella* infections in humans

The implication of animal feed as an indirect source of human salmonellosis has been described in several case studies (Table 2), where outbreaks in animals and/or humans have been traced back to contaminated animal feed. However, determining the overall contribution of contaminated animal feed to human illness, relative to other sources of contamination, is difficult with currently available data. Hald et al. (2006) estimated in a Danish study that up to 2.1% of the domestically acquired human salmonellosis cases in the period 1999-2003 could be attributed to feedborne serovars. Differences in serovars isolated from humans and from feedstuffs have been used as argument for feed not contributing substantially to human food-borne illness (Jones et al. 2004). However, several aspects should be considered in order to account for these differences such as sampling sensitivity at the feed producing facilities, the multiplication of *Salmonella* in feed and host related differences in serovars related to the pathogenesis (Crump et al. 2002).

5. Strategies to control *Salmonella* in the feed-chain

Control of *Salmonella* in feed has primarily been in focus and implemented in the poultry production, where much effort has been put into the elimination of non-feed borne *Salmonella* from the production. Compared to the pig- and cattle productions strict biosecurity measures and eradication of *Salmonella* in the poultry breeding stock has in many countries successfully led to a low frequency of vertical *Salmonella* transmission in the egg and broiler production, which is why introduction of *Salmonella* to the poultry flocks through feed is particularly undesirable.

Neither the concentration of *Salmonella* in feed nor the dose sufficient for infection of production animals is well known, but outbreak investigation data suggest that as little as 2 *Salmonella* per g feed may be sufficient to infect farm animals (Sauli et al. 2005). Large quantities of feed are consumed per animal and herd which increases the risk for herds or animals to become exposed to infectious levels of *Salmonella* during lifetime despite the generally low contamination levels and apparently an infrequent contamination.

Two main strategies have been used to control *Salmonella* in the feed chain. One is to prevent contamination and re-contamination of feed and the other is to reduce or eliminate an existing or suspected contamination. Comprehensive guidelines for production and control of *Salmonella* free feed has been set up in several countries (Butcher et al. 1995). Some countries have implemented mandatory programmes for control of *Salmonella* in the commercial feed production (e.g. heat treatment and process control) (Sauli et al. 2005).

Contamination with *Salmonella* of feed for Danish food production animals may occur along the entire feed production chain abroad as well as in Denmark (see section 3), and most raw feed components must be considered potentially contaminated with *Salmonella* (Sauli et al. 2005). Contamination may also occur during transport, processing and storage of feed, and in particular recontamination occurring after steps to reduce *Salmonella* contamination may occur.

A combination of process control and decontamination steps is needed to prevent or reduce survival and growth of *Salmonella* in animal feed and the exposure and infection of food animals with *Salmonella* from feed (Sauli et al. 2005). The following paragraphs deals with the prevention or reduction of survival and growth of *Salmonella* in animal feed.

Due to the low infection level in the poultry production in several countries, most studies on reduction of *Salmonella* in feed is obtained from the production of poultry feed in order to avoid or reduce feedborne introduction of *Salmonella*. When parallels are drawn to feed for other food production animals, differences in feed type, ingredients and physical/chemical properties should be considered.

The physical and chemical conditions in practically all non-acidified dry feeds allows *Salmonella* to persist for at least several months, and in moistened dry feed and in non-acidified wet feed *Salmonella* are also able to proliferate (Berends et al. 1996). Generally storage of feed for 56 day period in itself results in a reduction of *Salmonella* of approximately 0.5 log₁₀ units, and *Salmonella* seems to survive storage somewhat better in feed with soy-protein than in feed with meat- and bone meal. Higher protein contents tend – probably through reduced humidity – to lower

or delay the reduction rate. But large numbers of *Salmonella* survive a 56 day storage time (Ha et al. 1998).

The physical and chemical requirements for *Salmonella* to survive and multiply have been known for decades (Hansen 1987b). *Salmonella* is able to multiply at relatively low water activity $\geq a_w$ 0.94 (Lunestad et al. 2007) and many *Salmonella* strains will grow at temperatures $\geq 7^\circ\text{C}$ (Lunestad et al. 2007).

Some uncertainty on the pH limits for growth of *Salmonella* exists. *Salmonella* are sensitive to low pH. A pH of 5 allows growth of *Salmonella* (Hansen 1987b). Others reports growth of *Salmonella* to be possible in feed stored at pH between 4.0 and 9.6. At lower or higher pH *Salmonella* are reported to die out during storage (Lunestad et al. 2007). *Salmonella* are able to grow in atmospheres with and without oxygen (Hansen 1987b). Co-existence of other bacteria does not affect survival and growth of *Salmonella* with the exception of coexisting flora with high acid production, which will limit survival and growth (Hansen 1987b).

The most widely used process steps to reduce or eliminate a contamination with *Salmonella* in feed are heat treatment or acidification by organic acids (Sauli et al. 2005). In Denmark, only heat treatment has been accepted until recently, where chemical treatment became allowed if the efficacy of the chemical substances is ensured and its safety have been approved by EU⁷.

5.1. Heat treatment

Salmonella can be eliminated by heat treatment, depending of treatment time, temperature and moist, but considerable resistance to heat is observed in dry materials particularly if wrapped in lipids (EFSA 2008b, Hansen 1987b).

The heat resistance of *Salmonella* is strongly influenced by the strain, the physiological state and the matrix in which the bacterium is found. Thus *S. Senftenberg* is known to be particularly resistant to heat, and heat treatment at 88°C at 15% moisture was suggested (Maciorowski et al. 2004). In other studies no difference in heat resistance was observed between the selected strains of *S. Typhimurium*, *S. Enteritidis* and *S. Infantis* (Gradel et al. 2003). *Salmonella* in biofilm is relatively resistant to heat and the heat resistance in chocolate which has a low a_w is higher than in milk, (Lunestad et al. 2007). Authors state, that it is not possible to predict the heat resistance of *Salmonella* in products with a low a_w . For such products, the kinetics of the heat destruction must be determined in the product itself (Lunestad et al. 2007).

In Denmark, commercial feed has been subject to heat treatment (81°C) since 1993 (see section 6) in order to avoid introduction of feed borne *Salmonella* types to the herds through contaminated feed, and the apparent occurrence of *Salmonella* in commercial feed is low ($\leq 0.1\%$ feed samples culture positive for *Salmonella* in 2001-2003, which were the last years where *Salmonella* in compound feed was monitored) (Anonymous 2004). Heat treatment is only mandatory for poultry feed. Control of *Salmonella* in feed through heat treatment, adding organic acids, strict hygienic measures or combinations hereof is implemented in several countries, including Denmark, as part of

⁷ Regulation (EC) No 1831/2003

the quality assurance to control *Salmonella* in animal feed and in particular feed for the poultry production (Lindqvist et al. 2007, Sauli et al. 2003).

In a release assessment model, the reduction of *Salmonella* following heating at 71.1°C alone was assessed to be 0.17, 0.85 and 3.90 log₁₀ units at heating times of 5-10 seconds, 25-30 seconds and 115-125 seconds respectively (Sauli et al. 2005). But despite the indisputable potential effect of heat treatment to reduce *Salmonella* in feed, other studies have indicated that heat treatment in feed mills, even at high temperatures, does not alone ensure absence of *Salmonella* in the feed at the end of the production line when dust around the mill is contaminated (Jones et al. 2004).

The effect of heat treatment on *Salmonella* depends on the temperature and the treatment time, and optimal heat reduction of *Salmonella* seems to require humidity (Lunestad et al. 2007, Doyle et al. 2006). For example a 4.5 log reduction of *Salmonella* in feed was obtained from heat treatment at 82.2°C, 15% moisture but only a 1.5 log reduction was obtained from same temperature at 5% moisture (Doyle et al. 2006). Other authors found the optimal temperature, time and moisture content for conditioning feed in order to reduce *E. coli* and *Salmonella spp.* to be 85.7°C, 4.1 min and 145 g moisture/kg feed (Maciorowski et al. 2007). Other authors report *Salmonella* to be readily heat destroyed in food or feed with a water activity $a_w > 0.97$ (Lunestad et al. 2007).

A laboratory study showed that heat treatment at temperatures $\geq 60^\circ\text{C}$ and 100% relative humidity for 24 hours was able to eliminate *Salmonella* from feed artificially contaminated with high numbers of bacteria. Heat treatment was found to be more efficient if the humidity of the feed was high before and during heat treatment (Gradel et al. 2003).

Combination of heat treatment and chemical treatments such as propionic acid seems to be more effective than these treatments alone (Maciorowski et al. 2004). In a laboratory study the combination of heat treatment up to 80 sec at 71.°C and propionic acid concentrations up to 0.2% showed significant and independent effects on *Salmonella* contamination from heating time and acidification. After 80 sec heating and 0.2% propionic acid (approx. 15% moisture) a 10,000 fold reduction of *Salmonella* was obtained, and at 0.1% propionic acid the survival of *Salmonella* was 2 log₁₀ higher than at 0.2% (Matlho et al. 1997).

Pelleting of animal feed increases feed conversion and growth rate and is done under increased temperature and pressure. Pelleting of feed can reduce *Salmonella* considerably (more than 80% reduction (Rusul et al. 1996), 99% = 2 log₁₀ units (Maciorowski et al. 2004)), and *Salmonella* and other enterobacteriaceae may be completely eliminated by the pelleting process at temperatures exceeding 83°C (Blank et al. 1996). But the significance of this reduction for infection of livestock (pigs) is suggested to depend on the contamination level in the raw feed components prior to pelleting, and may not be sufficient at high contamination levels (Fedorka-Cray et al. 1997).

The efficiency of different techniques for heat treatment of feed in the conditioning and pelleting process have been tested, in order to overcome e.g. condensation and excess moisture in pellets after using raw feed ingredients stored in extremely cold environments like the Canadian prairies during winter. Thus in a study using heavily contaminated mash lots, a direct-fired steam conditioner (78-82°C, 3.5-4 min) appeared to perform equally good or slightly better than the conventional indirect-fired boiler-generated-steam conditioner (66 or 82 °C, 18-20s) to remove

Salmonella in the pellets. Both methods were sufficient to render the finished feed free from detectable levels of *Salmonella* (Blank et al. 1996).

In a field study of commercial animal feed Myint et al. (2007) found non-pelleted feed 13 times more likely to be contaminated with *Enterococcus* than pelleted feed, but this effect was not seen for the gramnegative/enterobacteriaceal indicator *E. coli* in the feed. The results indicate that the pelleting process alone may only have limited influence on the *Salmonella* contents in feed.

At least in pig herds, the effect of pelleting and heat treatment to reduce *Salmonella* contamination in the feed, must be seen in the perspective of the general and strongly increased risk for high *Salmonella* occurrence in pig herds feeding pelleted feed compared to pig herds feeding meal feed. Meal feed reduces *Salmonella* in pigs through increased production of organic acids and lowered pH in the gut, and thus outweigh the probably higher occurrence of *Salmonella* in non-pelleted feed (EFSA 2008c). Coarser grinding and barley rather than wheat is associated with production of organic acids in the gut and lowered risk for *Salmonella* in pigs.

5.1.1. Effect of feed heat treatments on organisms other than *Salmonella*

In Denmark, indicator bacteria, such as coliforme counts, have been used for many years in the feed control system for *Salmonella* alone or in parallel to culture for *Salmonella* (Det Danske Fjerkræråd 2008, Kjeldsen 2001).

A study found Enterobacteriaceae counts to be higher in feed samples positive for *Salmonella* than in negative samples and suggest that counts of Enterobacteria may be a useful indicator to assess the likelihood of *Salmonella* contamination in feed (Jones et al. 2004).

A laboratory study showed that heat treatment at temperatures $\geq 60^{\circ}\text{C}$ and 100% relative humidity for 24 hours is able to eliminate *Salmonella* inoculated onto faecal material as well as naturally occurring faecal *E. coli*. It was concluded, that *E. coli* could be used as a reliable and convenient indicator for presence or absence of *Salmonella* after heat treatment. The authors points to the fact, that *Enterobacteriaceae* are used as indicators for conditions allowing survival of *Salmonella* in feed mills, and that only few (no mentioned) scientific publications give statistical evidence for this (Gradel et al. 2003).

Other studies found that conditioning and pelleting of feed had similar effects on *E. coli*, *Salmonella* and *Listeria*, and that standard plate counts was not a good indicator as to the presence of pathogens after conditioning and pelleting (Blank et al. 1996).

5.2. Chemical treatment of feedstuffs

A wide range of chemical components have been evaluated for their efficiency to control *Salmonella* in feed or in production animals when added to animal feed. Besides their antimicrobial activity, consideration must be given to their corrosive effect on equipment, their feed damaging side effects, and their impact on animal growth and health (Sauli et al. 2005, Doyle et al. 2006).

Also they must efficiently reduce *Salmonella* in the presence of large amounts of organic matter and competing microflora, and ideally prevent recontamination. Finally they shall be easily and conveniently stored without posing serious threat to environment and persons (Maciorowski et al. 2004). For these reasons, buffered organic acids, a natural and toxic component of intestinal digesta are generally favoured for use in animal feed (Maciorowski et al. 2004).

5.2.1. Acidification-based treatments

The effect on *Salmonella* in feed from adding a range of acids have been evaluated (e.g. formic acid (HCOOH), hydrochloric acid (HCl , H_2O), nitric acid (HNO_3), phosphoric acid (H_3PO_4), propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$) and sulphuric acid (H_2SO_4)) (Doyle et al. 2006). Buffered organic acids are generally preferred to unbuffered acids (Doyle et al. 2006) and concentrations of hydrochloric, sulphuric, phosphoric and nitric acids greater than 0.1M are deleterious to birds growth (Maciorowski, et al. 2004). The effect of different organic acids seems to be species specific, and medium chained fatty acids are more anti-bacterial against *Salmonella* than short chain fatty acids (Van Immerseel et al. 2006).

The effect of organic acids on *Salmonella* in the feed has been demonstrated repeatedly. The effect is dependent on storage time, temperature and moisture. Since the water content of commercial feed is generally low, the action of the acids is not always optimal and it is not clear whether in-feed or gastrointestinal effect against *Salmonella* is the major reason for protection when fed to animals (Van Immerseel et al. 2006).

Optimal fermentation of liquid feed (sufficient moisture, temperature and time) leads to lowered pH and increased concentration of organic acids, which in a synergic action is capable of controlling *Salmonella* in feed and in animals (pigs). Organic acids may be added to the feed in order to reduce or eliminate *Salmonella* in the liquid feed, where natural fermentation does not lead to a sufficient drop in pH. The limits for growth of *Salmonella* for e.g. lactic acid and acetic acid are pH 4.05 and pH 5.40 respectively, and a pH below 4.5 in fermented liquid feed is recommended for control of *Salmonella* in pigs (Bysted et al. 2005). The amount of supplementary acid needed to achieve a pH of 4.5 varies with the natural fermentation and the feed composition (buffer capacity) (Hansen 1994). It is suggested, that diets designed to stimulate the production of organic acids in the gut may be an easier and more cost-effective measure, than addition of acids to feed or water (Van Immerseel et al. 2006). To improve acidification of fermented broiler feed, acidification with organic acids has been evaluated. Fermented feed acidified with 5.7% lactic acid ($\text{C}_3\text{H}_6\text{O}_3$) or 0.7% acetic acid (CH_3COOH) reduced *Salmonella* below detectable levels in contaminated broiler feed within 20 min and 2 h respectively (Doyle et al. 2006). Other studies fail to confirm these results indicating that other factors than presence of organic acids influence the *Salmonella* levels in fermented feed. In a release assessment model (Sauli et al. 2005) the reduction of *Salmonella* in feed from adding 1.2% propionic acids alone was 0,84 log¹⁰ units, and if added to the three heat treatment regimes in 5.1, the reduction of *Salmonella* was increased by approximately 40% (Sauli et al. 2005).

There seems to be a synergistic effect of 1-monoglyceride of capric acid (monocaprin, MC) and acids in killing *Salmonella*, and a combination of MC and organic acids added to the feed might be a feasible approach to control *Salmonella* in feed (Thormar et al. 2006).

Lactic acid added to liquid fermented feed spiked with *Salmonella* Typhimurium DT104:30 results in a dose dependent reduction of *Salmonella* with a \log^{10} reduction time from 10 hours (50 mmol/l) to 20 min (300 mmol/l). Acetic acid has proven to reduce *Salmonella* similar to lactic acid when added to the feed in same concentration (150 mmol/l). Copper (Cu^{2+}) has a significant effect on the survival of *Salmonella* in acidified liquid feed, but not in liquid feed without acidification. This effect of copper is dose dependent with a \log^{10} reduction time from 115 min (3.4 ppm) to 27 min (50 ppm) in feed acidified with acetic acid (200 mmol/l) compared to 142 min without copper. In acidified feed the effect of Cu^{2+} is independent of the source (CuSO_4 or CuCl). Substitution of copper with zinc ions (50 ppm) had no effect on *Salmonella*. Thus *Salmonella* seems to be extremely sensitive to lower quantities of free copper ions at lower pH values (Beal et al. 2004).

Adding organic acids (propionic, acetic, or formic) to feed components is suggested for sanitation of the process lines in feed mills as part of the guidelines for *Salmonella* free feed production in USA (Butcher et al. 1995).

5.3. Alternatives to heat or acid treatments

5.3.1. Formaldehyde-based feed treatments

In a study of *Salmonella* in consecutive turkey flocks in a production facility, isolation of *Salmonella* from animals, litter and drinkers dropped dramatically after adding a formaldehyde-based additive “Termin-8” to the feed at a rate of 0.2-0.3%. As this product was not able to eliminate *Salmonella* from the feed, the effect may be primarily in the gut of the birds. The study had no control group which leaves the possibility that the apparent effect may be due to pure coincidence (Nayak et al. 2003).

5.3.2. Sodium Chlorate

Chlorate is toxic to some bacteria because of an intracellular enzyme they possess (i.e., nitrate reductase), but does not kill all bacteria species. Enterobacteriaceae including *Salmonella* spp. possesses nitrate reductase and, therefore, are killed by chlorate treatment (Callaway et al. 2008). Chlorate administered to feed for broilers, swine and cattle is able to reduce *Salmonella* in the animals (Doyle et al. 2006). This review did not identify references to extra-animal effects of chlorate on pathogens in feed.

5.3.3. Stabilized oxychloro-based sanitizers (SOC)

SOC have proven efficient to decontaminate contaminated mung beans and prevent occurrence of *Salmonella* in the mung bean sprouts. Damaged beans may still carry *Salmonella* (Hora et al. 2007).

This review did not identify references to a decontaminating effect of SOC in animal feed.

5.3.4. Ammonia treatment

A pilot laboratory study showed ammonia treatment of contaminated wheat straw, corn grain and cottonseed to cause a 5 log₁₀ reduction of zoonotic bacterial pathogens (among these *Salmonella* Newport). Corn silage was shown to be actively antibacterial even without ammonization. The findings need follow up in large scale and under farm/production plant conditions. (Cliver (CRIS)).

5.3.5. Other chemical feed disinfectants

Halogenes are generally considered very sensitive to presence of organic material (Anonymous 2002a) and chlorinated water does not appear to be effective, thus 2 mg chlorine/ml was necessary to eliminate *S. Stanley* from alfalfa seeds (Maciorowski et al. 2004). Acid salts as formate (HCOO⁻), propionate (C₂H₅COO⁻) and trisodium phosphate (Na₃PO₄) have also been evaluated as *Salmonella* reducing feed additive (Maciorowski et al. 2004, Doyle et al. 2006).

5.3.6. Storage and composting

Recycled poultry bedding (RPB) is a protein and mineral supplement and nitrogen source for cattle in some countries (not in Denmark). RPB may contain *Salmonella* and may infect cattle. Although wastes may be heat treated, deep-stacking of wastes and fermentation through ensilage are commonly recommended as a means of elimination pathogens. When stacked and stored properly, the poultry bedding develops heat and increases the content of ammonia and under ideal circumstances is thereby able to reduce the contamination in the stack by several log¹⁰ units (Bush et al. 2007). Wastes are often deep-stacked or fermented through ensilage, and the heat production and/or lowered pH especially obtained from ensiling will under ideal circumstances and kill most infectious organisms (Bush et al. 2007). In practice changes in acidity and temperature may be far from ideal due to insufficient fermentation and the buffering effect of the litter. Thus, long term survival of *Salmonella* has been observed during fermentation of poultry litter (Haapapuro et al. 1997). Studies found the C/N (carbon/nitrogen) ratio to affect the survival of *Salmonella* inside heaps of compost (sawdust and manure) with increased reduction of *Salmonella* at higher C/N ratios. The C/N ration did not affect the survival of *Salmonella* on the heap surface (Erickson et al. (CRIS)).

5.4. Irradiation

The effectiveness of gamma-irradiation as a pathogen intervention treatment for feed has been reviewed (Doyle et al. 2006). Some reduction of the nutritional values of the feed must be expected from irradiation, thus irradiation doses at 25 kGy can destroy amino acids and lower doses (10 kGy) can destroy thiamine and riboflavin and requires additional supplementation (Maciorowski et al. 2004). Irradiation changes the structure of the bacterial DNA (Maciorowski et al. 2004). A maximum dose of 15-35 kGy would be sufficient to produce *Salmonella* free feed under

commercial conditions, and lower doses of 10-15 kGy would reduce *Salmonella* below detectable levels of routine testing (Doyle et al. 2006). A synergistic combination of heat, irradiation and chemical treatment was suggested to reduce the microflora on animal feed and maximum of 20 kGy would be sufficient for irradiation of pelleted feed (Maciorowski et al. 2004).

5.5. Examples of effectiveness of reducing *Salmonella* contamination under industrial scale

Only a few brief reports on this were found. Some referred second hand to the Nordic countries, in particular to the Norwegian and Swedish control systems.

6. *Salmonella* control of feed in Denmark – general considerations

By the implementation of the feed hygiene regulation (EU/183/2005)⁸, statutory control was in general significantly reduced and substituted by control managed by the feed producers. From 2006 it became the responsibility of the managers of feed producing companies to ensure feed quality and feed safety. The focus of the official control was changed to support the development of efficient quality systems within the companies, and subsequently to assess the quality management, and implementation of the quality systems. Less official samples were taken to assess the *Salmonella* risk of feed and feed processing equipment and facilities. Thus, prevention and control of *Salmonella* consequently became the responsibility of the companies. The official control concept has changed towards more risk based assessment, and thus the official *Salmonella* control changes every year to assess new potential risk areas, to monitor a certain area of interest, to assess quality management of *Salmonella* risk, or to compare official results to the company data.

It is emphasised that due to low test sensitivity (Maciorowski et al., 2005) and high volume of feed used, it will be impossible to guarantee *Salmonella*-free batches of feed (Davies et al., 2004). The real challenge lies therefore with the risk managers, to define an acceptable level of contamination so that batches with a contamination level above that limit can be handled in a cost-effective manner, where the obtained risk reduction bears comparison with the cost of intervention

According to the feed hygiene regulation, microbiological criteria should be established for feed. This has not happened yet. In order to focus on preventive and control measures of the risk of *Salmonella* of feed for animals and human in Denmark, the Plant Directorate decided in July 2010 to publish a note on the expectations of which serovars, the feed producers as a minimum should report to the Plant Directorate, inform their customers about (except end users), and consequently treat feed to kill *Salmonella*. There have been a few incidences in the fall 2010, where companies have notified the Plant Directorate about findings of these more health hazard serovars and appropriate actions have been taken. The Plant Directorate has decided only to publish Rapid Alerts if products are forwarded to other countries from Denmark (this happened in January 2011, where products were sent to Iceland).

Heat treatment to eliminate *Salmonella* has up till now been the only accepted method in Denmark. The method has proved its efficacy. Because companies have shown interest in other methods to eliminate *Salmonella*, the Plant Directorate has changed the Danish legislation⁹ and has introduced an opportunity to use other methods as long as the company can show that the method ensures efficient effect on *Salmonella*. Chemical methods should be approved in EU before use primarily to ensure that there is no hazard to animal, environment and human.

6.1. Monitoring and control of incoming feed materials

In 2010 the official control primarily addressed incoming feed materials in order to monitor for potentially health hazards and new serovars as well as increasing the level of knowledge about the

⁸ Regulation (EC) No 183/2005 of 12 January 2005 laying down requirements for feed hygiene

⁹ Order nr. 775 of 28th June 2011. Guidance on feed and feed mills, Plant Directorate, September 2010.

sampling procedures and handling of test results by the companies. Imported feed (primarily soy products) are tested consequently by the companies whereas other traded products as rape are tested more infrequently because rape products often are traded within the EU or are produced locally.

6.1.1. Current practice in Denmark

Companies test the products of imports upon arrival at ports. They should sample at least 1 pr. 2000 tons material and the sampling should be representative for the whole shipment. Testing results should be obtained 1 week after the sampling (indicating the presence or absence of *Salmonella*) and the serovar should be reported as soon as possible. If more than 10% of the total numbers of samples are positive, it was agreed in 2010, that the feed material should be treated to eliminate *Salmonella* (either in the production of compound feed or as the raw feed material). When the serovar is known, treatment of the product to eliminate *Salmonella* is mandatory if the serovars are amongst the one decided to be of most health concern for animals and humans. If other serovars are found, it is up to the company to decide whether they want to continue treatment. Even if the number of positive samples with *Salmonella* is less than 10% of the total, treatment to kill *Salmonella* should be initiated as soon as possible if one of the serovars of potentially more hazard to animals and human are found.

Poultry raw material is not tested before use because the efficacy of heat treatment has been trusted to be sufficient to eliminate *Salmonella*.

Feed materials are delivered to farmers (end users) before the *Salmonella* status is known. The end users are not informed, if *Salmonella* has subsequently been found in the material. Currently approx. 50% of feed for pig production is mixed on farms.

6.2. Process control

Process control is largely developed at feed production plants. Statutory process control is significantly reduced since 2006. In 2010 approx. 600 samples were taken at the majority of feed producers at approx. 100 production plants. In the official controls, findings were very few in 2010. No matter the detected serovar, cleaning and disinfection is mandatory when *Salmonella* is found in equipment or facilities.

6.2.1. Current practice in Denmark

Poultry feed producers are obliged to do weekly sampling and testing of process samples in order to be able to deliver feed to commercial poultry meat and egg producers. Official sampling at these plants was shut down in 2010.

Quarterly all poultry feed producers are inspected by a private company that assess management of heat treatment, hygiene themes, and takes samples for *Salmonella* testing to verify the hygiene standard. Findings of *Salmonella* at poultry feed producers are very infrequent and consequences of findings are taken care of immediately before production can be continued.

All feed producing companies tested approx. 8000 samples in 2010. Most samples were from the poultry feed producing plants. Other feed producing plants sample 2-4 times a year.

6.3. Monitoring and control of end products

The official routinely control of end products was terminated in 2006. Up till then results had shown that the prevalence of *Salmonella* in end products was very low. This was the result of the efficiency of the heat treatments (81 C) of feed containing products from oil rich seeds to kill *Salmonella*. The few positive findings were in products from companies that had persistent contamination of equipments i.e. in house-infections.

6.3.1. Current practice in Denmark

Companies do end products tests. In 2010, approx. 350 samples were taken. Feed for poultry, cattle, and pigs constituted approx. 1/3 each. There were no positive findings.

7. Conclusions and recommendations

1: Assessment of the association between *Salmonella* in animal feed and *Salmonella* infection of Danish broilers, table-egg layers, cattle, farmed fish, slaughter pigs and humans.

- Feedstuffs constitute a source of *Salmonella* infection in animals as supported by many studies.
- Considering the prevalence of *Salmonella* in feedstuffs and the amount of feedstuff consumed, it is assessed that contaminated feed on most occasions does not give rise to infections in food-producing animals.
- Which particular factors or combination of factors that determine whether an introduction of *Salmonella* via feed become established in a farm is not known, but storage conditions of feed, the prevalence and concentration of *Salmonella* in the feed and feeding strategies are considered contributing factors.
- In regions and/or animal populations in which *Salmonella* infections occur endemically, other factors for introduction and spread of *Salmonella* are considered more important than contaminated feedstuffs. In Denmark, this is assessed currently to be the case in pig production.
- In low prevalence situations, an introduction of *Salmonella* via contaminated feed can result in large outbreaks which may spread to humans via contaminated food of animal origin. Such outbreaks are observed from time to time in e.g. Sweden and Finland, and similar outbreaks in Denmark can be expected in low prevalence animal populations such as laying hens and broilers.
- In cattle in Denmark, *S. Dublin* and *S. Typhimurium* are the most important serovars and feedstuffs do not appear to play a major role for their introduction and dissemination. Feed contaminated with other serovars has been described as the source of infections in cattle in several studies, some of which also documented a spread to humans via contaminated food.
- Only a very few studies on the role of *Salmonella* contaminated fish feed could be found through this review and none provided any evidence for *Salmonella* transmission from fish feed to humans. Consequently, the risk is assessed to be negligible.
- Several studies comparing serovars found in feed with those found in animals and humans conclude that the most frequently occurring *Salmonella* serovars in humans are rarely isolated from animal feedstuffs. However, many serovars found in feed are also found in humans and studies have estimated that around 2% of human infections can be attributed to feedborne serovars.
- The implication of animal feed as an indirect source of human salmonellosis has been described in several case studies, where outbreaks in animals and/or humans have been traced back to contaminated animal feed.
- However, determining the overall contribution of contaminated animal feed to human illness, relative to other sources of contamination, is difficult with currently available data.

2: Identification of factors, associated with animal feed (pH, structure etc.), that determine whether exposure to *Salmonella* lead to infection in broilers, table-egg layers, cattle, farmed fish and slaughter pigs.

- Based on available data, oil-based feed materials such as soy-, rapeseed- and sunflowerseed products are considered the most importance sources of *Salmonella* contamination from feed. Animal derived protein sources are also frequently contaminated with *Salmonella*, but their use except for fish meal is currently very limited. In contrast, non-processed cereals are considered to be of very low importance. In general, however, data on *Salmonella* occurrence in feed materials are scarce.
- Many studies have shown a significantly higher risk for *Salmonella* occurrence in pig herds using heat treated and pelleted feed as compared to pig herds fed meal feed. The protective effect of meal feed is attributed to the increased production of organic acids and lowered pH in the pigs' gut. This association is assessed to outweigh the likely higher occurrence of *Salmonella* in feed materials (i.e. non-pelleted) used by farmers mixing their own feed e.g. oil-based products. Only few studies on the occurrence of *Salmonella* in home-mixed feed are available.
- Coarser grinding and barley rather than wheat is associated with production of organic acids in the gut and lowered risk for *Salmonella* in pigs.
- In Denmark, poultry are only given dried feed. For pigs approximately 40% of the feed is applied as wet feed. In cattle most feed is fed as a mixture of fodder concentrates and coarse fodder. Additional pelleted feed is supplied for milk-producing cattle.

3: Assessment of available preventive measures, control methods and methods to reduce *Salmonella* in animal feed.

- Compared to pig- and cattle production strict biosecurity measures and eradication of *Salmonella* in the poultry breeding stock has in many countries successfully led to a low frequency of vertical *Salmonella* transmission in the egg and broiler production, which is why introduction of *Salmonella* to the poultry flocks through feed is particularly undesirable and heat treatment of feed for poultry meat production is routinely applied in many countries including Denmark.
- The effect of heat treatment on *Salmonella* depends on the temperature, the treatment time, the humidity and the initial *Salmonella* concentration. However, the effect of heat treatment in feed mills may be hampered due to the risk of recontamination from e.g. dust in the mill environment after processing. Persistent contamination of feed mill equipment has also been identified as a significant source of feed contamination leading to outbreaks in animals.
- *E. coli* has been proposed as a reliable indicator for the presence or absence of *Salmonella* after heat treatment. However, only few scientific publications provide statistical evidence for this.
- The effect on *Salmonella* of adding organic acids to the feed has been demonstrated repeatedly. The effect depends on storage time, temperature and moisture. Since the water content of commercial feed is generally low, the action of the acids is not always optimal and it is not clear whether it is an in-feed or a gastrointestinal effect against *Salmonella* that is the major reason for protection when fed to animals.

- Due to low test sensitivity and high volume of feed used, it will be impossible to guarantee *Salmonella*-free batches of feed and the currently applied sampling procedures can only reliably identify highly contaminated lots of feed materials and compound feed. The real challenge lies therefore with the risk managers, to define an acceptable level of contamination so that batches with a contamination level above that limit can be handled in a cost-effective manner, where the obtained risk reduction bears comparison with the cost of intervention.
- Feed producers should strive to reduce the occurrence of *Salmonella* in compound feed for all food-production animals. HACCP based programs and establishment of microbiological criteria (as laid down by the feed hygiene regulation) along the feed production chain should prevent (re-)contamination of feed and thereby ensure the quality of the end product.

4: Evaluation of the systematic review process as a tool to address the public health impact of *Salmonella* in animal feed.

The purpose of this review was to evaluate and summarize the evidence for an association between *Salmonella* occurrence in animal feed and human salmonellosis. We chose the systematic review process in order to evaluate the available information, using transparent and repeatable methods. The goal was to minimize the impact of study biases on the review conclusions and to convey to the reader not only the conclusion, but also enough information for the reader to appraise the value contained in the conclusion.

The studies on which we based our answers to the study questions were of a very varied nature including everything from simple descriptive studies of monitoring data to randomized controlled-trial studies. In addition, very few studies attempted to answer the same question. This made it very difficult to perform a strict systematic review, where the purpose is to appraise and compare studies providing evidence for and against a specific hypothesis (i.e. answer to a study question), respectively.

This was further complicated by the fact that most studies providing evidence for an association between *Salmonella* contaminated feed and infections in animals and/or humans were case-based studies (i.e. case stories) mainly describing outbreaks caused by contaminated feed. Obviously, studies providing no evidence for such an association cannot be found in the literature, although every incidence of animals being fed *Salmonella* contaminated feed without being infected, in theory, could be considered as such. Still, it is also possible that many of the observed infections in animals and humans actually do originate from contaminated feed. The association has just not been identified due to the complexity of the transmission pathways and the limited amount of data on *Salmonella* in feed, or the association has simply not been reported in the available literature. This means that the available literature most likely gives a biased picture of the true situation.

So although we from the beginning of the study were aware that this systematic review could only be a qualitative appraisal (as opposed to e.g. a meta analysis) of relevant literature, we found even this to be very difficult. We conclude that study questions to be addressed by systematic reviews should be very specific, and studies to be included should preferably have the same objectives, be conducted using well-described and appropriate study designs, and provide statistical measures for the investigated association. Studies based on a description of monitoring data or case-based studies

can very well provide evidence for the association under investigation, but they are not suitable for a systematic review due to the reasons discussed above.

Exclusion of seemingly relevant research findings due to poor quality is a major concern to readers of systematic reviews. During the quality assessment step, we excluded 32 references, which we consider not to have influenced the conclusions drawn. However, it cannot be excluded that useful references may have been excluded during the title screening, if the title did not indicate its relevance for the subject.

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APPENDIX 1: Literature search strategy

Systematic review of the human-health impact of *Salmonella* in animal feed and an evaluation of existing strategies for prevention, control and reduction of *Salmonella* in animal feed

**Report on literature search
03.04.2008**

**By
Katrine Lundsby**

**National Food Institute, Technical University of Denmark
Mørkhøj Bygade 19, 2860 Søborg, Denmark**

Search process

No year or language limits were set in any of the searches performed.

Electronic databases

Food Science and Technology Abstracts (FSTA), BIOSIS and CAB International

Name of host/system:	ERL WebSPIRS 5.12 SilverPlatter
Date of search:	14.02.08
Type of search:	Advanced search
Fields searched in:	Abstracts and titles
Subjects searched in:	-
Search string 1:	("Food animal*" OR "production animal*" OR Broiler* OR avian OR Chicken* OR Chick* OR poultry OR Hen OR hens OR Fowl* OR Pullet* OR Cock* OR Turkey* OR Layer* OR Egg* OR bovine OR Cattle* OR dairy OR dairies OR beef OR Ruminant* OR calf OR calves OR Cow* OR Heifer* OR Bull* OR Steer* OR aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet* OR Gilt* OR Hog* OR Barrow* OR Boar* OR swine OR Sow* OR Dam OR dams OR finisher*) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein concentrate" OR "fish protein" OR "Malt sprout" OR culm OR malt) AND (<i>Salmonella</i> OR salmonel* OR enteropathogen OR enterobacter*)
Hits of search string 1 (abstracts only):	2743
Hits of search string 1 (title only):	414
Total in RefMan database:	2190 (duplicates removed)

ScienceDirect

Name of host/system:	ScienceDirect
Date of search:	14.02.08
Type of search:	Expert search-Journals
Fields searched in:	Title, abstract, keywords and full text (i.e. not in reference list)
Subjects searched in:	"Agricultural and Biological sciences", "Biochemistry, Genetics and Molecular biology", "Immunology and Microbiology", "Medicine and Dentistry" and "Veterinary Science and Veterinary Medicine"
Document types:	Article, review article, short survey and short communication
Search string 1:	TITLE-ABSTR-KEY(({Food animal*} OR {production animal*} OR Broiler* OR avian OR Chicken* OR Chick* OR poultry OR Hen OR hens OR Fowl* OR Pullet* OR Cock* OR Turkey* OR Layer* OR Egg* OR bovine OR Cattle* OR dairy OR dairies OR beef OR Ruminant* OR calf OR calves OR Cow* OR Heifer* OR Bull* OR Steer* OR aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet* OR Gilt* OR Hog* OR Barrow* OR Boar* OR swine OR Sow* OR Dam OR dams OR finisher*) AND ({Ready-mixed} OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR {Fish flour} OR {Fish protein concentrate} OR {fish protein} OR {Malt sprout} OR culm OR malt) AND (<i>Salmonella</i> OR salmonel* OR enteropathogen OR enterobacter*)) or FULL-TEXT(({Food animal*} OR {production animal*} OR Broiler* OR avian OR Chicken* OR Chick* OR poultry OR Hen OR hens OR Fowl* OR Pullet* OR Cock* OR Turkey* OR Layer* OR Egg* OR bovine OR Cattle* OR dairy OR dairies OR beef OR Ruminant* OR calf OR calves OR Cow* OR Heifer* OR Bull* OR

Steer* OR aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet* OR Gilt* OR Hog* OR Barrow* OR Boar* OR swine OR Sow* OR Dam OR dams OR finisher*) AND ({Ready-mixed} OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR {Fish flour} OR {Fish protein concentrate} OR {fish protein} OR {Malt sprout} OR culm OR malt) AND (*Salmonella* OR salmonel* OR enteropathogen OR enterobacter*))

Hits of search string 1:

2488

Total in RefMan database:

2431 (duplicates removed)

PubMed

Name of host/system:

NCBI Entrez retrieval system

Date of search:

11.02.08

Type of search:

-

Fields searched in:

All

Subjects searched in:

-

Search string1:

("Food animal*" OR "production animal*" OR Broiler* OR avian OR Chicken* OR Chick* OR poultry OR Hen OR hens OR Fowl* OR Pullet* OR Cock* OR Turkey* OR Layer* OR Egg* OR bovine OR Cattle* OR dairy OR dairies OR beef OR Ruminant* OR calf OR calves OR Cow* OR Heifer* OR Bull* OR Steer* OR aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet* OR Gilt* OR Hog* OR Barrow* OR Boar* OR swine OR Sow* OR Dam OR dams OR finisher*) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflowerseed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein concentrate" OR "fish protein" OR "Malt sprout" OR culm OR malt) AND (*Salmonella* OR salmonel* OR enteropathogen OR enterobacter*)

Hits of search string 1:

1482

Total in RefMan database:

1477 (duplicates removed)

ISI Web of Knowledge 4.0

Name of host/system:

Thomson Cooperation

Date of search:

14.02.08

Type of search:

Advanced search

Fields searched in:

Title, Abstract, Author, Keyword and Keywords Plus®

Subjects searched in:

-

Search string 1:

TS=((("Food animal*" OR "production animal*" OR Broiler* OR avian OR Chicken* OR Chick* OR poultry OR Hen OR hens OR Fowl* OR Pullet* OR Cock* OR Turkey* OR Layer* OR Egg* OR bovine OR Cattle* OR dairy OR dairies OR beef) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein concentrate" OR "fish protein" OR "Malt sprout" OR culm OR malt) AND (*Salmonella* OR salmonel* OR enteropathogen OR enterobacter*))

Hits of search string 1:

1121

Search string 2:

TS=((Ruminant* OR calf OR calves OR Cow* OR Heifer* OR Bull* OR Steer* OR aquaculture OR Fish) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein concentrate" OR "fish protein" OR "Malt sprout" OR culm OR malt) AND (*Salmonella* OR salmonel* OR enteropathogen OR enterobacter*))

Hits of search string 2:

234

Search string 3:

TS=((Pig OR pigs OR porcine OR Piglet* OR Gilt* OR Hog* OR Barrow* OR

Boar* OR swine OR Sow* OR Dam OR dams OR finisher*) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein concentrate" OR "fish protein" OR "Malt sprout" OR culm OR malt) AND (*Salmonella* OR salmonel* OR enteropathogen OR enterobacter*))

Hits of search string 3: 306

Total in RefMan database: 1383 (duplicates removed)

AGRICOLA

Name of host/system: United States Department of Agriculture. National Agricultural Library (NAL) Catalog

Date of search: 12.02.08

Type of search: Advanced search

Fields searched in: All

Subjects searched in: -

Search string 1: ("Food animal?" OR "production animal?" OR Broiler? OR avian OR Chicken? OR Chick? OR poultry OR Hen OR hens OR Fowl? OR Pullet? OR Cock? OR Turkey?) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflowerseed OR rape OR rapeseed OR canola) AND (*Salmonella* OR salmonel? OR enteropathogen OR enterobacter?)

Hits of search string 1: 395

Search string 2: ("Food animal?" OR "production animal?" OR Broiler? OR avian OR Chicken? OR Chick? OR poultry OR Hen OR hens OR Fowl? OR Pullet? OR Cock? OR Turkey?) AND (bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein" OR "fish protein concentrate" OR "Malt sprout" OR culm OR malt) AND (*Salmonella* OR salmonel? OR enteropathogen OR enterobacter?)

Hits of search string 2: 60

Search string 3: (Layer? OR Egg? OR bovine OR Cattle? OR dairy OR dairies OR beef OR Ruminant? OR calf OR calves OR Cow? OR Heifer? OR Bull? OR Steer?) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflowerseed OR rape OR rapeseed OR canola) AND (*Salmonella* OR salmonel? OR enteropathogen OR enterobacter?)

Hits of search string 3: 205

Search string 4: (Layer? OR Egg? OR bovine OR Cattle? OR dairy OR dairies OR beef OR Ruminant? OR calf OR calves OR Cow? OR Heifer? OR Bull? OR Steer?) AND (bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein" OR "fish protein concentrate" OR "Malt sprout" OR culm OR malt) AND (*Salmonella* OR salmonel? OR enteropathogen OR enterobacter?)

Hits of search string 4: 40

Search string 5: (aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet? Or Gilt? OR Hog? OR Barrow? OR Boar? OR swine OR Sow? OR Dam OR dams OR finisher?) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflowerseed OR rape OR rapeseed OR canola) AND (*Salmonella* OR salmonel? OR enteropathogen OR enterobacter?)

Hits of search string 5: 123

Search string 6: (aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet? Or Gilt? OR Hog? OR Barrow? OR Boar? OR swine OR Sow? OR Dam OR dams OR finisher?) AND (bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein" OR "fish protein concentrate" OR "Malt sprout" OR culm OR malt) AND (*Salmonella* OR salmonel? OR enteropathogen OR enterobacter?)

Hits of search string 6: 39

Total in RefMan database: 659 (duplicates removed)

References in Silverplatter:	2190
References in Agricola:	659
References in PubMed:	1477
References in ScienceDirect:	2431
References in ISI Web of Knowledge:	1383
Collective number in databases:	8140
Number of duplicates removed:	2817
Total number in final database:	<u>5323</u>

Conference proceedings

International Society for Veterinary Epidemiology and Economics (ISVEE)

The full historical archive of ISVEE proceedings from 1976 to 2006 was searched on-line at the SciQuest web site: <http://www.sciquest.org.nz>. *SciQuest*[®] is a fully indexed and searchable e-library of quality New Zealand and Australian veterinary and animal science and veterinary continuing education publications.

URL:	http://www.sciquest.org.nz
Date of search:	07.03.08
Type of search:	Publications
Fields searched in:	Non per-reviewed publications
Years:	All (1952-2008)
Subjects searched in:	"Companion animal proceedings", "Dairy cattle proceedings", "Food Safety Biosecurity, epidemiology and industry", "ISVEE" and "Sheep and beef proceedings".
Search string1:	("Food animal*" OR "production animal*" OR Broiler* OR avian OR Chicken* OR Chick* OR poultry OR Hen OR hens OR Fowl* OR Pullet* OR Cock* OR Turkey* OR Layer* OR Egg* OR bovine OR Cattle* OR dairy OR dairies OR beef OR Ruminant* OR calf OR calves OR Cow* OR Heifer* OR Bull* OR Steer* OR aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet* OR Gilt* OR Hog* OR Barrow* OR Boar* OR swine OR Sow* OR Dam OR dams OR finisher*) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR seed OR "Fish flour" OR "Fish protein concentrate" OR "fish protein" OR "Malt sprout" OR culm OR malt) AND (<i>Salmonella</i> OR salmonel* OR enteropathogen OR enterobacter*)
Hits of search string 1:	30
Total in RefMan database:	30

International Pig Veterinary Society (IPVS) Congress Proceedings

Year:	1998	Year:	2002
Congress number:	15 th	Congress number:	17 th
Search string:	Salmonel*	Search string:	Search in titles
Results:	6 (0 imported)	Results:	2 imported
Year:	2000	Year:	2004
Congress number:	16 th	Congress number:	18 th
Search string:	Salmonel* and feed	Search string:	<i>Salmonella</i> &feed
Results:	38 (6 imported)	Results:	3 imported
Total in RefMan database:	11		

I3S International *Salmonella* and Salmonellosis

Year: 1997
Search method: Hand searching
Results: Nothing of interest

Year: 2002
Search method: CD ROM
Results: 5 references

Year: 2006
Search method: Hand searching
Results: 1
Total in RefMan database: 7

International Symposium on the Epidemiology and Control of *Salmonella* and other food borne pathogens in Pork (Salinpork/Safepork)

Year:	2001	Year:	2003
Congress number:	4 th	Congress number:	5 th
Search method:	Hand searching	Search method:	Hand searching
Results:	3 (6 duplicates)	Results:	11 (1 duplicate)

Year:	2005	Year:	2007
Congress number:	6 th	Congress number:	7 th
Search method:	Hand searching	Search method:	Hand searching
Results:	12	Results:	1

Total in RefMan database: 26

International Symposium on Ecology of *Salmonella* in Pork Production

Year: 1996
Congress number: 1st
Search method: Hand searching
Results: 2

Total in RefMan database: 2

Unpublished literature

Current Research Information System (CRIS)

URL: <http://cris.csrees.usda.gov/search.html>
Date of search: 27.03.08
Type of search: CRIS Professional search (Standard Technical Format)
Fields searched in: Fulltext terms (Title, Objectives, Approach, Non-Technical Summary, Keywords, Progress, and Impact)
Subjects searched in: -
Search string1: ("Food animal*" OR "production animal*" OR Broiler* OR avian OR Chicken* OR Chick* OR poultry OR Hen OR hens OR Fowl* OR Pullet* OR Cock* OR Turkey* OR Layer* OR Egg* OR bovine OR Cattle* OR dairy OR dairies OR beef OR Ruminant* OR calf OR calves OR Cow* OR Heifer* OR Bull* OR Steer* OR aquaculture OR Fish OR Pig OR pigs OR porcine OR Piglet* OR Gilt* OR Hog* OR Barrow* OR Boar* OR swine OR Sow* OR Dam OR dams OR finisher*) AND ("Ready-mixed" OR premixed OR diet OR feed OR soy OR soya OR soybean OR soyabean OR sunflower OR sunflower seed OR rape OR rapeseed OR canola OR bean OR flake OR cake OR meal OR husk OR hulls OR

	seed OR "Fish flour" OR "Fish protein concentrate" OR "fish protein" OR "Malt sprout" OR culm OR malt) AND (<i>Salmonella</i> OR salmonel* OR enteropathogen OR enterobacter*)
Hits of search string 1:	303
Total in RefMan database:	266 (38 duplicates)

Other internet search sites

Dansk Svineproduktion

URL:	http://www.dansksvineproduktion.dk/Publikationer/Publikationer.html
Date of search:	13.03.08
Type of search:	Faglige publikationer
Fields searched in:	Nøgleord søgning
Search string 1:	<i>Salmonella</i>
Hits of search string 1:	93
Search string 2:	<i>Salmonella</i> typhimurium
Hits of search string 2:	5
Search string 3:	<i>Salmonella</i> DT 104
Hits of search string 3:	3
Total in RefMan database:	93 (8 duplicates)

Faculty of Agricultural Sciences, University of Aarhus

URL:	http://pure.agrsci.dk:8080/front.do
Date of search:	03.04.08
Fields searched in:	Publications from DJF
Search string 1:	Salmonel*
Hits of search string 1:	49
Total in RefMan database:	47 (2 duplicates)

Total number of references in RefMan database: 5803

APPENDIX 2: Questions addressed for evaluation of each reference

All questions involved in the steps of the systematic review.

1. Refid
2. Reviewer
3. Please write your initials
4. Does the paper only relate to the question 4 in the relevance screening: Does the reference describe factors associated with feed (structure, pH etc.) or feed additives (probiotics, antibiotics, etc), that determines whether exposure to *Salmonella*
5. Does the paper only describe testing of diagnostic methods for i.e. testing for *Salmonella* in spiked feed? If yes, do not perform the quality assessment?
6. How relevant is the study for answering the key questions of the systematic review (including studies on prevalence of *Salmonella* in feed)?
7. Clearly focused and appropriate question?
8. Which animal feed type(s) does the study describe? - Cereals (e.g. oat, barley, corn)
9. Which animal feed type(s) does the study describe? - Oil containing (e.g. soy, rape)
10. Which animal feed type(s) does the study describe? - Legumes (e.g. peas, beans, alfalfa)
11. Which animal feed type(s) does the study describe? - Roots (e.g. turnips, potato)
12. Which animal feed type(s) does the study describe? - Dairy (e.g. whey, milk powder)
13. Which animal feed type(s) does the study describe? - Land animal (e.g. bone meal)
14. Which animal feed type(s) does the study describe? - Aquatic (e.g. fish meal, fish oil)
15. Which animal feed type(s) does the study describe? - Minerals (e.g. calcium)
16. Which animal feed type(s) does the study describe? - Miscellaneous (e.g. waste products, bakery, fatty acids)
17. Which animal feed type(s) does the study describe? - Straw, hay and grass
18. Which animal feed type(s) does the study describe? - Mixed/compound feed (e.g. pelleted feed)
19. Which animal feed type(s) does the study describe? - Other
20. Comment
21. Which animal feed type(s) does the study describe? - None
22. Which animal feed type(s) does the study describe? - Question not applicable
23. Will this feed type ever be relevant under Danish conditions?
24. Is the study relevant for Danish production?
25. Which bacteria does the study describe? - *Salmonella*
26. Which bacteria does the study describe? - Enterobacteriaceae
27. Which bacteria does the study describe? - Non-enterobacteriaceae
28. Which bacteria does the study describe? - None
29. Which serovars are in the paper?
30. Does the paper refer to antimicrobial resistance in *Salmonella* in feed?
31. What is the size of the setting? - International
32. What is the size of the setting? - National
33. What is the size of the setting? - Regional
34. What is the size of the setting? - Herds
35. What is the size of the setting? - Herd
36. What is the size of the setting? - Pen
37. What is the size of the setting? - Individual
38. What is the size of the setting? - Other

39. Comment
40. Which part(s) of the feed-human chain does the study cover? - Feed production
41. Which part(s) of the feed-human chain does the study cover? - Primary animal production
42. Which part(s) of the feed-human chain does the study cover? - Slaughter
43. Which part(s) of the feed-human chain does the study cover? - Food processing
44. Which part(s) of the feed-human chain does the study cover? - Human consumption
45. Which part(s) of the feed-human chain does the study cover? - Human illness
46. Which part(s) of the feed-human chain does the study cover? - Other
47. Comment
48. Is the study a qualitative or quantitative study i.e. what type of results is provided?
49. Which of the following does best describe the design of the study? - Controlled trial:
50. Which of the following does best describe the design of the study? - Randomized
51. Which of the following does best describe the design of the study? - Non-randomized
52. Which of the following does best describe the design of the study? - Observational:
53. Which of the following does best describe the design of the study? - Cross-sectional
54. Which of the following does best describe the design of the study? - Case-control
55. Which of the following does best describe the design of the study? - Cohort
56. Which of the following does best describe the design of the study? - Case-based
57. Which of the following does best describe the design of the study? - Primarily based on surveillance and monitoring data (e.g. prevalence study)
58. Which of the following does best describe the design of the study? - Outbreak description
59. Which of the following does best describe the design of the study? - Risk assessment
60. Which of the following does best describe the design of the study? - Review
61. Which of the following does best describe the design of the study? - Other
62. Comment
63. Are the outcomes of the study clearly described?
64. Are one or more representatives from public institutions (national institutes, ministries, university, etc.) included on the author list?
65. Should the paper pass to the next step?
66. Comment
67. Clear description of study population?
68. Is the sample size sufficient and are the samples representatives of the study population?
69. Are the primary (measured) study outcomes clearly defined?
70. Are the secondary (calculated) study outcomes clearly defined?
71. Is the statistical analysis adequate according to sample size and study design?
72. Is this study an analytical epidemiological study estimating risk-based outcomes?
73. Should the paper pass to the next step?
74. Comment
75. Are the study group(s) comparable to the population of interest with regard to confounding factors?
76. Are controls/non-exposed similar to cases/exposed except for the condition/exposure of interest?
77. Is there a clear case definition?
78. Is there a clear definition of the exposure(s)?
79. Is there a clear definition of the intervention(s)?
80. Does the study consider relevant confounding factors in the analysis (if not already done so in the study design by e.g. matching)?
81. Should the paper pass to the next step?

82. Comment
83. Is it an experimental or “real life”- intervention study?
84. Is randomization considered and handled appropriately?
85. Are the study groups similar at baseline?
86. Are the interventions clearly detailed for all study groups (e.g. dose, route, etc.) i.e. is the study clearly reproducible?
87. Should the paper pass to the next step?
88. Comment
89. Are the sources of the input data sufficiently described or referenced?
90. Are the assumptions clearly described and are they adequate and appropriate?
91. Is the model clearly described and appropriate?
92. Is the model qualitative (i.e. no quantitative measures provided; may be described in words e.g. low, medium and high)), semi-quantitative (i.e. some form of quantitative measures are used to rank the risks e.g. scoring on a scale) and/or quantitative?
93. Is the model deterministic (using point estimates only) or stochastic (using distribution; providing uncertainty)?
94. Should the paper pass to the next step?
95. Comment

APPENDIX 3: Complete list of references that passed the quality assessment

Ref. id. Reference.

2. Siegford, J.M.; Powers, W.; Grimes-Casey, H.G. 2008. Environmental Aspects of Ethical Animal Production. *Poult Sci.* 87. 2. 380-386.
21. Bucher, O.; Holley, R.A.; Ahmed, R.; Tabor, H.; Nadon, C.; Ng, L.K.; D'Aoust, J.-Y. 2007. Occurrence and Characterization of *Salmonella* from Chicken Nuggets, Strips, and Pelleted Broiler Feed. *J Food Prot.* 70. 10. 2251-2258.
28. Al-Zenki, S.; Al-Nasser, A.; Al-Safar, A.; Alomirah, H.; Al-Haddad, A.; Hendriksen, R.S.; Aarestrup, F.M. 2007. Prevalence and Antibiotic Resistance of *Salmonella* Isolated from a Poultry Farm and Processing Plant Environment in the State of Kuwait. *Foodborne Pathog Dis.* 4. 3. 367-373.
31. Callaway, T.R.; Edrington, T.S.; Anderson, R.C.; Byrd, J.A.; Nisbet, D.J. 2008. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. *J Anim Sci.* 86. 14_suppl. E163-172.
33. Kaneko, Y.; Katagiri, Y.; Ota, T.; Sakata, F.; Kobayashi, K. 2006. Plural infection by *Salmonella* O7 group that hospital infection was doubted. *Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi.* 17. 1. 33-39.
63. Lindqvist, N.; Pelkonen, S. 2007. Genetic surveillance of endemic bovine *Salmonella* Infantis infection. *Acta Vet Scand.* 49. 1. 15.
80. Harrison, T.M.; Harrison, S.H.; Rumbeiha, W.K.; Sikarskie, J.; McClean, M. 2006. Surveillance for selected bacterial and toxicologic contaminants in donated carcass meat fed to carnivores. *J Zoo Wildl Med.* 37. 2. 102-107.
86. Hora, R.; Kumar, M.; Kostrzynska, M.; Dixon, M.A.; Warriner, K. 2007. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* on artificially or naturally contaminated mung beans (*Vigna radiata* L) using a stabilized oxychloro-based sanitizer. *Lett Appl Microbiol.* 44. 2. 188-193.
93. Hald, T.; Wingstrand, A.; Brondsted, T.; Wong, D.M.A. Lo Fo. 2006. Human Health Impact of *Salmonella* Contamination in Imported Soybean Products: A Semiquantitative Risk Assessment. *Foodborne Pathog Dis.* 3. 4. 422-431.
95. McCrea, B.A.; Macklin, K.S.; Norton, R.A.; Hess, J.B.; Bilgili, S.F. 2006. A Longitudinal Study of *Salmonella* and *Campylobacter jejuni* Isolates from Day of Hatch through Processing by Automated Ribotyping. *J Food Prot.* 69. 12. 2908-2914.
125. Morita, T.; Kitazawa, H.; Iida, T.; Kamata, S. 2006. Prevention of *Salmonella* cross-contamination in an oilmeal manufacturing plant. *J Appl Microbiol.* 101. 2. 464-473.
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APPENDIX 4: Background information on feed production and consumption in Denmark

Total consumption of feed by the major food animal species (tons), 2009/2010 data

	Pigs	Cattle	Poultry	Total
Total	na	na	na	na
From feed producers (tons)	2.897.000	905.000	535.000	Na
Other feed	3.213.000 Minerals, soy, cereals, and suppl. feed	na	na	na
Cereals (tons)	na	na	na	6.058.000
Concentrated fodder (mio. FE) includes cereals from above)	na	na	na	9630
Coarse Fodder (mio. FE)	na	na	na	4929

na: Not available

Compounded feed production (tons) 2010

Pigs	Cattle	Poultry
2.888.000	905.000	535.000

The production and use of oil seed meals (tons)

2009	Soy products	Rape products	Products from other oil containing seed
Domestic production	0	346.400	49.100
Imports	1.463.200	293.600	239.100
Used for feeds	1.385.500	598.700	242.800

Imports of feedstuffs and feed ingredients (tons)

	2009
Soy products	1.463.200
Rape products	293.600
Other products of oil containing seeds	239.100
Fish products (meal, silage etc.)	378.700
Grain	64.100

Turnover and flow of feed ingredients per animal species

Figure A 4.1. shows the turnover and flow of feed ingredients in Denmark. They are used in the production of compound feed at feed mills and directly mixed by the farmer ("homemixers"). The figure is organized by animal species, but within the species the type of feed is further differentiated according to age and other criteria. The map is based on information from the feed industry and farmer advisors. The numbers in mio tons is the volume used as feed in 2005. However, no exact numbers are available for the amounts of feed. Stated here is a qualified estimation.

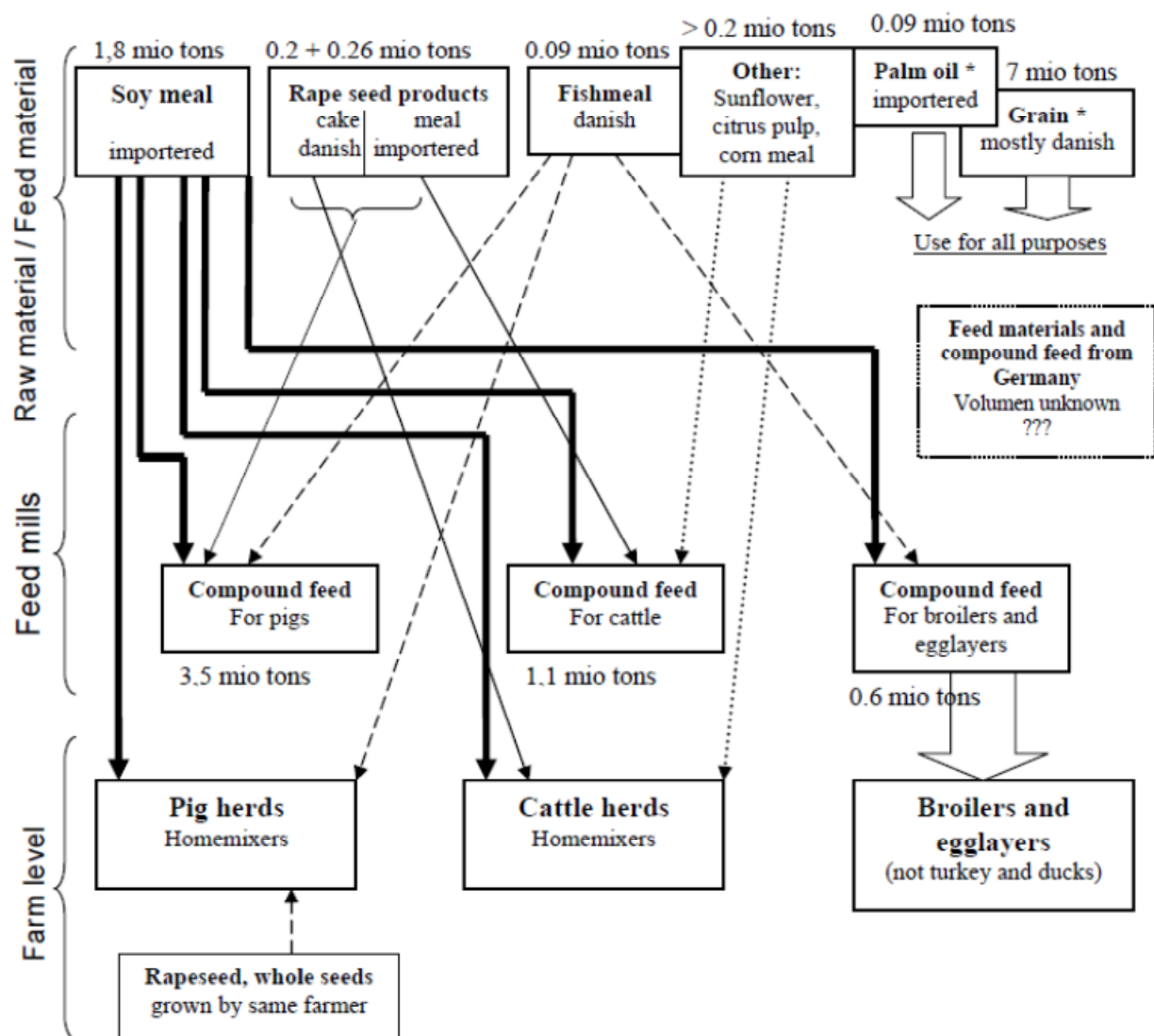


Figure A 4.1: Turnover and flow of feed ingredients in Denmark, 2005.

Processing of feed in Denmark

Feedstuffs ingredients are transported to the feed mills by trucks, railway and tankers, and deposited in storage bins. From the storage bins it is brought into the feed mills by pneumatic transport or in bags.

Most feed produced at feed mills is pelleted. Other feed is made by expansion, pellet cross or by intermixing with grain products.

Feed for poultry is heat treated at 81 C according to the industry code; “Regelsæt for god produktion af fjerkræfoder”. Feed for pig and cattle are produced according to legislation and most frequently also heat treated at 81 C.

Approximately 50% of the feed used for pig is home mixed based on soy products, cereals and mineral mixes or as supplementary feed supplemented with cereals. For cattle approximately 40 % is produced in mixers on farms, pelleted feed is supplemented to milk producing cattle. For poultry feed approximately 20 % whole grains is supplemented to the pelleted feed.

Feed and feeding systems for major farm animal species

Poultry are only given dried feed. For pigs approximately 40 % of the feed is applied as wet feed. In cattle most feed is fed as a mixture of fodder concentrates and coarse fodder. Additional pelleted feed is supplied for milk producing cattle.