The National Salmonella Control Programme for the Production of Table Eggs and Broilers

1996-2002

Ministeriet for Fødevarer, Landbrug og Fiskeri
Fødevaredirektoratet

The National Salmonella Control Programme for the Production of Table Eggs and Broilers 1996-2002

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The Danish Veterinary and Food Administration is part of the Danish Ministry of Agriculture, Food and Fisheries. The Danish Veterinary and Food Administration is responsible for the administration and control within food and veterinary areas "from farm to fork", as well as practical matters relating to animal protection (otherwise under the Ministry of Justice).

Making of regulations and co-ordination, take place in the Administrations center in Moerkhoej. The 10 Regional Authorities handle the practical inspection of food and veterinary matters, including import/export etc.

The central administration of The Danish Veterinary and Food Administration employ a staff of approx. 350 full-time employees, whilst the 10 regional authorities employ a further approx.1 500 full-time employees.

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1. Preface

The National Salmonella Control Programme for expanded control of salmonella in the broiler and table egg production processes is part of the Danish Parliament and Government's general objective of improving the quality of Danish foodstuffs, including their microbiological quality.

The editorial staff wishes to thank everyone who has contributed to the implementation of the National Salmonella Control Programme from 1996 to 2002. We wish to extend our gratitude to everyone who has contributed written material for this report, participated in the compilation and proofreading processes, and participated in the comprehensive practical work related to the National Salmonella Control Programme.

Our appreciation goes to sector representatives and producers, the staff of the Danish Poultry Council, Division Six of the Department of the Ministry of Food, Agriculture and Fisheries, Eurofins, the Danish Institute for Food and Veterinary Research, the Danish Zoonosis Centre, the Royal Veterinary and Agricultural University, Statens Serum Institut, the Danish Plant Directorate, members of the technical task groups for the broiler and table egg sectors, members of the steering committee, the district veterinary officers, the eleven regional veterinary and food control authorities (formerly the district veterinary offices), and the Food Department FA2 under the Danish Veterinary and Food Administration.

Division of Zoonoses, Veterinary Department VA02, Danish Veterinary and Food Administration
Mørkhøj, Denmark
6 February 2004

2. Summary

The National Salmonella Control Programme was launched in December 1996, and the first samples were solicited in the spring of 1997. An allocation of DKK 188.1 million was earmarked for the National Salmonella Control Programme that was planned to run for three years. The poultry industry contributed DKK 30 million of the DKK 188.1 million. DKK 62 million were still left in 1999, and the National Salmonella Control Programme was extended by another three years. The National Salmonella Control Programme was designed to be a 'top-down' control effort based on an elimination strategy, whereby the infected flocks were eradicated by means of compulsory destruction or slaughter. The National Salmonella Control Programme was revised throughout the process. The funding for the National Salmonella Control Programme expired on 31 December 2002, after which the poultry sector took over the administrative and financial responsibility, while salmonella control and prevention will continue to be under public surveillance. This means that the Danish Veterinary and Food Administration will retain control of the National Salmonella Control Programme in relation to the industry and the regional veterinary and food control authorities, just as the public sector shall set the goals of the continued efforts. For instance, the regional veterinary and food control authorities are responsible for the practical aspects of taking suspect samples, public inspection and sanctioning of any deviations from the National Salmonella Control Programme.

The National Salmonella Control Programme comprises all salmonella serotypes (except for the host-specific *Salmonella* Pullorum and *S.* Gallinarum, which cause fowl typhoid) in all links of the production chain, in the breeding and table egg sectors (including farm-gate sellers). The sample-taking programme includes serological and bacteriological analyses for obtaining maximum certainty that infected flocks are detected as early as possible. All serotypes arouse suspicion, after which the regional veterinary and food control authority takes suspect samples, though suspect samples are not taken from broiler flocks, however. If salmonella is found in these samples, the flock is declared infected and forced to comply with various restrictions.

After the expiry of the National Salmonella Control Programme on 31 December 2002, compensation will in future only be paid to parent flocks that are declared infected with *Salmonella* Typhimurium or *S.* Enteritidis. These flocks will be destroyed and the eggs will either be destroyed or sent to heat treatment. Parent flocks infected with other serotypes will be placed under public supervision and may not continue normal production, as the eggs must be sent to heat treatment. After the flock has been removed, cleaning and disinfection are compulsory in every situation (regardless of the salmonella status). The cleaning and disinfection procedures have to be approved by the regional veterinary and food control authority. Regardless of serotype, the infection of pullet rearing flocks for table egg production will cause the flock to be put under supervision without compulsory destruction or slaughter; they will not be allowed to continue to table egg production, to be sold or similar. Infected table egg flocks will be allowed to produce eggs, under the supervision of the regional veterinary and control authority, until they are slaughtered, but all eggs must be sent to heat treatment from the date of suspected infection.

The results in both sectors have been good: the percentage of positive broiler-production flocks at *ante mortem* (AM) inspection has declined from 12.9% in 1997 to 1.5% in 2002. Since the launch of the National Salmonella Control Programme, the percentage of flocks infected with salmonella in the breeding and parent flock segments has hovered around 1.2%, and as the detection of infection

has resulted in flock eradication, none of these flocks have spread salmonella down through the production pyramid. This is very important, because in Denmark we have only a few breeding flocks supplying many production flocks. The percentage of infected flocks in the table egg sector has declined from 13.4% in 1998 to 2.6% in 2002; the dominant serotype has been *S.* Enteritidis FT8.

This improvement in the primary production segment is reflected in a striking 59% decline in the number of registered human salmonellosis cases, from 5,015 in 1997 to 2,071 in 2002. The source-of-infection statistics show that the total number of human salmonellosis cases attributable to eggs has been reduced by 80% from 1997 to 2002. While 60% of the salmonella cases (totalling 3,009) in Denmark were egg-related in 1997, only 31% (totalling 636) in 2002 were attributable to eggs. The dominant serotype in this context is *Salmonella* Enteritidis.

DKK 110 million of the DKK 188.1 million were spent on indemnification for destroyed flocks, consequential loss, etc. This does not include the funding provided by the industry. Assuming that the incidence of human salmonellosis had remained the same as in 1997, the financial loss resulting from hospitalisation, lost working days, etc., would have cost society almost DKK 650 million, as up to 150,000 more persons would have been infected with salmonellosis from 1998 to 2001. It is worth remembering in this context that the actual incidence of human salmonellosis is estimated to be ten times greater than the number of cases reported to Statens Serum Institut by general practitioners. It is reasonable to conclude from this that the funds invested in the salmonella control effort have more than covered the expenses.

Even if the original goal of the Ministry of Agriculture and Fisheries to drastically reduce the number of human salmonellosis cases by reducing the level of salmonella in broiler flocks and table egg flocks to less than 5% had been met by the end of the National Salmonella Control Programme, the effectuation of additional improvements in primary production and in human health is still a political objective. Consumers who observe normal, sound kitchen hygiene in their cooking routines should not be threatened will illness from the food they eat.

3. Goals of the National Salmonella Control Programme

I. Original Primary Goals (1996-2002):

►to reduce the level of infected table egg flocks to less than 5%.

Subsidiary goal: To reduce the occurrence of salmonella in flocks by one-third every year.

►To reduce the percentage of infected broiler flocks to less than 5%.

Subsidiary goal: To reduce the occurrence of salmonella in flocks by one-third every year.

►To reduce significantly the occurrence of human salmonellosis related to Danish-produced poultry products.

II. Additional Goals (1998-2002):

■ To reduce the percentage of broiler flocks infected with *S*. Typhimurium to less than 1%.

■ To reduce the percentage of broiler flocks infected with *S*. Enteritidis to less than 1%.

■ to reduce the percentage of broiler flocks infected with exotic strains of salmonella to less than 2%.

4. Introduction

4.1 Public Institutions

The Danish Ministry of Agriculture was merged with the Ministry of Fisheries under the name of the Ministry of Agriculture and Fisheries by Royal Resolution of 27 September 1994. The name of the Ministry of Agriculture and Fisheries was changed to the Ministry for Food, Agriculture and Fisheries by Royal Resolution of 30 December 1996. On 1 July 1997, the Danish Veterinary Services and the National Food Agency were merged into the Veterinary Services and Food Administration. In 1999, the name was changed to the Danish Veterinary and Food Administration. On 1 January 2000, eleven regional veterinary and food control authorities were formed, thus replacing the district veterinary offices. The Danish Veterinary Laboratory merged with the National Veterinary Institute for Virus Research under the new name of Danish Veterinary Institute as of 1 January 2002. On 1 January 2004, the institute merged with the Institute of Food Safety and Nutrition under the Danish Veterinary and Food Administration, forming the Danish Institute for Food and Veterinary Research.

4.2 Background

The incidence of human salmonellosis rose in Denmark during the 1990s, attracting political attention to the problem. The Minister of Agriculture and Fisheries appointed a task group in 1994 to evaluate whether any precautionary measures were necessary to reduce human illness. The task group recommended that all links in the production chain should be tightened and that the salmonella level should be reduced to less than 5% as quickly as possible.

The poultry industry's voluntary control programme aimed at Danish broilers had been jointly established in the late 1980s with the Danish Veterinary Laboratory and Danish Veterinary Services. As a result broilers have been routinely inspected since 1989 through bacteriological *ante mortem* (AM) analysis roughly three weeks before slaughter. The public programme of AM inspection of all broiler flocks was implemented in 1992. This was followed in 1992 by a voluntary control programme for the Danish table egg sector. At the time, the voluntary control programme involved the testing of table egg layer flocks in conjunction with placement, as well as the testing of day-old parent chicks imported to Denmark. Danish table egg hatcheries contracted agreements with import enterprises, which stipulated that the importer had to pay the costs and deliver a replacement flock if salmonella bacteria or anti-salmonella antibodies were detected. This voluntary model has also continued after 1996, whereby day-old parent chicks in the broiler and table egg sectors are required to be salmonella-free at the time of purchase. This is effectuated through contractual demands made on the suppliers, various tests of the chicks on arrival to Denmark and by means of structural and work-flow requirements made on the non-Danish enterprises that supply these birds. Industrial approval of the enterprises is temporary and is to be followed up by additional inspection.

On 1 January 1994, Denmark became the first EU Member State to implement Council Directive 92/117/EEC, also known as the 'Zoonosis Directive'. This directive prescribes the routine bacteriological testing of all parent flocks for broiler and table egg production for *Salmonella* Enteritidis and *S.* Typhimurium, as well as the subsequent destruction of positive flocks. The EU reimburses 50% of member state expenditures for the destruction of flocks and hatching eggs.

Prompted by the sharp rise in the incidence of human salmonellosis, a hearing on the Danish salmonella situation was held in early 1996 (Pedersen, 1996; Wegener; 1996; Salmonella Hearing, 1998). The hearing was organised by the Danish Ministry of Agriculture and Fisheries and participants included representatives from the poultry industry, the Danish Consumer Council, the Danish Veterinary Laboratory, Statens Serum Institut, other authorities and politicians. The current situation for salmonella control efforts was presented and a specific action plan – far more comprehensive than the voluntary control programmes – was presented to solve the salmonella problems in the poultry industry. The National Salmonella Control Programme was approved by the Finance Committee of the Danish parliament by Act No. 325 of 29 May 1996. An overall budget of DKK 188.1 million was allocated for the implementation of the National Salmonella Control Programme over a three-year period. The purpose of the State's commitment was to assist the industry in solving a serious human health problem caused by poultry meat and table eggs contaminated with salmonella. During the course of 1996, the Danish Veterinary Services worked out specific legislation to effectuate the National Salmonella Control Programme. The Ministry of Agriculture and Fisheries laid down the condition that the industry should be responsible for paying

the cost of upholding the improvement of the salmonella situation that was expected to be achieved by the end of the three-year period and be given full responsibility for the salmonella control effort.

The first drafts of the 'Order on controlling *Salmonella* Typhimurium and *Salmonella* Enteritidis in table egg flocks and pullet reared for them' and the 'Order on controlling salmonella in hatching egg layer hens' were completed in February 1996. The National Salmonella Control Programme was implemented on 9 December 1996. The National Salmonella Control Programme expanded the monitoring to include all serotypes (except for *Salmonella* Pullorum and *S.* Gallinarum, as these are covered by special rules) in the parent flock segment, and at the time, a 'zero-tolerance strategy' was in effect for all production links that included the killing (destruction or slaughter) of flocks in return for full compensation, as well as the subsequent cleaning and disinfection of houses. Table egg and pullet-rearing flocks infected with *S.* Enteritidis and *S.* Typhimurium were similarly eradicated. The National Salmonella Control Programme did not include any new initiatives affecting broiler flocks as a salmonella investigation programme had already been initiated as part of the AM inspection procedure.

The original goal of the National Salmonella Control Programme was to limit the percentage of broiler and table egg flocks infected with salmonella to less than 5% at the end of the National Salmonella Control Programme. In addition, a subsidiary goal stipulated that the salmonella level in flocks had to be reduced by one-third every year.

A steering committee and two technical task groups, for the table egg and broiler sectors respectively, were appointed in 1996. A project manual was also compiled (1996) to serve as a scenario for the implementation. The manual was distributed to the members of the steering committee, both technical task groups, the Danish Veterinary Laboratory and Division Six of the Department under the Ministry of Food, Agriculture and Fisheries. In January 1997, announcements were published in various periodicals and newspapers informing producers of the new National Salmonella Control Programme for the prevention of salmonella. The first samples were solicited in March 1997.

Already during the first year of the National Salmonella Control Programme, it became apparent that salmonella was more widespread in the table egg sector than assumed at the start of the National Salmonella Control Programme. The destruction of pullet rearing flocks and table egg layer flocks was discontinued on 10 September 1997 owing to the fact that an acute shortage of Danish eggs would arise if the eradication strategy continued. Moreover, it was likely that adhering to the control strategy would soon drain the indemnification funding. As a result, the decision was made to focus exclusively on serological monitoring of the pullet rearing and table egg productions for a while, to chart the salmonella situation in these two areas.

This made it necessary to amend the National Salmonella Control Programme so that efforts could concentrate on ensuring salmonella-free hatching egg production, i.e., a 'top-down' model. An amended National Salmonella Control Programme was available in March 1998, which included the monitoring of all serotypes (except *Salmonella* Pullorum and *S.* Gallinarum), continuing the eradication of infected rearing flocks and intensifying the analysis of table egg flocks. From June 1998, table eggs for the retail sector came exclusively from flocks that had been analysed for salmonella. After the amendment of the National Salmonella Control Programme, infected table egg flocks were put under supervision which included the compulsory heat-treatment of eggs. The

programme was additionally tightened in late 1999, by requiring eggs to be sent to heat treatment already from the date of the suspected infection.

After a tragic event in 2000 in which two persons died from salmonella poisoning after eating a cake made with raw, infected eggs from a barnyard flock, the interest of farm-gate sellers and barnyard flock owners in knowing the salmonella status of their flocks increased dramatically. This meant that by the end of 2000, some 800 farm-gate sellers and roughly 1700 barnyard flock owners were registered under the programme, which was a respective four-fold and ten-fold increase over the year before. In 2001, the interest in participating in the National Salmonella Control Programme declined at the same time that sample-taking went from being publicly funded to a system based on user charges. In this conjunction, a reimbursement scheme was established that refunded 75% of the analysis costs for farm-gate sellers and table egg producers with fewer than 500 and 1000 birds respectively. This scheme was implemented because it was feared that many small producers would not register because of the high costs involved. The chronological sequence of the National Salmonella Control Programme is shown in Figure 1.

Figure 1: Timeline for the National Salmonella Control Programme and other initiatives

1989	1992	1992	1994	1996	1997	2000	2003
Voluntary plan, broiler sector	Public AM inspection, broilers	Voluntary plan, table egg sector	Implementation, Zoonosis Directive	Development of the National Salmonella Control Programme	The three-year National Salmonella Control Programme begins	New three-year period, National Salmonella Control Programme	The National Salmonella Control Programme is transferred to the poultry industry

4.2 Organisational Structure

The project organisation included a steering committee, two technical task groups and a project group (including representatives from the Veterinary Department of the Danish Veterinary Services).

The steering committee was given the executive responsibility for the wording and scientific veterinary content of the National Salmonella Control Programme, for approving all amendments as well as for the project budget. The steering committee was comprised of representatives from the project group, the Danish Veterinary Services, the Danish Veterinary Laboratory, Statens Serum Institut (SSI), the Danish Poultry Council and the Department of the Ministry of Agriculture and Fisheries. The Chief Veterinary Officer was appointed chairman.

The two technical task groups for the table egg and broiler sectors respectively were chaired by the Danish Veterinary Laboratory and the members came from the project group, the Food Department of the Danish Veterinary Services and the Danish Poultry Council, and also included producers. The technical task groups were given the responsibility for the technical aspects related to the preparation and implementation of the National Salmonella Control Programme, including sample

taking, analysis techniques, hygiene, cleaning, disinfection, training, improvement suggestions and more.

The project manager (from the Division of Zoonoses, VA02) was put in charge of the project group and of implementing practical activities related to the National Salmonella Control Programme. A project manual was also prepared. Samples were solicited and registered in a database developed by the Danish Veterinary Services. The project group was also put in charge of implementing compulsory destruction and compensation payments. The project group initially comprised a project manager, three veterinary officers and two clerks, and was subsequently enlarged to include a financial manager.

The consulting unit at the Danish Veterinary Laboratory was staffed with two veterinary surgeons and two laboratory technicians and functioned from 1997 to 2000. The purpose of the unit was to provide consultancy to salmonella-afflicted enterprises and holdings, as well as provide technical assistance to the project group related to training, eradication descriptions and good manufacture practices. The unit worked closely together with the project group and the industry's consultancy institution, i.e., the National Agricultural Advisory Service in Skejby, which was given responsibility for consultancy tasks during the course of 2000 under the National Salmonella Control Programme.

After the funding expired for the National Salmonella Control Programme at the end of 2002, the steering committee and the technical task groups continued their work, thereby providing continuous monitoring of the continuation of the National Salmonella Control Programme. The Danish Veterinary and Food Administration is represented in these groups by an employee from the Food Department (FA2) and by an employee from the Veterinary Department (VA2). After the project group is disbanded, an employee of the Veterinary Department (VA2) of the Danish Veterinary and Food Administration will still be assigned to manage the industry's take-over and continuation of the National Salmonella Control Programme.

4.3 Poultry Production Structure

Poultry production is divided into the table egg sector and the broiler sector. A common feature of these two production forms is that only a few flocks of élite and parent birds foster the chicks which become either table egg layers or broilers. Poultry production is a pyramidal system with only a few birds at the top and many at the bottom of the pyramid (Fig. 2 and Table 1).

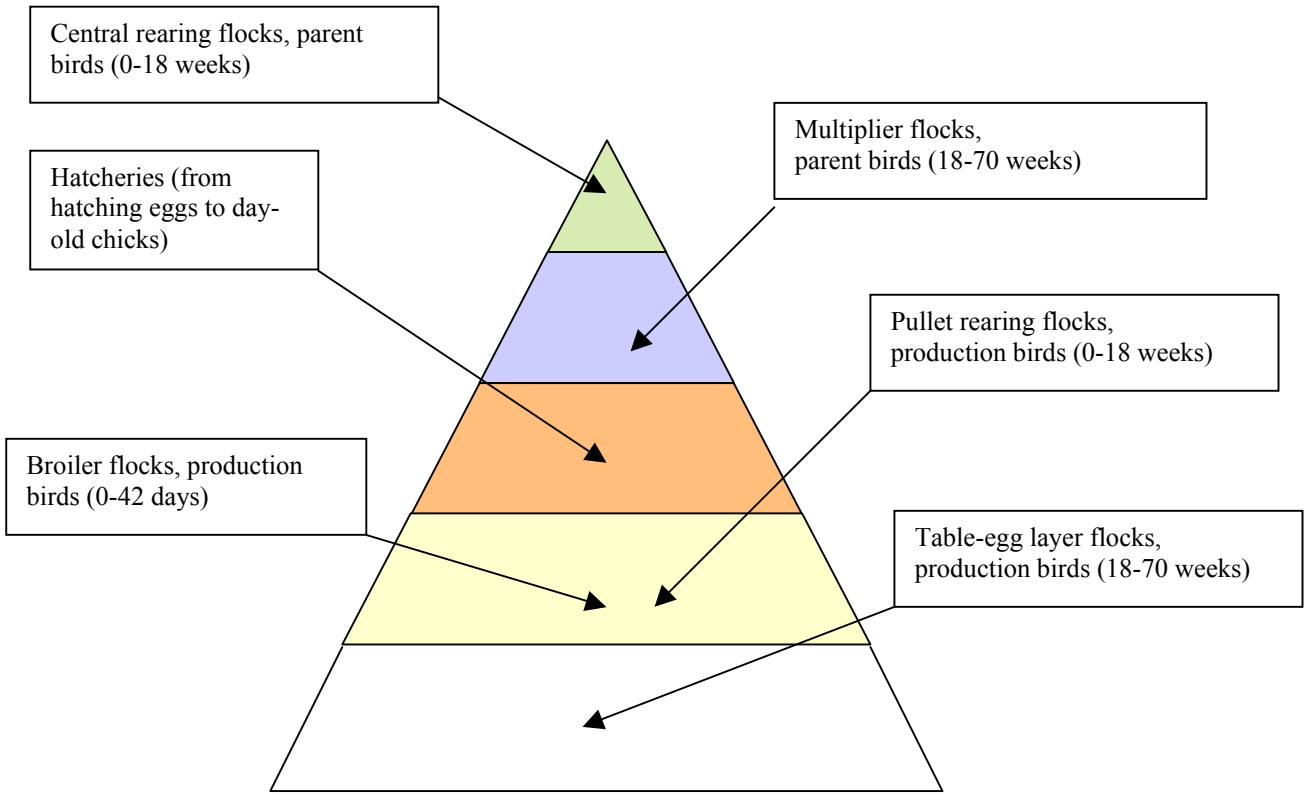


Figure 2: Pyramidal structure of poultry production

The poultry breeding sector currently consists of only a few multinational companies, in which two broiler-rearing companies are responsible for most of the chickens produced world-wide. The market is almost equally as concentrated in table egg poultry breeding, but on a worldwide level, 3-4 of the largest breeding companies are responsible for most of the layers used for egg production.

More than 90% of the day-old parent chicks for broiler production are imported from Sweden. Sweden imports the élite birds from a breeding company in the UK, but as Sweden cannot fully exploit the production from these imports, the surplus birds are sold to Denmark. At the same time, this provides great certainty that salmonella bacteria are not present in the imported flocks and is financially beneficial for Sweden and Denmark alike. The remaining birds are imported as day-old parent chicks from the UK, where the industry must comply with special salmonella requirements. All purchases are made via a small company that was founded for this purpose.

For table egg production, day-old parent chicks are imported from two companies, either in Germany or from France and/or the UK.

The industry has laid down special conditions related to the control of salmonella, and samples are taken from all flocks of parent chicks before they are placed in Danish poultry houses to ensure that the chicks are salmonella-free when they arrive in Denmark.

Day-old layer chicks are also imported, i.e., the same chicks that are subsequently used as layers in the table egg production process. These chicks are imported from a Swedish hatchery which via the industry is subject to the same strict salmonella control as in Denmark, as the Swedish control programme is not as comprehensive as its Danish counterpart. The purchases are not centrally organised as in the broiler sector.

The distribution of holdings, houses and birds is shown in Table 1. It is worth noting in this regard that the top of the pyramid consists of a few large enterprises with a relatively large number of houses. Hatching-egg production in the table egg sector in particular has always been executed by only a few companies.

Table 1: Number of holdings, houses and birds in the broiler and table egg sectors, 2002.

	Holdings	Houses	Birds
Broiler sector			
Central rearing	28	117	1,244,000[a]
Multiplier flocks	68	207	1,180,000
Hatcheries	7	-	-
Broilers	350	839	143,287,000[b]
Table egg sector			
Central rearing	7	8	60,000[a]
Multiplier flocks	7	12	60,000
Hatcheries	5	-	-
Pullet rearing	112	177	3,500,000[b]
Egg layers, except farm-gate sales	305	429	3,624,787[c]

a) Annual purchases. Source: Danish Poultry Council.
b) Hatched for use in Denmark.
c) Including hens imported as day-old chicks.

5. Salmonella: Characteristics and Epidemiology

5.1 Aetiology

Salmonella consists of two species, *S. enterica* and *S. bongori*. *Salmonella enterica* is subdividable into some 2,500 serotypes of significance to the National Salmonella Control Programme, from which the two host-specific serotypes for poultry, i.e. *Salmonella* Pullorum and *S.* Gallinarum, are excepted, however. Salmonella is a gram-negative bacillus that grows on agar and other simple media. Salmonella ferments glucose but not lactose and can reduce nitrate to nitrite (Jordan & Pattison, 2001).

With the aid of rabbit antisera, salmonella bacteria are classified into serotypes based on the epitopes of two surface antigens, O and H. The O antigen is attached to the surface (the cell wall), while the H antigen is attached to the bacterium's motility appendage (the flagella). Each salmonella bacterium has a specific pattern of O and H epitopes. Approximately 3000 different serotypes have now been identified on the basis of these antigen and epitope combinations. Serotype analysis usually takes two or three days, yet sometimes even longer. In a few instances, the serotype defies identification. This occurs if a bacterium's cell wall or flagella are damaged, for instance. Serotype identification makes it possible to rule out or confirm various hypothetical sources of a given salmonella outbreak.

The serotypes *S*. Enteritidis and *S*. Typhimurium can be additionally classified in phage types, depending on their reaction patterns to bacteriophages, i.e., virus-like particles that selectively attack bacteria (cf. 6.3.2.1).

5.2 Pathogenesis

The number of salmonella bacteria in a food product that are needed to induce illness (i.e., the infective dose) varies. In some instances, 10 bacteria per gram of foodstuff are sufficient to give an infection, but usually the dose must be far greater (i.e., > 100,000 bacteria/gram). The infective dose of fatty foods is low, as the salmonella bacteria are protected by the fat, enabling them to resist being acted on by the gastric juices. A person's general health is also important to the amount of the infective dose. A small dose can induce illness in persons who are weakened to start with, while the symptoms are not as pronounced in persons who are in good health.

5.3 Clinical Symptoms

Salmonella bacteria can cause salmonellosis in humans and animals.

Although poultry disease is rarely caused by salmonella, sub-clinical infection is common (Jordan & Pattison, 2001). However, poultry production has previously had considerable problems with *S.* Gallinarum and *S.* Pullorum (fowl typhoid). Salmonellosis is most common in chicks less than two weeks old and is relatively rare in birds after the age of four weeks. Although morbidity and mortality rates vary, they are usually less than 20%. The symptoms include depression, reduced motility, closed eyes and dishevelled plumage. Infected birds develop diarrhoea and dirtied plumage around the cloaca. When poultry are infected with salmonella after the age of four weeks, the

bacteria can colonise the intestines. In some hens, the infection is latent, and if a stressful situation arises later on, the bird may start to excrete bacteria again, such as around the start of egg laying (Skov *et al.*, 2002).

Salmonellosis is one of the most frequent, food-borne infections in humans. Although all salmonella serotypes are potentially pathogenic, the host-specificity and ensuing clinical symptoms vary greatly. The most frequently isolated serotypes pathogenic to human beings are *S.* Enteritidis and *S.* Typhimurium, which are also the most widespread types in Danish livestock production. The symptoms of human salmonella infection are pyrexia, stomach pains, headache, vomiting and diarrhoea. In a few instances, the infection can lead to complications such as blood poisoning (septicaemia) and can also prolong the course of the illness.

5.4 Epidemiology

Humans, rodents, wild birds, insects, feedingstuffs, bedding, water and farm implements can all transmit the bacterium to chickens. Salmonella infects the intestinal tract of animals and humans from where it is excreted in the faeces. *S.* Enteritidis in particular is capable of infecting the egg-laying hen's ovaries, which can cause the salmonella to be localised inside the egg, whereas other types of salmonella are usually found in the faecal residue on the outer surface of the eggshell. If salmonella becomes established in a multiplier flock, for instance, it can infect through the eggs and move on to the hatched chicks by means of ovarian transmission. As a result, *Salmonella* Enteritidis can infect day-old chicks, table eggs or ready-made foods made from the eggs. The occurrence of *S.* Enteritidis inside the eggs is believed to be the cause of the vast majority of human infections of this serotype. It is generally assumed, therefore, that salmonella can spread through a multitude of possible channels, as the bacterium can be transmitted vertically (from mother to potential offspring) and horizontally, i.e., faeces-borne (direct from person to person or indirect via tools, implements, etc.).

5.5 Prevention

Salmonella is very resistant to drying. For this reason it is very important to properly clean and disinfect the houses between each poultry flock and after removing an infected flock, to prevent the infection from continuing. It is also important to optimise biosecurity (e.g., pest control), feedingstuffs monitoring, manure handling, and more.

5.6 Multi-resistant *Salmonella* Typhimurium DT104

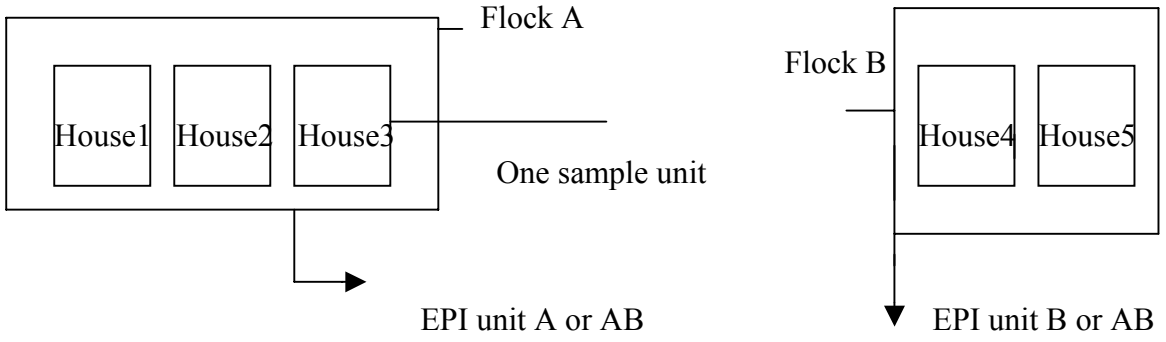
A multi-resistant strain of *Salmonella* Typhimurium DT104 has been a problem in Europe and the USA since the 1990s. The bacterium's resistance to several types of antibiotic may bring about treatment failure when treating persons with ordinary antibiotics. The bacterium often develops additional resistance. It has also proven its ability to spread quickly throughout flocks and from one flock to another. For this reason control programmes have been carried into effect for all livestock species, including cattle, pigs and poultry. Although the destruction of all infected flocks and herds is no longer required, the regional veterinary and food authorities can order a herd/flock to be slaughtered, with subsequent heat treatment of the meat.

6. Materials and Methods

6.1 Terminology: Animal Units and Types of Production

A flock is defined as a group of chickens that constitute an operational and age-related unit. The flock can be comprised of many birds placed in different houses. Each house is the equivalent of one basic sample unit. The concept of consequential unit (epidemiological unit) is used in the hatching egg and table egg segments alike, because it has been decided that the detection of salmonella in one house can affect one or possibly several flocks. By definition an epidemiological unit is a group of poultry that do not have infective contact with other hens. The epidemiological units of each enterprise are defined by the Danish Veterinary and Food Administration (Fig. 3).

The National Salmonella Control Programme involves salmonella analysis of all hatching egg flocks, pullet rearing flocks and table egg layer flocks for the resale of eggs, including farm-gate sales. Owners of barnyard flocks are not included in the National Salmonella Control Programme, and the analysis of this poultry category is based on voluntary participation.



In terms of the parent-animal segment, flocks A and B may belong to separate EPI units or the same EPI unit.

Figure 3: The concepts of sample unit, house, flock and epidemiological unit.

6.2 Sample Material

Under the National Salmonella Control Programme, bacteriological and serological analyses are used for detecting salmonella. The submitted samples include faeces samples, sock samples with faecal matter, blood, eggs, hatching dust, crate litter and destroyed hens:

Sample material is taken from the intestines and organs of the chickens and is bacteriologically analysed.

Sock samples consist of moistened tube gauze pulled on over clean rubber boots, after which the wearer walks through the poultry houses until the 'socks' have absorbed faecal matter. Faeces is taken as samples of fresh faeces (a pooled faeces sample is made up of a number of separate samples of fresh faeces). Both sample types are bacteriologically analysed. During the course of the National Salmonella Control Programme, the sample type was changed in favour of sock samples. Several studies have shown that 5 pairs of sock samples are just as sensitive as 12 pooled faeces samples of 50 samples each (Skov *et al.*, 1999). In addition, the sock procedure is far more practical to carry out than the taking of faeces samples.

Hatching dust samples are taken at the hatcheries after each hatching. The sample consists of dead chicks, as well as eggshells and meconium residue (the first faeces excreted by chicks). Crate material samples may consist of the linings of crates for day-old chicks and of destroyed day-old chicks. Both of these sample types are bacteriologically analysed.

Egg and blood samples are serologically analysed to detect anti-salmonella antibodies.

6.3 Methods

The Danish sample-taking programme has employed a combination of serological and bacteriological analyses to obtain maximum certainty that positive flocks would be detected as quickly as possible. Flocks that are serologically positive do not always excrete sufficient amounts of bacterial matter to enable bacteriological detection. Conversely, some bacteriologically positive flocks never induce a serological response. These differences can be explained by the variation in time when the infection was introduced, the amount of the infective dose, the resulting level of infection and other factors (Skov *et al.*, 2002). In conclusion, while the less sensitive process of bacteriological analysis can obviously detect the actual presence of the bacterium, the more sensitive process of serological analysis reveals whether the bird has been infected with salmonella (Hald & Ekeroth, 2001).

6.3.1 Serology

The serological method is based on the fact that an organism infected with salmonella bacteria will try to combat the infection by producing antibodies aimed at the invading micro-organism. Antibodies and other mediators are involved in combating bacteria. As antibodies have relatively long half-lives, measuring their presence serves as a viable indicator of whether the bacterium is or has been present.

Antibodies are found in relatively high concentrations in the blood, but they can also be measured in other tissue fluids. Antibodies can therefore also be measured in egg yolks, although in a lower concentration.
The serological tests are based on the underlying principle that the salmonella antigen lipopolysaccharide (LPS) adheres to the bottom of a plastic well. Next, a serum sample or a sample from an egg is added, and if they samples contain antibodies against the antigen, they will adhere to it. When the well is subsequently washed, only the bonded antibody will remain. Then a reagent (conjugate) is added which is capable of binding to the antibody and catalysing a colour reaction at the same time. After one more wash and the admixture of a colour reagent, the enzyme will catalyse

a colouring reaction (if it is bound in the well) which can be terminated after a while using a stop reagent. Thus, the intensity of the colour becomes an indicator of the positive intensity of a sample. By isolating a surface antigen from salmonella – which is specific to this bacterium and at the same time has a texture which makes the host animal produce antibodies against it – it becomes possible to design tests that are specifically aimed at salmonella, for instance.

The serological tests applied under the National Salmonella Control Programme have used lipopolysaccharides (LPS) from *S.* Typhimurium, *S.* Enteritidis and *S.* Infantis. The advantage of LPS is that the host animal reacts strongly to some of the antigen structures (epitopes) on this substance. A possible drawback is that these epitopes can appear in several strains of salmonella, which means that the test detects not only one serotype. Some of these structures have been described and numbered. They are used in the bacteriological typing of salmonella. As a result, *S.* Typhimurium has 1,4,5 and 12, *S.* Enteritidis has 1,9 and 12 and *S.* Infantis 6 and 7. As *S.* Typhimurium and *S.* Enteritidis clearly have 1 and 12 in common, it would be impossible to separate these two types serologically using the applied antigens.

6.3.2 Bacteriology

The purpose of the bacteriological study is to detect the presence of salmonella bacteria in the analysed material. If the material contains bacteria, these are typed. A knowledge of which salmonella types have been detected in various contexts is used to illustrate modes of transmission, which is a prerequisite for successful control.

Salmonella is traditionally detected through cultivation. Although salmonella apparently thrives well in livestock, barn environments, livestock faeces, feedingstuffs, foodstuffs and human beings, the bacteriological detection of salmonella involves a relatively difficult, lengthy analysis procedure. The analysis consists of five steps and is performed in accordance with standard international methods for detecting salmonella:

In the first step (Day 1), the sample material is transferred to a liquid substrate devoid of bacteria inhibitors. The cultured substrate is incubated under temperature conditions that promote the ideal growth of pathogenic bacteria (i.e., non-selective enrichment). The first step is necessary because the bacteria in the materials being analysed under the National Salmonella Control Programme often occur in such low numbers that they cannot be detected through direct cultivation. In addition, this step enables any bacteria that are damaged by the action of disinfection agents, etc., to become capable of growing normally. In the first step, many other bacteria from the sample material besides salmonella will also grow. This particularly involves other bacterial species that are closely related to salmonella (such as *E. coli*, etc.).
In the second step (Day 2), an attempt is made to remove as many of the other bacteria as possible by transferring the cultured material obtained in the first step to one or more liquid substrates. These substrates contain substances that inhibit almost all other bacteria except salmonella (i.e., selective enrichment) and some substrates do so under temperature conditions that also inhibit the growth of many other bacteria. The choice of substrates and cultivation temperature depends on the material to be analysed. Cultivation during the second step also has an inhibiting effect on salmonella bacteria, but they are not inhibited to the same extent as many other bacteria. This step is necessary for removing as many of the other bacteria as possible that might be able to disturb the next step.
In the third step (Day 3), the cultured material is transferred from the substrates of the second step to agar plates containing substrates in which salmonella and salmonella-like bacteria take on an

unusual colour or dye the surrounding substrate with an unusual colour. This step makes it possible to see whether the sample contains bacteria that could be salmonella.
In the fourth step (Day 4) the above-mentioned agar plates are read. If bacteria resembling salmonella are not visible on the agar, then the result is negative and the analysis is concluded. If salmonella-like bacteria are detected on the agar, additional analyses must be made to rule out or confirm the suspected presence of salmonella. Using a variety of salmonella antisera that can detect a broad spectrum of various salmonella types (polyvalent antisera), it is possible to test whether the bacteria are salmonella. If this test is positive, a typing is carried out to identify the isolate.
In the fifth step, a more detailed identification process is carried out on the detected bacteria strains. The identification process primarily consists of serotyping.

The EiaFoss® salmonella method consists of an enrichment step followed by an automated ELISA-analysis. The analysis is a quick method in which a negative result is available within 1-2 days. The ELISA method involves an immunochemical assay of antigens, in this case the flagella of the salmonella bacteria. For this reason the culture in the enrichment medium is killed by boiling before the actual ELISA analysis begins. This means that bacteria cannot be isolated for confirmation and identification if the results are positive. This is why parallel cultivation using the EiaFoss® analysis method is carried out.

6.3.2.1 Phage typing

If the detected serotype is either *S.* Typhimurium or *S.* Enteritidis, it can be further identified by performing phage typing. Bacteriophages are virus-like particles that attack bacteria. Bacteria are resistant in different degrees to a specific type of bacteriophage. This is apparent by spreading a bacterial colony on the surface of an agar plate and then adding successive drops of a suspension of each the bacteriophages that can attack the given bacterial species. The following day, the agar surface will be blanketed by bacteria. But wherever bacteriophages have been added, an entire range of reactions will appear, from no effect of the bacteria within the diameter of the drop, to the killing of all the bacteria within the area. Combinations of qualitative and quantitative reactions to a panel of specific bacteriophages make it possible to classify bacteria into phage types. Based on the bacterium's phage type, other transmission modes can be rendered probable.

6.3.2.2 Sensitivity test

A sensitivity test to antibiotics is performed on all isolates of *S.* Typhimurium. This is done to detect the multi-resistant phage type DT104.

6.3.2.3 Other methods

In special cases, various salmonella isolates undergo further analysis by means of genetic engineering and biochemical methods. This enables some serotypes and phage types to be classified even further. These methods are not routinely used in conjunction with the National Salmonella Control Programme, but have been included in various infection-tracking investigations.

6.3.3. Analyses applied in the National Salmonella Control Programme

Two different serological tests have been used in the National Salmonella Control Programme.

1) In mix-ELISA, antigens from *S.* Typhimurium and *S.* Enteritidis have been combined thereby constituting a relatively broadly applicable salmonella test (Feld *et al*., 2000; Gradel *et al*., 2001). The test, which also covers *S.* Gallinarum and *S.* Pullorum, has been used for all flocks that had to be serologically analysed.

2) The ELISA test for *S.* Infantis has been used in the case of rearing flocks and hatching egg flocks to ensure that this serotype would also be detected serologically. This analysis procedure was discontinued in early 2000, as *S.* Infantis was rarely detected in poultry flocks.

In conjunction with the National Salmonella Control Programme, two methods of bacteriological testing were used, i.e., the cultivation method and the EiaFoss® method. The EiaFoss® salmonella method is primarily used for analysing hatching dust. The poultry industry has contracted an agreement with Eurofins A/S, formerly 'Alfred Jørgensen's Laboratories', that puts Eurofins A/S in charge of the bacteriological analyses of routine samples. If salmonella is detected in the samples, the isolate is sent to the Danish Institute for Food and Veterinary Research to be serotyped.

7. The Salmonella Database

At the beginning of the National Salmonella Control Programme, the Danish Veterinary Services developed a database in which all analysis results were entered on an ongoing basis.

In 2001, the industry, represented by the Danish Poultry Council, assumed responsibility for the Salmonella Database. The present database is based on the original version developed by the Danish Veterinary Services. The Danish Poultry Council receives files every day from the Danish Veterinary and Food Administration and from Eurofins containing laboratory results and adds them to the database, a procedure which was performed by the project organisation at the beginning of the National Salmonella Control Programme. At present, the database is accessible via the Internet, and can be accessed by anyone with a username and password. The database is a daily tool in the work of the Veterinary Department of the Danish Veterinary and Food Administration and Denmark's eleven regional veterinary and food control authorities. All poultry producers are registered in the database under a CHR number (a unique number of the Central Husbandry Register). A CHR number is affiliated with a specific geographic locality. An enterprise with an affiliated CHR number is usually subdivided into houses. At the time of registration, each house is assigned a unique number. Each flock placed in a house is assigned a flock identification number, also referred to as the rotation number. This number is used in the database for identifying the flock concerned.

Samples are solicited automatically via the database for table egg flocks, farm-gate flocks and barnyard flocks. Samples from a flock are entered under the flock's rotation number, so it is always possible to go back and find samples from a specific flock. Sample-taking reminders are also sent via the database. If necessary, the regional veterinary and food control authority takes the compulsory samples at the producer's. When entering a positive laboratory result, a suspected infection is created under the CHR and house number concerned. A summary of suspected flocks is presented in a special view, making it possible to follow the flocks at all times until they are either declared infected or the suspected infection is withdrawn. The Danish Poultry Council updates weekly lists of producer status for any packing enterprises that have requested them. This list parallels the database and can also be accessed via the Internet.

Data from the Salmonella Database is updated every week in a parallel database at the Danish Veterinary and Food Administration, where additional searches can be performed as needed.

8. Consultancy for Poultry Producers

8.1 Consulting Unit

In conjunction with the implementation of the monitoring and eradication initiatives against salmonella under the National Salmonella Control Programme, a consulting unit for poultry producers was founded in early 1997. The consulting unit included two veterinary surgeons working with and in charge of broiler and table egg production respectively, and the veterinary surgeons were based in Århus at the Danish Veterinary Laboratory's division for poultry, fish and fur-bearing animals. The purpose of the consulting unit was to provide advice regarding problem identification, destruction procedures and the prevention of spread of infection from flocks infected with salmonella.

In conjunction with the continuation of the National Salmonella Control Programme in 2000-2002, it was agreed that the consulting function should be gradually turned over to the poultry industry. This transfer of functions took place during the first half of 2000, whereby the Advisory Service Centre for Agriculture (now the Danish Agricultural Advisory Services, National Centre for Poultry), Skejby, assumed responsibility as from 1 July 2000 for the consultancy aspects concerning the National Salmonella Control Programme. As a result of this, the consulting unit at the Danish Veterinary Laboratory was phased out at the same time and discontinued its activities in 2000.

8.2 Consultancy Database

Early in the process, an Access database was set up at the Danish Veterinary Services in which all case information regarding positive and negative salmonella results (bacteriological and serological) was registered along with other information, such as which hatching egg flocks had supplied chicks to broiler flocks infected with salmonella. This type of database was necessary for getting a fast, general idea of the possible sources of infection in the event of new salmonella outbreaks. Bacteriological file information was continuously entered in the database in conjunction with journal approvals, whereas serological salmonella-positive file information (i.e., involving at least 2 birds with an antibody value of OD% > 40) was sent by telefax from the project organisation. The creation of a consultancy database was essential for providing effective consultancy in the initial phase of the National Salmonella Control Programme. The need for an independent consultancy database was reduced by the ongoing development of the database at the Danish Veterinary and Food Administration, and the salmonella consultancy services are now based on the information in the Salmonella Database, which as mentioned is run by the Danish Poultry Council.

8.3 Consultancy in the Broiler Sector

The implementation of the National Salmonella Control Programme in December 1996 did not include new sample-taking procedures for broiler flocks, but because they are already included in an integrated "farm to fork" concept and because much of the consultancy has involved these flocks,

they are included in the description. But one should bear in mind in this context that the occurrence of salmonella at the bottom of the production pyramid among broiler producers greatly depends on the top of the breeding pyramid, where parent flocks and hatcheries have played integral roles in the National Salmonella Control Programme.

AM samples have been taken from all broiler flocks since 1992, and samples pursuant to the Zoonosis Directive (92/117/EEC) have been taken from parent flocks since 1994. As a result, the implementation of the National Salmonella Control Programme can be viewed as a tightening of the existing sample-taking programme in a sector that is accustomed to participating in a monitoring process. There have been systematic procedures and openness regarding all information that was required for making an effective infection-tracking effort.

The consultancy for the broiler sector is divided into three general functions:

1. Monitoring the salmonella situation, including relations between specific types of salmonella and possible sources of infection, e.g., hatcheries or feed enterprises.
2. Tracking down the sources of infection by detecting salmonella infection in the breeding segment and/or at the hatchery.
3. Consultancy regarding the elimination of an already detected infection at a holding.

8.3.1 Parent flocks

The infection sources were tracked down after the infection had been officially confirmed in breeding, central rearing or multiplier flocks. In direct continuation of this, the flock owner, the affiliated hatchery and the district veterinary officer were contacted and offered a consultancy visit at the flock.

The consultancy visits were often carried out with the above-mentioned parties to facilitate a coordinated effort. The surroundings and the actual poultry houses were inspected and the person in charge of the flock was asked – using a systematised questionnaire – about various matters and procedures. If needed, samples were taken for the purpose of tracking down the source of infection. After the visit, and following any sample results and/or additional analyses, a report was prepared, which primarily illustrated the possible sources of infection and circumstances that would enable salmonella to persist, and which also included recommended measures for preventing this. The report was sent to the flock owner, the hatchery and the district veterinary officer. Consultancy visits and subsequent drafting of reports were made at 1 élite flock and 11 parent flocks from 1996 to 2000.

8.3.2 Broiler flocks

As a result of the intensified sample-taking procedures and monitoring, as well as the phage-typing of *S.* Enteritidis and *S.* Typhimurium under the National Salmonella Control Programme, it was discovered that new infection of broiler flocks with these two types of salmonella often originated at the hatchery. Most broiler houses contaminated with *S.* Enteritidis or *S.* Typhimurium, however, could produce salmonella-free broilers one or a few rotations after this infection had occurred. As new infection cases from other sources were only sporadic, it was concluded that most finds of salmonella in broiler flocks that were not type *S.* Enteritidis or *S.* Typhimurium were traceable to

samples from the same houses or enterprises (i.e., persistently infected broiler houses or broiler enterprises).

8.3.3 Persistently infected broiler flocks

The persisting types of salmonella, usually *S.* Infantis, S. 4.12:b:- or *S.* Indiana, had often persisted for years despite optimised cleaning and disinfection procedures, rodent control and hygiene barriers.

Therefore a control programme was outlined in early 1997 for broiler flocks that were persistently infected with salmonella. Under the programme all flock owners afflicted with persistent salmonella infections were offered a consultancy visit. The visits were often held in cooperation with hatchery consultants and a few times also with slaughterhouse representatives or the district veterinary officer. In principle, the consultancy visits followed the same procedure as visits to parent flocks infected with salmonella. Less attention was paid to the sources of infection, however, and more to the initiatives related to the elimination of the salmonella infection. From April 1997 to December 1998, consultancy visits and subsequent reporting were carried out at 46 broiler flocks, 5 of which involved follow-up visits. In addition to this and in conjunction with an initiative by a slaughterhouse, 4 of these visits to persistently infected broiler flocks included consultants from the slaughterhouse.

All data from each consultancy visit was entered in a statistics database in order to identify general trends that could explain why certain types of salmonella persisted in some flocks but not in others. Even so, dividing them into problem houses and control houses proved difficult, owing to the different times of infection and to the many rotations in which a type that had not been detected, would often reappear later on. For this reason, the visits were expanded to include all broiler houses that were infected with *S.* Enteritidis FT8, and/or *S.* Typhimurium DT66 in spring 1997. In this case the source of infection had been recognised as being two infected parent flocks and it was possible to subsequently classify the houses as problem houses (infected with one or both types in two or more rotations) and control houses (that only had been infected with one or both types in one rotation). A total of 78 broiler houses dispersed over 42 enterprises were included in this study, which concluded with a report and a scientific article (Gradel & Rattenborg, 2003).

8.4 Consultancy in the Table Egg Sector

The primary element of the consultancy offered by the Danish Veterinary Laboratory to owners of flocks infected with salmonella was free assistance in eradication planning.

8.4.1 Parent flocks

Shortly after the start of the programme, intensive sample-taking showed that many salmonella outbreaks could be traced back to flocks related to the same hatchery. At first most of the consultancy effort was therefore targeted here in an attempt to clarify the transmission modes and risks and to draw up suggestions for eradication and the prevention of infection re-entry (‘biosecurity’). The effort was made in cooperation with the district veterinary officer involving meetings with the hatchery management and staff, the inspection of houses, facilities and movement to and from the hatchery, sample-taking in and around the infected houses, as well as the drafting of reports with recommendations for ideal elimination of and protection against infection. The first

experiments involving the heat-treatment of battery cage systems took place in one of the hatchery's parent flock houses fitted with family cages. Samples were taken for bacteriological analysis during the process, and the experiment eventually formed the basis of subsequent heat-treatment projects (cf. chapter 15). The flock visits were dispersed as indicated in Table 2.

Table 2: Distribution of flock visits in the table egg sector

Year	Central rearing/ multiplier flocks	Pullet rearing/table eggs
1997	3	30
1998	2	33
1999	1	15
2000		4

8.4.2 Table egg layer flocks

In terms of the production enterprises, the consultancy effort was coordinated by the Danish Veterinary Laboratory's consultants who contacted the owner when a positive analysis result of suspect samples had been issued. The district veterinary officer and the Danish Poultry Council's consultant at the Agricultural Advisory Service in Skejby were then contacted to arrange a joint meeting at the flock. Because of the large number of infected flocks scattered throughout Denmark in 1998, however, it was no longer possible to continue this active consultancy effort for each flock owner.

Flocks infected for the first time were given top priority, and it was hoped that owners with previously infected flocks would contact the consultants if they needed assistance.

Flock visits took place after the hens had been removed and involved inspection of the production facilities followed by planning of the eradication effort. The problematic parts of the facilities were singled out and suggestions were made for improvements, replacements, refurbishments, cleaning and disinfection involving the specific parts of the facilities, as well as for preventing infection in future.
The district veterinary officer who subsequently had to approve the cleaning, handled the legal aspects and consequences. From the outset, the Danish Poultry Council's consultant was a building expert who could advise on possible renovation and improvements of the facilities, and the Danish Veterinary Laboratory's consultant advised on the most ideal hygiene measures to implement and on future protection against infection, including the fitting up of an anteroom and pest control. The Danish Veterinary Laboratory's consultant prepared a report that included the observations, suggestions and decisions, as well as any special eradication requirements of the district veterinary officer. The report was sent to the consultants involved for any remarks before the approved version was distributed to all meeting participants.

During the course of 2000, the Danish Poultry Council's veterinary consultant joined the team of salmonella consultants in anticipation of the gradual inclusion and take-over of the Danish Veterinary Laboratory's consultancy unit on 1 July 2000.

8.4.3 Persistently infected table egg flocks

When responsibility for the National Salmonella Control Programme was turned over to the industry on 1 January 2003, the salmonella problems in the table egg sector had not been conclusively solved. Hatchery improvements have been achieved, and hatcheries now have a very high hygienic standard and seem capable of controlling potential sources of infection. In the production segment, it was clear that there are persistently infected facilities where the infection is difficult or impossible to eradicate. Many older facilities have shut down, also prompted by the final phase of the battery-cage scrapping scheme, and considerable efforts have been made involving a few of the very large battery cage systems to achieve a salmonella-free status beyond the first months after the placement of new birds. These flocks have a relatively great impact on the statistics, and in cooperation with the technical task groups, control programmes have been drawn up for any flocks that cannot eradicate salmonella, despite cleaning and disinfection. The designation 'persistently infected' means that a holding has had salmonella infection in two or more successive rotations.

Ten persistently infected flocks have been identified in cooperation with the technical task group for the table egg sector. Each of these production facilities has been inspected, and the industry has subsequently prepared control programmes for them in cooperation with the producer. The technical task group has assessed the control programmes for each producer and made recommendations to the Danish Veterinary and Food Administration, which has been in charge of final approval and the granting of exemptions. The goals of the control programmes are either to eradicate or to convert to a different production form and/or to implement hygienic measures. In continuation of initiatives that are implemented to achieve an enduring reduction of the occurrence of salmonella in these problem flocks, exemptions have been granted – on request – for putting birds into houses that have not been cleaned and disinfected. Eggs from these flocks are exempted from the sample-taking programme and sent direct to heat treatment. After the rotation in question, the holdings must either convert the production or discontinue it. The effect of this will not be entirely clear until all the identified flocks have completed the control programmes.

9. Salmonella-free Poultry Feed

In 1989, a thorough assessment of the occurrence and control of salmonella in the feed industry was carried out. This work resulted in a report entitled 'Proposals for the manufacture of salmonella-free poultry feed' (Skovgaard, 1989). Based on this report, the Danish Poultry Council worked out guidelines called 'Good production practices in the manufacture of poultry feed'. These guidelines were subsequently included from 1 October 1990 as part of the guidelines prepared by the industry for broiler production and table egg production respectively.

The primary aims of these guidelines have concentrated on achieving an expedient organisation of the facilities at feed mills in terms of the buildings, machinery and conveyance equipment. There should also be a distinct separation between raw materials and finished products, as well as an HACCP programme for the production line. Each feed mill is also required to have cleaning programmes tailored to the feed mill. If a feed mill also produces feed for other livestock than poultry, the mill must comply with special requirements, and there are also limitations on which animals may receive the feed. Stricter rules are imposed on parent flocks, which have a greater risk

of transmitting possible infection, in terms of the feedingstuffs that may be purchased, compared to the requirements made on so-called commercial flocks of egg-laying hens.

During the manufacturing process the feedingstuffs must be heated to at least 81º C, and this temperature must be continuously registered. In addition the ventilation system must fulfil special requirements such as where and how cooling air intake is organised. All of these measures are intended to prevent the introduction of or eliminate salmonella that is already present in the feedingstuffs.

Inspection visits are made to each feed mill at least four times a year to inspect the production facilities and take samples at critical control points for the purpose of detecting whether salmonella and/or coliform bacteria are present. If salmonella is detected, the mill immediately loses its permit to supply feed until the problem has been solved. If high levels (more than 10,000 colony forming units) of coliform bacteria are detected on two successive visits to the feed mill, the mill loses its permit to supply feed until the problem has been solved. Delivery vehicles must also meet cleaning and disinfection requirements, and basic standards for loading procedures and sites have been laid down.

Since their initial adoption in 1990, the guidelines have been amended a few times and adapted to the situation concurrent to a general tightening of the requirements everywhere.

A follow group has been appointed for making quarterly assessments of the technical reports prepared by an impartial laboratory for the purpose of giving the mill a renewed permit or determining whether the mill should be downgraded or possibly lose the right to supply feedingstuffs to poultry flocks entirely. Since 1995, elements of this scheme have been included in legislation concerning requirements for feed mills in general for the purpose of reducing the occurrence of salmonella in feedingstuffs. As a result, the feed guidelines are also incorporated in the legislation, as special rules apply to flocks that follow these guidelines. In order to achieve continuity and coordination with the public authorities, reports from the industry's scheme must be submitted to the authorities, and the authorities are also represented in the follow group. The scheme's effectiveness is reflected in the exceedingly low occurrence of salmonella at the feed mills that are governed by this scheme. These results are evident in the Plant Directorate's reports that are published at regular intervals. The industry's feed guidelines have been described in conjunction with the salmonella control programmes in affect at the time (Bisgaard, 1992). They clearly show that ensuring that the animals receive salmonella-free feed has a great impact on efforts to keep poultry products salmonella-free.

10. Sample-Taking Programmes

10.1 Hatching Egg Layer Flocks

As previously mentioned, the Danish poultry industry voluntarily implemented a control programme in 1989 to combat salmonella in the broiler sector. In recognition of the great significance of infection from breeding and parent animals, the programme targeted the top of the breeding pyramid. As a result, parent flocks were monitored by taking samples of newly hatched chicks at the hatcheries. Whenever salmonella was found in the chicks, the infected parent flocks were destroyed, regardless of salmonella type.

In 1992, the table egg sector of the Danish poultry industry started a voluntary control programme, which at the time involved the testing of central rearing flocks imported as day-old flocks. The birds were subjected to bacteriological and serological analysis, and the hatcheries contracted agreements with import enterprises on the terms and conditions in the event of salmonella or antibody finds. The voluntary initiatives have continued after the implementation of the National Salmonella Control Programme.

After the European Council's 'Zoonosis Directive' took effect in 1994, the voluntary initiatives were followed up by the analysis of central rearing flocks at the ages of one day and four weeks and then two weeks before the start of the egg-laying period. The multiplier flocks were analysed during the egg-laying period at two-week intervals in the form of meconium samples taken from 250 chicks or samples taken from each flock in the form of 50 destroyed or dead birds. If *S.* Typhimurium or *S.* Enteritidis was found, the flock was either destroyed or slaughtered. From 1994 until the National Salmonella Control Programme took effect, the sample-taking programme thus consisted of meeting the minimum requirements of the 'Zoonosis Directive' combined with voluntary initiatives.

Through the establishment of the National Salmonella Control Programme in December 1996, the parent flocks were subjected to a much tighter sample-taking programme. Analyses of central rearing flocks were added at the ages of one and two weeks in the form of samples taken from dead pullets. In addition, the taking of faecal samples at the age of eight weeks was introduced. The programme was expanded in the multiplier segment to include bacteriological and serological sample-taking every four weeks. From now on, the sample-taking programme had been significantly improved because all serotypes were now included in the programme and because a more sensitive detection method, i.e., serological analysis, had been implemented.

When the National Salmonella Control Programme was revised in 1998, the sample-taking programme for the multiplier segment was left unchanged.

In 1999, modifications were proposed for the sample-taking programme involving the multiplier segment. The changes were intended to increase the analysis frequency for multiplier flocks to every two weeks, instead of every four weeks as before, and to replace the bacteriological analysis of faecal samples with 'sock samples', also seen as an improvement. Instead of examining hatchlings every week, it was suggested that samples be taken of all hatchlings after each hatching, but with the option of taking samples from up to 4 hatchlings in one sample.

When the original National Salmonella Control Programme was extended, the sample-taking programme for parent flocks was modified in 2000. Thus, the sample material taken from central rearing flocks in the second and fourth weeks was changed from dead chicks to two pairs of 'sock samples'. In multiplier flocks, the sample-taking interval was shortened from every four weeks to weekly, and the sample material was changed from 60 faecal samples and 60 blood samples to two pairs of sock samples (thus eliminating serological samples). From May 2000, all hatchlings were analysed after every hatching. A problem arose in September 2000, however, when the European Commission announced that the introduction of two pairs of sock samples in the hatching egg

production segment was not in keeping with the wording of the Zoonosis Directive. The sample-taking at the age of four weeks and two weeks before moving in central rearing flocks was subsequently reverted to the taking of 60 faecal samples, which is still in effect today. The current sample-taking form is shown in Table 3.

CENTRAL REARING FLOCKS		**Central rearing flocks**	**Breeding segment, central rearing flocks**
Time	**Sample-taking**	**Material**	**Material**
Day-old	Per delivery	10 crates and 20 chicks (ZD)*	10 crates and 20 chicks (ZD)*
1st week	Per unit	40 chicks	-
2nd week	Per unit	2 pairs of sock samples (in one bag)	-
4th week	Per unit	60 samples of fresh faeces (ZD)*	60 samples of fresh faeces (ZD)*
8th week	Per unit	2 pairs of sock samples (in one bag)	2 pairs of sock samples
2 weeks before being moved	Per unit	60 samples of fresh faeces (ZD*) and 60 blood samples	60 samples of fresh faeces (ZD)*
HATCHING-EGG PRODUCTION		**Hatching egg production**	**Breeding segment, hatching egg production**
Every two weeks	Per flock	250 meconium samples or 50 dead-chick samples taken from the hatchery (ZD)*	250 meconium samples or 50 dead-chick samples taken from the hatchery (ZD)*
		Regional Veterinary and Food Control Authority (RVFCA) takes samples every 8 weeks (ZD)*	RVFCA takes samples every 8 weeks (ZD)*
Every week	Per unit	2 pairs of sock samples (in one bag)	-
		RVFCA is in charge of sample-taking once every quarter	
HATCHERY			
Every hatching, every hatchling	Up to 4 hatchlings can be pooled	At least 25 grams of wet dust per hatchling	At least 25 grams of wet dust per hatchling

*Compulsory samples pursuant to the Zoonosis Directive.

Table 3: Sample-taking programme for the parent segment.

10.2 Table Egg Layer Flocks

The establishment of the National Salmonella Control Programme in late 1996 marked the start of the actual monitoring of table egg flocks. A tightened requirement on the registration of table egg flocks led to the registration of 80 new table egg producers, solely from the effectuation of the National Salmonella Control Programme in December 1996 to late January 1997.

The strategy was based on the elimination of all table egg and pullet rearing flocks if *S.* Enteritidis and *S.* Typhimurium were detected as a way of protecting consumers against infected eggs. The pullet rearing flocks were analysed as day-old chicks, at the ages of 3 weeks and then at the earliest 12 weeks or not later than 2 weeks before they were to be moved. It was originally intended to establish the sample-taking procedure at specific ages in commercial table egg flocks; the idea had to be abandoned, however, in favour of fixed time intervals. If more than one egg-laying period was involved, the flock was required to be analysed in the middle of the period. At the beginning of the National Salmonella Control Programme, there were two categories of table egg producers, i.e., commercial table egg flocks and table egg flocks whose eggs were not delivered to certified packing houses or product manufacturing enterprises. The flocks in the second category were analysed twice a year at 5-7 month intervals. As a result, no attempt was made to distinguish between what we now refer to as farm-gate flocks and barnyard flocks.

During the course of 1997, the strategy involving the destruction of any flocks that tested positive for *S.* Enteritidis or *S.* Typhimurium turned out to be financially unsound, and supplies of Danish table eggs to consumers were endangered. Because the extent of salmonella infection was far greater than anticipated, the National Salmonella Control Programme was suspended for table egg and pullet rearing flocks in September 1997. Up to the time when the amended National Salmonella Control Programme took effect in March 1998, the investigation of pullet rearing and table egg flocks was therefore limited to the monitoring of salmonella by means of serological samples. The sample results were only forwarded if so requested by the producer. Routine follow-up of positive flocks was not performed. The consequence of a suspected flock infection was amended from being a requirement for verification of the suspicion into an optional verification. The possibility was also retained of ordering the destruction/slaughter of flocks or putting flocks in which *S.* Enteritidis or *S.* Typhimurium had been detected under public supervision.

The original National Salmonella Control Programme was amended by a new act of parliament. From March 1998, a decision was made to concentrate resources on controlling salmonella in the parent-flock segment and in rearing flocks. The rearing flocks were again analysed pursuant to the programme from 1997. New requirements were implemented regarding the fitting up of rearing-flock houses to minimise the risk of introducing infection. From then on, table egg layer flocks had to be monitored, and the heat treatment of eggs became compulsory if infection was detected. From this date, the effort against salmonella was intensified, however, as all types of salmonella were covered by the programme from then on. At the same time, it also became possible to order more frequent sample-taking of non-infected flocks at holdings where infection had been detected in one or more houses.

Due to a case of human infection originating from a table egg flock during a suspicion interval (i.e., the time interval between a positive result of a routine sample and the final verification of the

infection in the form of a positive result of a suspect sample), the programme was additionally tightened, making heat treatment of eggs compulsory already from the date of the suspected infection, and it became possible to verify suspected flocks serologically. In contrast with previous practice under the National Salmonella Control Programme, whereby a flock could only be verified as being infected by isolating salmonella bacteria pursuant to the requirements stipulated in the EU's 'Zoonosis Directive'.

As previously discussed in the section on hatching egg layer flocks, the sample material was changed in May 2000. This also applied to the table egg sector. Samples of fresh faeces were changed to sock samples, which were far easier to take.

In December 2000, the industry suggested that it should only be possible to declare a flock infected on the basis of bacteriological analyses and, if necessary, through more comprehensive analyses of 300 destroyed chickens. An epidemiological assessment was made of bacteriological (300 chickens) versus serological samples, which clearly documented that serological analysis is by far the most sensitive method of the two. As a result, the possibility of declaring a flock infected on the basis of a positive results from serological suspect samples was upheld.

From January 2001, user charges were introduced for routine analyses. This caused many small producers as well as non-commercial flocks to deregister from the monitoring programme. As a result, the Ministry of Food, Agriculture and Fisheries decided to pay compensation for 75% of the analysis costs for producers with flocks of less than 1000 hens who deliver to a packing enterprise, and for farm-gate sellers with flocks of up to 500 hens. The costs funded by the Danish Veterinary and Food Administration amount to about DKK 1 million a year. The previous year, non-commercial flocks had been divided into two groups, i.e., farm-gate sellers and barnyard flock owners respectively, based on information submitted on rearing flocks from the producers. From this date, the Danish Veterinary and Food Administration effectuated a signposting scheme for farm-gate sellers to document to consumers that these flocks were registered under a public inspection scheme. Subsequently, the programme was only compulsory for farm-gate sellers (selling eggs direct to consumers) and not for barnyard flock owners (producing eggs for personal consumption). Farm-gate sellers were required to purchase pullets from certified, registered rearing flocks. Based on the previously mentioned assessment of serological versus bacteriological analysis, the sample-taking programme was changed to involve serological analysis only, and the frequency was increased to three times a year (Table 4). The sample-taking programme for farm-gate sellers is still in effect. Barnyard flock owners were not required to participate in a salmonella analysis programme. A voluntary scheme resembling the farm-gate model was offered to them, however. These measures took effect in September 2001. The sample-taking programme has not been changed since then.

REARING STOCK		
Time	**Sample-taking**	**Material**
Day-old	Per delivery	10 crates and 20 chicks
Week 3	Per flock	5 pairs of sock samples (in 5 bags) or 300 samples of fresh faeces (in 5 bags), if the taking of sock samples is not possible. Flocks of less than 200 birds: only 2 pairs of sock samples (in one bag) or 60 samples of fresh faeces (in one bag).
Week 12	Per flock	Flocks of 500 and more: 60 blood samples and 5 pairs of sock samples (in 5 bags) or 300 samples of fresh faeces (in 5 bags) if the taking of sock samples is not possible.* Flocks of 200-499: 55 blood samples and 5 pairs of sock samples (in 5 bags)* Flocks of less than 200 birds: blood samples, cf. Table 1 of govt. order, as well as 2 pairs of sock samples (in one bag) or 60 samples of fresh faeces (in one bag).*
TABLE EGG PRODUCTION FOR CERTIFIED EGG PACKING STATIONS		
Every 9 weeks	Per flock	A number of eggs per Table 1 of govt. order 44 and 2 pairs of sock samples (in one bag) or samples of fresh faeces (in one bag) equalling the number of eggs where it is not possible to take sock samples.
FARM-GATE FLOCKS AND BARNYARD FLOCKS		
3 times a year	Per flock	A number of eggs pursuant to Table 1 of the govt. order.

*The regional veterinary and food control authority is in charge of taking and sending in the samples indicated.

Table 4: Sample-taking programme for rearing stock and table egg production and farm-gate flocks.

11. Procedures for Suspected Flocks

Through the establishment of the National Salmonella Control Programme in 1996, an action plan was prepared for the detection of salmonella-positive isolates. The plan was distributed to the district veterinary officers. The plan outlined the guidelines for handling flocks suspected of being infected with salmonella.
Lists of positive laboratory results were automatically printed at the Division of Zoonoses, VA02 of the Danish Veterinary Services, which informed the district veterinary officers (positive sample results not encompassed by the National Salmonella Control Programme were sent by fax to the VA02). All positive and negative laboratory results were distributed to the submitting enterprise. The model has continued according to the same principle after the industry's take-over, though the VA02 and the regional veterinary and food control authorities are still informed of positive laboratory results by e-mail and fax.

11.1 Hatching Egg Layer Flocks

Before the National Salmonella Control Programme was adopted, no distinction was made between the breeding segment and the parent segment. As a result, it had been possible to suspect a breeding flock and a parent flock if *S.* Enteritidis or *S.* Typhimurium were bacteriologically detected in routinely submitted material, or if a flock had received birds or hatching eggs from an infected flock. The implementation of the National Salmonella Control Programme involved a distinction between the breeding segment and the parent segment, as breeding flocks are exclusively produced for export. The analyses followed roughly the same guidelines, except that the one-week and two-week samples were not taken from the breeding segment's central rearing flocks. After this, suspected infection by *S.* Enteritidis and *S.* Typhimurium was only triggered in the breeding segment, if the suspicion was triggered by routine samples. The first government order resulting from the National Salmonella Control Programme made it possible to suspect central rearing flocks and multiplier flocks in the parent segment if the suspicion was based on:

- bacteriological and serological detection in routine samples,
- bacteriological detection in material from the flock's habitats,
- introduced birds from a flock in which salmonella infection has been detected,
- epidemiological analysis of salmonella results from broilers or pullets reared for table egg layer flocks hatched from eggs from the flock which makes it plausible that the flock has been infected with salmonella bacteria,
- the detection of salmonella in feedingstuffs delivered to the flock,
- the detection of salmonella in the dust from the machine in which the flock's hatching eggs were hatched.

When the sample-taking programme was revised in 1998, the government order included the option of suspecting flocks in the parent segment, if bacteriological and serological analysis showed that salmonella had been detected in material submitted for reasons different than those outlined above. This same option was also given in the breeding segment.
In 2001 it was decided that from now on the breeding segment could be suspected if any type of salmonella or antibody was detected in routine samples or in material submitted for a different reason.

Prior to the National Salmonella Control Programme, a suspected flock in the breeding or parent segment was placed under the supervision of the district veterinary officer. The district veterinary officer was in charge of taking suspect samples and imposing restrictions on poultry, eggs, faeces and implements, machinery and the like from the holding.
Through the first government order under the National Salmonella Control Programme, i.e. No. 1059 of 9 December 1996, a suspected hatching egg flock no longer had to be put under supervision, but only had to have suspect samples taken.
Material for ruling out or verifying a suspicion was taken according to guidelines that distinguished between direct versus indirect detection and respective finds of *Salmonella* Enteritidis / *S.* Typhimurium versus exotic strains of salmonella. As a result, if *S.* Enteritidis and *S.* Typhimurium were directly detected in a flock (routine bacteriological and serological samples and bacteriological samples taken from flock habitats), then 60 samples were taken for serological analysis, 60 hens for bacteriological analysis and 300 faecal samples. For the detection of exotic strains or indirect detection (e.g., through introduced birds, retracing from an infected flock, salmonella-infected feed and positive hatching samples) the sample-taking would only consist of 300 faecal samples. The amendment of the government order on parent flocks in 1999 introduced the definition of a flock. From now on, a flock could consist of several epidemiological units. The suspicion samples were changed from 300 faecal samples to 5 pairs of sock samples per epidemiological unit. In addition, the possibility of declaring a flock infected on the basis of serological analysis disappeared. This model is still in effect and is illustrated in Table 5.

11.2 Table Egg Flocks

As previously mentioned, the control of salmonella in table egg layer flocks and pullet rearing flocks for this segment was limited to *S.* Enteritidis and *S.* Typhimurium at the beginning of the National Salmonella Control Programme. Suspicion was triggered by bacteriological or serological reaction to these types in routine samples or samples submitted for a different reason. Retracing human infection to a flock as well as infection in a flock could trigger suspicion of the other flocks at the holding.

At the start of the National Salmonella Control Programme, the strategy for pullet rearing and table egg flocks was based on eradication. Suspected infection did not bring about any flock restrictions, but only the taking of suspect samples by the district veterinary officer. The material to be analysed depended on the size of the flock, as sample-taking for flocks with up to 200 birds had to consist of faecal samples, as well as blood or eggs as instructed by the district veterinary officer. For flocks of up to 500 birds, suspect samples consisted of 30 killed hens, while in flocks of more than 500 birds, the samples had to consist of 60 hens.
During the period when the original National Salmonella Control Programme was suspended, the control programme was changed into monitoring, and it was only possible to suspect a flock with salmonellosis. The possibility of placing a flock under suspicion if human infection could be retraced to the flock was also retained, however. The routine samples no longer triggered suspicion.

The amendment of the National Salmonella Control Programme in 1998 realigned the strategy. From now on, all salmonella types could trigger suspicion and eradication was changed to compulsory heat treatment of eggs from the time of infection. This also meant that from now on a distinction had to be made when taking suspect samples as to whether the find that triggered the suspicion was *S.* Enteritidis / *S.* Typhimurium or an exotic strain of salmonella. Finds of *S.*

Enteritidis or *S.* Typhimurium still triggered suspect samples as before, but finds of exotic salmonella led to the taking of faecal samples as instructed by the district veterinary officer for flocks of up to 300 birds and the taking of 300 faecal samples for flocks of more than 300 birds.

In late 1999, the guidelines regarding suspected infection were tightened, whereby a suspected flock was either ordered to send eggs to heat treatment or destruction. In addition, it was now possible to declare a suspected flock as being infected with *Salmonella* Enteritidis or *S.* Typhimurium on the basis of serological analysis. Once again, flocks with up to 200 birds had to send in eggs or blood for serological identification, in addition to the faecal samples. For flocks with up to 500 animals, the suspect samples were to be supplemented with 60 eggs or 60 blood samples, and for flocks of more than 500 birds, the 60 birds were to be accompanied by 60 eggs or blood samples.

This was followed in 2000 by the possibility of placing other flocks at other holdings under suspicion if birds had been delivered or received from flocks that were found to be infected with salmonella. This model is still in effect.

In 2000, sock samples were introduced instead of the faecal samples. Suspect samples were now taken as two and five pairs of sock samples, respectively. Furthermore, the new government order included a table that made it possible to compute the number of eggs or blood samples to be taken from flocks with up to 500 birds. The last change affecting suspected infection in pullet rearing and table egg flocks was made in 2001. In the event of a suspected infection with *S.* Enteritidis and *S.* Typhimurium, the previous number of suspect samples was supplemented to include two pairs of sock samples if the flock was pullet-rearing stock. This model is still in effect (Table 6).

Direct detection of *S.* Enteritidis or *S.* Typhimurium.	60 birds and 5 pairs of sock samples
Direct detection of exotic salmonella type	5 pairs of sock samples
Indirect detection of every salmonella type	5 pairs of sock samples

Table 5: Guidelines for taking of suspect samples from hatching egg flocks.

	S. Enteritidis or *S.* Typhimurium*	Exotic strains of salmonella
Table egg flocks, 1-199 birds	Sock samples, eggs or blood	Sock samples
Table egg flocks, 199-499 birds	Hens, eggs or blood	Sock samples
Table egg flocks, 500 birds and above	Hens, eggs or blood	Sock samples

*Sample-taking among pullet rearing stock is additionally supplemented by 2 pairs of sock samples.

Table 6: Guidelines for taking suspicion samples from pullet rearing and table egg flocks.

12. Procedure for Controlling Salmonella Infection

12.1 Hatching Egg Layer Flocks

Since the implementation of the Zoonosis Directive, the strategy for controlling salmonella in hatching egg production has been based on eradication. This has meant that the costs of combating salmonella have primarily been borne by the Member State, 50% of which has been reimbursed by the European Commission if *S.* Enteritidis or *S.* Typhimurium had been found in a suspected flock. The scope of the National Salmonella Control Programme, however, was to control all types of salmonella, and the costs were to be borne partly by the state and partly by the industry. After the funding for the National Salmonella Control Programme ended, the eradication expenses involving parent flocks were taken over by the industry, in so far as exotic strains of salmonella were concerned.

12.1.1. Public supervision/orders

If one or more suspect samples receive positive laboratory results, the holding with the infected flock is placed under public supervision and restrictions are imposed on the owner concerning destruction, isolation from non-infected flocks, precautionary hygienic measures for coming and going among infected and non-infected flocks, the handling of feedingstuffs and manure, as well as cleaning and disinfection procedures before the placement of new birds.

12.1.1.1 Destruction/slaughter

If a find of salmonella is confirmed in hatching egg production, the flock is declared infected. This has led to the required destruction or slaughter of a flock until the allocated funding ceased on 31 December 2002. After this date, this requirement is only imposed on flocks infected with *S.* Enteritidis and *S.* Typhimurium. Several restrictions, which are still in effect, are imposed on flocks infected with exotic strains of salmonella.

12.1.1.2 Modification of salmonella status

In early 2002, the possibility of changing the salmonella status of a flock infected with an exotic strain of salmonella was introduced. This decision was based on a summary of suspected flocks from 2000 to 2002, which showed that suspect samples rarely re-detected the bacterium in flocks if the suspicion involved an exotic strain of salmonella. Similarly, the exotic strains found in parent flocks could not be re-detected in the broiler segment. The technical task group assessed that finds of exotic strains in poultry flocks that could not be subsequently verified by finds of the same type involve a low-grade infection, and on this basis the technical task group proposed a model to the steering committee in which the salmonella status of parent flocks suspected of infection with an exotic strain of salmonella could be modified on the basis of the already taken suspect samples. The new model took effect in October 2002. The salmonella-status modification requires the daily sending of two pairs of sock samples for a period of two weeks, in addition to samples of hatching dust or chicks, for bacteriological analysis. None of the samples may contain salmonella. The new model also allows the selling of day-old chicks from such a flock, on the condition that a quick test of hatching dust from day-old chicks does not contain salmonella.

12.1.1.3 Heat treatment of eggs

If infected, several restrictions are imposed on hatching egg flocks, such as heat treatment or destruction of eggs, and that any hatching eggs received by the hatchery from an infected flock must be removed from the incubator and stores and be destroyed. By making it possible as from October 2002 to modify the salmonella status of multiplier flocks in which exotic strains of salmonella have been detected, it also became possible to use eggs from these flocks, if the eggs were placed in pre-incubators and incubators before the date of infection. As previously mentioned, this is only allowed on the condition that the result of a certified salmonella quick-test of hatching dust is negative. Infected hatching eggs to be used in food products may only be heat treated if the eggs have not been disinfected. This has only been used to a limited extent, however, as the flocks have been slaughtered or destroyed shortly after an infection has been confirmed.

12.1.1.4 Internal control

In 1998, it became possible to order parent-flock holdings to prepare an action plan that aims to reduce the risk of salmonella infection. At the same time this made the placement of new poultry or the receipt of hatching eggs dependent on the approval of these action plans by the Danish Veterinary Services and Food Administration.

12.1.1.5 Fertiliser and feedingstuffs

In addition to the above-mentioned initiatives, manure left after an infected flock has been required to be burned, deposited or buried. Rules on the treatment of feed as instructed by the district veterinary officer have also been laid down.

12.1.1.6 Cleaning and disinfection

Throughout the National Salmonella Control Programme, cleaning and disinfection have been required before any new birds could be placed in hatching egg houses, regardless of whether a flock was infected. Through the implementation of the Zoonosis Directive, rules were also laid down stipulating a minimum period of 14 days following cleaning and disinfection before a new flock could be placed in the cleaned and disinfected house. This requirement was withdrawn in the first government order issued under the National Salmonella Control Programme, but is upheld in the 'Order on the fitting up and operation of hatcheries and hatching egg producing enterprises, etc.' which makes an exception for hatcheries, however. Cleaning and disinfection must be approved by the veterinary officer from the regional veterinary and food control authority on the basis of a visual and bacteriological assessment. Swab samples are to be taken from induction valves, walls, floors, feed systems/equipment (cages, nest boxes), anteroom/packing room, exterior platform and egg room. As requirements have not been made for the practical implementation of cleaning, this has been performed individually by each holding (cf. Chapter 15).

12.2 Table Egg Layer Flocks

The control of salmonella in pullet-rearing and table egg flocks is a national initiative and was initially funded through the National Salmonella Control Programme exclusively. In early 2001, user charges were introduced for the taking of routine samples, and the industry took over other costs in late 2002. When the National Salmonella Control Programme ended, all costs for eradicating pullet-rearing flocks as well as losses incurred from heat treatment of eggs were transferred to the industry.

12.2.1 Compulsory measures

12.2.1.1 Destruction/slaughter

In the early stages of the National Salmonella Control Programme, the salmonella control strategy included the destruction/slaughter of pullet-rearing and table egg flocks infected with *S.* Enteritidis or *S.* Typhimurium.
After March 1998, the strategy was upheld for pullet-rearing flocks for all types of salmonella, but was altered to the heat treatment of eggs from infected table egg flocks, as these flocks are allowed to continue the production of eggs until the end of the rotation.
If multi-resistant *Salmonella* Typhimurium DT104 is found in a table egg flock, then the eggs from the flock must be heat treated, and the meat must be subjected to heat treatment after slaughter.

12.2.1.2 Heat treatment of eggs

The scope of the original National Salmonella Control Programme was amended to include all types of salmonella in the programme, and the strategy was changed to the heat treatment of eggs from infected table egg flocks. In late 1999, a requirement was introduced whereby the heat treatment of eggs was compulsory already from the date of a suspected infection. Eggs for heat treatment were not allowed to be delivered by way of the packing station to the product enterprise but had to be sent directly to the product enterprise.

12.2.1.3 Isolation of infected flocks

Infected flocks have been put under public supervision, if destruction or slaughter was not imposed. This supervision has included orders to isolate infected flocks, as well as special precautionary hygienic measures for coming and going between infected and uninfected flocks. Prohibitions and special conditions have also been introduced regarding the removal of and placement of birds in the flock.

12.2.1.4 Intensified sample-taking

The revision of the National Salmonella Control Programme additionally tightened the rules by implementing requirements for intensified sample-taking in flocks in which no infection has been detected if infected flocks have been found at the same holding. As a result, samples are taken under public supervision every four weeks instead of every nine weeks from all uninfected flocks of a holding.

12.2.1.5 Manure and feedingstuffs

Requirements have also been made for special handling and treatment of manure and feedingstuffs left over from destroyed flocks and flocks under public supervision.

12.2.1.6 Cleaning and disinfection

The cleaning and disinfection of houses and implements after the detection of an infection have been required since the start of the National Salmonella Control Programme. Like the parent-flock segment, there are no specific requirements for the practical implementation of cleaning and disinfection procedures. The approval of cleaning and disinfection is performed by the veterinary officer from the regional veterinary and food authority and is based on a visual assessment and an analysis of swab samples. In 2000, it became possible for producers who did not wish to continue production after an infected table egg layer flock had been destroyed to submit a written statement to this effect. If a producer makes this statement, the regional veterinary and food control authority will retract the requirement of cleaning and disinfection if birds are not placed at the holding within the next two years.

Because of the various housing systems in the table egg sector, the cleaning and disinfection results have varied. Experience shows that battery cage systems are particularly difficult to clean and disinfect. In order to provide specific advice on cleaning and disinfection, a research project has been carried out to illustrate the effect of heat treatment on an infected flock's habitats (cf. Chap. 15).

13. Legislation

Before 16 December 1996, the date on which the National Salmonella Control Programme took effect, only breeding, central rearing, multiplier and hatchery enterprises were covered by the salmonella control efforts stipulated in 'Order No. 1022 of 15 December 1993 on the investigation of salmonella bacteria, as well as the control and prevention of *Salmonella* Enteritidis and *S.* Typhimurium in hens'. The order implemented the EU's Zoonosis Directive (92/17/EEC), which prescribes monitoring analyses of hatcheries and hatching egg layers.

13.1 The Zoonosis Directive

Denmark was the first country to implement the directive. The initiatives should have been implemented by each Member State in 1994. The deadline for implementing the directive was subsequently postponed till 1998 at the request of several countries. Some countries have still taken no action in this regard, however.

EU's Member States are reimbursed by the EU for 50% of the expenditures for the destruction of parent flocks infected with *S.* Enteritidis or *S.* Typhimurium and for the destruction of hatching eggs from these flocks. The Member States must submit a quarterly report and issue an annual technical and financial review. Each year, the Member States are to apply for approval and seek financial support for a national salmonella control programme the following year.

The most common serotypes in each Member State differ from country to country. The nine countries which submitted a Trend report[1], in 2001 have indicated the following serotypes as the most frequent causes of human salmonellosis in the EU and Norway: *S.* Enteritidis (71.49%), *S.* Typhimurium (16.19%), *S.* Virkow (1.90%), *S.* Hadar (1.62%), *S.* Infantis (1.05%), *S.* Agona (0.95%), *S.* Newport (0.80%) and *S.* Braenderup (0.72%).

An amendment of the Zoonosis Directive has been requested on several occasions. The European Commission did not submit a new proposal for amending the Zoonosis Directive [2] and the regulation[3] until August 2001. The regulation lays down the rules for preventing the significant types of salmonella which are important to general public health, hereby enabling the expansion of salmonella control measures implemented by Member States till then. The new regulations make it possible for each Member State to get additional trade guarantees. The proposals were adopted by the European Council of Ministers in November 2003. Denmark has exerted considerable influence on the wording and scope of the new rules, as a result of the experience gained by Denmark from the implementation of the National Salmonella Control Programme. The revised Zoonosis Directive

[1] 'Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in the European Union and Norway in 2001'.
[2] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents.
[3] Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents.

and the new regulation took effect on 12 December 2003, whereby the latter will apply from 12 June 2004.

13.2. Government Orders, 1996 -2002

'Order No. 1022 of 15 December 1993 on the monitoring of salmonella bacteria, as well as the control and prevention of *Salmonella* Enteritidis and *S.* Typhimurium in hens' was replaced by 'Order No. 1059 of 9 December 1996 on the control of salmonella in hatching egg layer hens' and by 'Order No. 1060 of 9 December 1996 on the control of *Salmonella* Typhimurium and *Salmonella* Enteritidis in table egg flocks and pullets reared for them'.
As previously mentioned, these government orders expanded the public control of salmonella. For example, they initiated the routine monitoring of salmonella in table egg flocks. If *S.* Typhimurium or *S.* Enteritidis was found in a flock, it was destroyed and the owner was indemnified for consequential loss and for the value of the birds. The National Salmonella Control Programme included both the commercial segment and the farm-gate segment of table egg production. The National Salmonella Control Programme was a continuation of the salmonella control programmes that had taken place since 1994 in accordance with the rules stipulated in the Council Directive 92/117/EEC, i.e., the Zoonosis Directive.
The orders were amended in late 1997 to modify the original strategy, i.e., the destruction of infected table egg flocks. Two new orders were issued in early 1998, resulting from a revision of the National Salmonella Control Programme. From then on, all types of salmonella were monitored in table egg flocks. An amending of the government order on table egg production was issued in late 1999 in conjunction with the tightening of rules for the handling of eggs from flocks suspected of being infected with salmonella. In 2000, the original National Salmonella Control Programme was extended by three more years, and the government orders were in this conjunction amended once again. This resulted in two new government orders that took effect in May 2000. Later that same year, amending orders were issued, after the European Union had stated that the sample-taking sock routines in force at the time were inconsistent with the faecal-sample requirements stipulated in the Zoonosis Directive. A proposal from the poultry industry to modify the sample-taking programme resulted in the issuing of two new orders in late 2001. At the last minute, however, the poultry industry decided to retract the new model, and as a result, the changes implemented by the new orders were insignificant. In the order for the control of salmonella in table egg flocks, a 75% refund scheme with retroactive effect was introduced for farm-gate sellers with up to 500 hens and for table egg producers with up to 1,000 hens who deliver to a packing station. A proposal from Dansk Slagtefjerkræ [the Association of Danish Broiler Producers] brought about an amendment of the hatching eggs order in 2002. This order made it possible to change the salmonella status of a hatching egg flock infected with an exotic strain of salmonella.
The strategy at the end of the National Salmonella Control Programme still involves eradication in the event of infection with *S.* Typhimurium and *S.* Enteritidis in hatching egg flocks, which are the only flocks that are eligible for compensation from public funds. The scheme of public refunding of expenses related to the eradication of hatching egg flocks infected with exotic strains of salmonella has ceased, and from now on the poultry industry bears all these costs. The rearing-flock strategy is still based on eradication, but here, too, the industry has to cover the costs, as the public compensation scheme has ended. Similarly, the poultry industry bears all costs for all types of sample-taking related to the salmonella monitoring programme in conjunction with the take-over by the industry of the day-to-day administration of the National Salmonella Control Programme as of 1

January 2003. The orders representing the industry's final take-over of the salmonella monitoring took effect in early February 2003.

13.3 European Approval of the National Salmonella Control Programme

The implementation of the National Salmonella Control Programme was conditional on the approval of the EU. Every amendment of the programme requires the subsequent submission of a report to the EU, which similarly waits for an annual application before approving next year's National Salmonella Control Programme. Every year, a technical and financial report for the previous year is sent to the EU, which is a prerequisite for EU's part-financing of the compensation payments.

14. Financial Basis of the National Salmonella Control Programme

14.1 Appropriations via the Danish Finance Bill: Origins and Financing of the National Salmonella Control Programme

By adopting Act No. 325 of 29 May 1996, the Finance Committee of the Danish Parliament acceded to the implementation of a three-year National Salmonella Control Programme for expanding the control of salmonella in broilers and table eggs at a total cost of DKK 188.1 million. The Ministry of Finance contributed DKK 87.7 million of this amount and the Ministry of Agriculture and Fisheries DKK 70.4 million, while the funding was conditional on the payment of DKK 30 million by the Fjerkræafgiftsfonden [Poultry Tax Fund]. The industry's contribution was dispersed as 5, 10 and 15 million DKK respectively over the three years (1996-1998) in which the National Salmonella Control Programme was intended to run. The National Salmonella Control Programme was notified to the EU in September 1996 as a new subsidy scheme, and it was named the 'National Salmonella Control Programme for the expanded control of salmonella in broilers and table eggs'.

The industry's participation in the funding of the National Salmonella Control Programme was obtained by an increase in production fees and a subsequent transfer from the Poultry Tax Fund to the Ministry of Agriculture and Fisheries.

The DKK 188.1 million stipulated in the act were dispersed over the years 1996-1998, and the expenditures for this effort were estimated to be DKK 79.0 million in 1996, on the condition that DKK 5 million of this amount would be paid by the Poultry Tax Fund, as mentioned above.

As a result of substantial amendments to the National Salmonella Control Programme, a new bill was subsequently submitted and adopted by the Finance Committee later on in 1996. In Act No. 441 of 10 September 1996, the expenditures were dispersed over the years 1996-1999, during which DKK 75.3 million were expected to be used, on the condition that DKK 1.3 million of this amount would be transferred from the Poultry Tax Fund.

Based on the experience gained during the initial phase, it became necessary to inform the Finance Committee of developments. This was done by Act No. 329 of 9 September 1997, which

announced a modified strategy and intended revisions of the National Salmonella Control Programme for the control of salmonella in table egg flocks.

By Act No. 154 of 25 February 1998, the Finance Committee adopted various amendments to the National Salmonella Control Programme for the remainder of the period up to the end of 1999.

When the National Salmonella Control Programme ended on 31 December 1999, DKK 62.9 million of the original allocation remained, and for this reason the government decided to propose the continuation of the National Salmonella Control Programme for a new period, if an agreement on the conditions for this could be reached with the industry. This effort succeeded and the provisions and financial framework are described in Act No. 229 of 10 May 2000. The act extended the National Salmonella Control Programme by three years, 2000-2002, during which the activities of the three-year period would be gradually transferred to the industry, so that the entire day-to-day operation of the National Salmonella Control Programme would be transferred to the industry by the end of 2002. A budget was prepared for the allocation of the DKK 62.9 million, and the Act details the allocation of the funds on the various activities. It was anticipated that the appropriation might be spent before the end of the National Salmonella Control Programme, and in this context, the indemnity served as a 'buffer'. At worst, therefore, there could have been a period in which indemnity and other costs related to infection could not be reimbursed. As it turned out, however, the expenditures were less than originally budgeted, and it has been possible to pay indemnity up to the end of the National Salmonella Control Programme in late 2002.

14.2 Reimbursement from the European Union (EU)

The EU's Zoonosis Directive has served as the basis for the 50% reimbursement of expenditures in conjunction with the destruction of parent flocks and pullets reared for them, which have been infected with *Salmonella* Enteritidis or *S.* Typhimurium. However, the EU does not refund expenses for consequential loss payments or VAT.

To be eligible for reimbursement from the EU, *S.* Enteritidis or *S.* Typhimurium must have been detected in dead birds, whereby the samples must have been taken not later than the date on which the infected birds were destroyed. With regard to the application of the received reimbursement, Act No. 154 of February 1998 stipulates the following:

"The Danish Finance Bill for 1998 includes a revenue allocation of DKK 1.5 million for the EU reimbursement of indemnification. The account concerns Community subsidy in the amount of 50% for destruction pursuant to Council Directive 92/117/EEC (the Zoonosis Directive). Community support in the amount of 50% shall be provided, after one year, in accordance with the Zoonosis Directive to compensate destruction, etc., but not for compensation provided for partial cover of consequential loss. For each calendar year, however, the European Commission determines the maximum amount of Community support that may be paid. If the income from the EU reimbursement exceeds the annual revenue appropriations, these revenues will be reapplied within the sphere of the National Salmonella Control Programme."

Although similar wording was not included in Act No. 229 of 10 May 2000, the plan is still managed in practice according to the mentioned guidelines. In past years, the following amounts have been specified in the Finance Bill, and the indicated items from the accounts have appeared in the national accounts (Table 7).

Table 7: Accounting items in the national accounts related to the salmonella programme in the table egg and broiler production segments.

Year	Finance Bill, revenue appropriation (MDKK)	EU reimbursement received, cf. accounts (MDKK)	Contingent transfer to the National Salmonella Control Programme (MDKK)
1998	1.5	1.5	0
1999	3.7	0.4	0
2000	1.5	1.2	0
2001	0.4	1.0	0.6
2002	0	(1.3 + 2.1) *	1.3 + 2.1
2003	0		

*) DKK 1.3 mill. are reimbursement for fiscal year 2001 and DKK 2.1 mill. are reimbursement for 2002. The reimbursement for 2002 is included because the Danish Veterinary and Food Administration now applies the acquisition of right principle.

14.3 Total Accounts

As shown in the above, the budgeted expenditures have not been identical to the realised expenditures owing to delays in and modifications of the planned action. As the budgets are of less interest at present, however, only realised expenditures are indicated in Table 7.

The distribution of expenditures on various activities in 1996-2004 in millions of DKK is shown in Table 8.

Table 8: Summary of the public expenditures for various purposes in conjunction with the National Salmonella Control Programme for broiler and table egg production (1996-2004).

Total, MDKK	Accounts (Accs.) 1996	Accs. 1997	Accs. 1998	Accs. 1999	Accs. 2000	Accs. 2001	Accs. 2002	Accs. 2003	Appro-priation 2004	Programme, total
Administration	0.754	2.242	2.239	3.201	2.589	1.894	2.126	0.400 *)	0.200 *)	**15.445**
Monitoring/Danish Veterinary Laboratory (DVL) analyses	0	5.107	15.639	10.564	11.748	0.666	0.456	0	0	**44.180**
Cleaning projects	0	0	0	0	0.289	2.120	1.218	0.970	0	**4.597**
DVL producer consultancy	0	3.500	2.900	1.700	1.000	0	0	0	0	**9.100**
Producer indemnification	1.500	40.151	22.872	12.801	15.484	8.233	8.916	0.046	0	**110.003**
Total expenditure	2.254	51.000	43.650	28.266	31.110	12.913	12.716	1.416	0.200	**183.525**

*) Report and symposium.

DKK 188.1 million was originally appropriated for the implementation of the National Salmonella Control Programme, DKK 30.0 million of which was contributed by the Danish Poultry Council, while the remainder was provided by the state. In addition, much of the available EU subsidy for destruction, etc., in the parent-flock segment has been infused into the programme. At the completion of the programme, an unused sum of DKK 6.4 million remained, which was allocated in relation to the original contributions, pursuant to Act No. 83 of 2 December 2003. As a result, DKK 1.0 million was returned to the Danish Poultry Council, while the Danish Veterinary and Food Administration received the rest.

'Administration' includes all expenditures for wages and other expenses incurred by the central and regional authorities alike.

Under 2001 and 2002, the item 'Monitoring/DVL analyses' only includes suspect-sample and control analyses, which were turned over to the industry as of 1 January 2001.

The item 'DVL producer consultancy' covers consultancy for producers in conjunction with cleaning after flock infection. The consultancy programme was gradually transferred to the industry during the course of 2000.

Seeing that 'Cleaning projects' are delayed, funds have been set aside to cover this expenditure in 2003. Funds have also been earmarked for compiling a report and holding a symposium.

14.4 Indemnity

The compulsory slaughter or destruction of flocks has been indemnified by the state.

Four indemnity categories are involved:

1. Loss of birds
2. Consequential loss, primary producer
3. Consequential loss, subsequent production segments
4. Loss of eggs and feed, as well as the cost of destruction.

Indemnity for the compulsory destruction or slaughter of birds is based on a series of scale values for the various production types, including the value of the birds from placement to removal. Indemnification for an entire flock is paid into a frozen account in the interests of claims from possible mortgagees. According to the Land Registration Act, a holding's livestock, including poultry, are included in the holding's value and will therefore be included in the holding's mortgage.

Indemnification is computed on the basis of the number of placed birds, so birds are usually not counted in conjunction with destruction, but it should be ensured that the number of destroyed animals is approximately the same as the number of placed animals.

In 1996, it was necessary to pay consequential loss indemnity in excess of the 8% consequential loss indemnity in effect at the time. This is because poultry production is organised according to the just-in-time principle, whereby it is virtually impossible to acquire birds for placement in the production facilities if a flock is removed in the middle of a production process. Full consequential loss indemnity was paid for destruction in the central rearing and pullet rearing segments and in the subsequent production segment, with a percentage-of-loss deductible of 10% until 3 March 1998. From 3 March 1998 till 31 May 2000, a 20% deductible clause applied to consequential loss indemnity, after which the deductible was increased to 30%. For immediately recurring infections involving the same salmonella serotype or phage type in the same locality, as well as other specified circumstances, the deductible percentage was increased.

Under the indemnity scheme, consequential loss in the subsequent production segment was also indemnified when a central-rearing or pullet-rearing flock was destroyed, if the owner of the rearing flock and the production site that should have received the eradicated flock was the same legal person. This indemnify was also conditional on the fact it was not possible to obtain birds for placement in the production facilities of the subsequent segment.

According to the principle of tort liability, it is the duty of the claimant to limit losses as much as possible. If it can be proven that the claimant has not fulfilled this obligation, then the indemnity for consequential loss can be reduced or withdrawn. The indemnity can be reduced, for instance, if it can be proven that the indemnified recipient has not reduced the consequential loss period by making use of an opportunity to obtain replacement birds for placement in an eradicated house.

Producers of eggs sent to heat treatment from table egg flocks owing to suspected infection with salmonella, which is subsequently disproved, were eligible for indemnity for the loss, cf. the government order of 31 May 2000, with retroactive effect to 11 September 1999. Eggs subjected to compulsory heat treatment or destruction had to be counted, and the amount of destroyed feedingstuffs, if relevant, was also jointly estimated with the owner. This indemnity scheme was abolished when the new government orders took effect in 2003.

15. Research Activities

As the National Salmonella Control Programme was gradually implemented, the problem of persistent salmonella infections in primary production became increasingly dominant in the table egg and broiler sectors alike. Apart from the obvious zoonotic significance, this also involved potentially great economic consequences for the poultry producers, partly as a result of reduced prices for egg or meat, and partly as a result of additional resources spent on extra cleaning and disinfection of poultry houses infected with salmonella. The consultancy unit at the Danish Veterinary Laboratory in Århus provided advice to poultry producers on measures that could eliminate persistent salmonella infections, but apart from general recommendations to clean as thoroughly as possible and disinfect all surfaces, research in this field was obviously lacking. Even though most of the advice was followed and procedures were optimised, infection with the house's persistent type of salmonella often re-appeared when the next poultry flock was placed in the house.

15.1 Cleaning and Disinfection Projects

Accordingly, it was widely agreed that research funds should be spent on documenting the effect of the cleaning and disinfection procedures. This resulted in the following two subsidiary projects:

- Sub-project I: Developing microbiological monitoring models in broiler houses: an assessment of the influence of cleaning and disinfection procedures on salmonella persistence.

- Sub-project II: Thermal disinfection as a method for eradicating salmonella infection in poultry houses.

During the time leading up to the official start of these research projects, experiments involving the heating of houses infected with salmonella had been carried out – particularly in the table egg sector. The house types in this sector (particularly battery-cage houses) are comparatively more difficult to clean than those of the broiler sector, and the financial consequences of persistent salmonella infections were often substantial. As a result, it was decided that Sub-project II should be implemented before Sub-project I.

Although the projects were officially implemented in July 2000, the Danish Veterinary Laboratory's consultancy unit in Århus had been involved in the problems beforehand, particularly with regard to heating poultry houses. This resulted in a theoretical article summarising relevant factors that via the heating could affect the elimination of salmonella bacteria (Gradel, 2000a). Results of field trials performed by representatives of the table egg sector were also published (Gradel, 2000b; Gradel, 2001; Olsen *et al.,* 2000).

The actual Sub-project II was divided into two phases: phase 1 consisting of laboratory experiments and phase 2 consisting of field trials that involved the testing of measures proven through laboratory experiments to facilitate the elimination of salmonella bacteria.

15.1.1 Thermal disinfection

15.1.1.1 Laboratory experiments

The laboratory experiments were designed to simulate the worst conceivable conditions in a house abounding with organic material and large quantities of stressed salmonella bacteria. The following factors – which would conceivably affect the thermal eradication of salmonella bacteria but which could also be implemented in field trials – were selected: organic material (faeces/pellet feed), salmonella type (*S.* Enteritidis FT8, *S.* Typhimurium DT 110, *S.* Infantis), drying before heating (yes/no), relative humidity during the heating (16-30% and 100% relative humidity (RH)), and the final temperature during the heating process (50/55/60/65/70º C). Faeces and feed were used in their original state and pre-equilibrated for ten days at 30% RH. All samples were heated from 20º C to the final temperature in increments of 1º C an hour, as slow heating would theoretically make the salmonella bacteria less sensitive to heat. In addition to the salmonella bacteria, naturally occurring *E. coli* in faecal samples were used as indicator bacteria. This bacterium is more abundant in poultry houses than salmonella and can be used to verify the effectiveness of the salmonella control measures. The experiments showed that heating to 60^{o} C at 100% RH for 24 hours could eliminate roughly 10^{5} CFU (Colony Forming Units) of salmonella per gram of organic material, and that the elimination of *E. coli* was highly correlated to the elimination of salmonella, which made the former bacterium a well-suited indicator (Gradel, 2002a; Gradel *et al.,* 2003a).The above-mentioned method was therefore considered to be an expedient standard for subsequent testing in field trials.

15.1.1.2 Field trials

Field trials were performed in four percheries (‘barn eggs’) and five battery houses (‘cage eggs’). Before the thermal disinfection, the houses concerned were cleaned using normal high-pressure cleaning methods. Various models were used, but the studies generally confirmed that the heating of the infected houses to 60º C at 100% relative humidity for 24 hours is an effective means – also in field trials – of eliminating salmonella bacteria, particularly when 30 ppm of formaldehyde are added at the beginning of the process. In addition, it is necessary to disinfect floor surfaces, as the air temperature at low floor height (approximately 10 cm) is about 5º C lower than elsewhere. The finds of salmonella bacteria correlated positively with finds of coliform bacteria, which explains why the latter could supplement the actual detection of salmonella to predict whether salmonella had actually been eliminated (Gradel, 2002b; Gradel *et al.,* 2002; Gradel *et al.*, 2003b). The estimated cost of thermal disinfection for a poultry house is in the area of DKK 30-40,000. As of November 2003, none of the disinfected houses had become re-infected.

15.1.2 Chemical disinfection

Two experiments have been carried out, dealing with resistance to disinfection agents and realistic surface experiments.

The conditions for whether a chemical disinfection succeeds are basically related to three main factors: the micro-organism (e.g., type and possible resistance to the disinfection agent), the disinfection agent (including type and concentration) and the surroundings (ambient temperature, amount and type of organic material, surface characteristics of livestock equipment frequently found in poultry houses and much more).

15.1.2.1 Resistance to disinfection agents

The aim of this experiment was to determine the MIC (Minimum Inhibitory Concentration) of five disinfection agents in relation to 'persistent' and 'non-persistent' salmonella serotypes frequently found in Danish poultry houses and compare them to serotype, salmonella persistence and use of disinfection agents. In addition, adaptation and de-adaptation studies were made involving five disinfection agents against salmonella isolates with high or low MIC respectively to study whether resistance could be developed and upheld.

MIC tests were performed on 286 salmonella isolates (269 from the Danish poultry sector, including 256 from broiler houses, and 17 from the UK) from 88 broiler houses in relation to 5 disinfection agents (glutaraldehyde / benzalconium chloride, formaldehyde, an oxidising agent, a phenol compound and an iodophor). The three first disinfection agents are the most frequently used agents in Danish poultry houses. The Danish salmonella serotypes included Enteritidis, Typhimurium, Tennessee, 4.12:b:-, Infantis, Indiana and Senftenberg. The results showed little MIC variation against commonly used disinfection agents, and they could not be epidemiologically related to salmonella persistence or the use of disinfection agents.

The 6 salmonella isolates selected for the adaptation and de-adaptation studies (3 with high and 3 with low MIC values) in relation to 5 disinfection agents showed no significant changes in MIC values after adaptation or de-adaptation.

In general, the results showed that the development of resistance to these five disinfection agents could not be induced in actual laboratory experiments and as a result this does not seem to be a contributing factor to the persistence of salmonella bacteria (Gradel, 2003; Gradel & Randall, 2003).

15.1.2.2 Realistic surface disinfection tests

Surface disinfection tests resembling worst-case scenarios in poorly cleaned poultry houses were carried out at low temperatures. A high number of bacteria (*S.* Enteritidis FT8, *S.* Senftenberg and *Enterococcus faecalis*) were dissolved in various types of organic material (feedstuffs, oil, egg yolks) and applied to relevant poultry house materials (concrete tiles, feeder chain links of non-stainless steel, wooden dowels, jute egg conveyors). After this, the materials were disinfected using three disinfection agents (formaldehyde, glutaraldehyde / benzalconium chloride and an oxidising agent)[2]. In general, the results showed that formaldehyde was better than glutaraldehyde, which in turn was better than the oxidising agent. Water yielded the worst results. However, the oxidising agent was the most effective agent for disinfecting greased feed chains at 30º C. With respect to the bacteria, there was no difference in the effect of formaldehyde and glutaraldehyde on *S.* Enteritidis and *S.* Senftenberg , whereas the oxidising agent was much more lethal against the latter bacterium. *Enterococcus faecalis* was at least as difficult to eliminate as the two salmonella isolates, making it an expedient indicator bacterium.

[2] The results of the cleaning and disinfection projects are part of a Ph.D. study programme. The guidance counsellors are Robert H. Davies, Veterinary Laboratories Agency, Weybridge, U.K., who has published articles on persistent salmonella-contaminated poultry houses, and Janet E. L. Corry, University of Bristol, U.K., who has published articles on the thermal tolerance of salmonella bacteria in foodstuffs.

16. Results

In conjunction with the industry's voluntary initiatives, the investigation of destroyed flocks of table egg hens started in 1992. The National Salmonella Control Programme was implemented later on, and the registration and sample-taking in pullet-rearing and table egg flocks was instituted from 1996 till the National Salmonella Control Programme was temporarily suspended in 1997. From September 1997 till March 1998, the National Salmonella Control Programme was temporarily suspended as regards pullet-rearing and table egg flocks, but after this the routine analysis of samples taken from these flocks was initiated. The figures included here are from January 1997, as very few data are available from December 1996, i.e., the start of the National Salmonella Control Programme.

The number of examined flocks is somewhat uncertain as some of the flocks may have converted from one type of production to another over time and therefore may be included in a different production type category than the one in which they were registered at the time of examination. The database is structured so that if the production type of one house changes, and this change is entered in the database, all previously entered data concerning the production type in the house concerned will also be modified. The number of examined and infected flocks have been continuously reviewed, however, for use at steering committee meetings, etc., and for publications such as the Danish Veterinary Laboratory's monthly report 'Zoonose-Nyt' and the Danish Poultry Council's 'Det Gule Fjerkræblad'. The figures from these reports form the basis of the number of infected flocks in all production types. The number of examined flocks has been computed by searching in the database by year. Although each flock is only counted once under this method, it deserves notice that more than one rotation may occur each year, depending on the type of production.

16.1 Prevalence of Salmonella in the Broiler Sector, 1996-2002

16.1.1 Live birds

The primary production of broilers has been subjected to salmonella testing since 1989.

In the infected flock figures in Table 9, a flock is defined as one epidemiological unit. This means that the infection of some flocks involves more than one house.

In conjunction with the revised National Salmonella Control Programme, which took effect in March 1998, new goals were set for salmonella prevalence. The goal for broiler production was to reduce the percentage of flocks infected with *Salmonella* Typhimurium or *S.* Enteritidis to less than 1% and the percentage of flocks infected with other serotypes to less than 2%. The original goal for broiler production had been to reduce the level of infection to less than 5%, which had been met.

Table 9: Number of examined and infected élite and parent flocks, 1997-2002 (broiler sector)

	1997		1998		1999		2000		2001		2002	
	Examined *	Infected **	Examined	Infected	Examined	Infected	Examined	Infected	Examined	Infected	Examined	Infected
Central rearing flocks, breeding * **	83	0	47	0	25	0	25	0	28	0	15	0
Multiplier flocks, breeding * **	8	0	1	0	2	0	2	1	3	0	3	1
Central rearing flocks	198	0	220	0	221	0	246	1	233	0	244	0
Multiplier flocks	392	8	347	2	363	2	345	3	325	6	330	2
Total	681	8	615	2	611	2	618	5	589	6	592	3

* The database is structured so that if the production type of one house changes and this change is entered in the database, all previously entered data concerning the production type in the house concerned will also be changed.
** In the figures involving infected flocks, a flock is defined as one epidemiological unit. This means that the infection of some flocks involves more than one house.
*** Breeding includes grandparent/élite birds.

Throughout the entire National Salmonella Control Programme, 1 élite flock and 8 parent flocks (dispersed over 18 houses) have been infected with *S.* Enteritidis, 1 élite and 13 parent flocks (dispersed over 25 houses) have been infected with *S.* Typhimurium and 4 parent flocks (dispersed over 18 houses) have been infected with an exotic strain of salmonella. Over the years, the total percentage of infected flocks of the flocks studied has varied from 0.3% to 1.2% (average: 0.7%) (fig. 4). It is worth noting, however, that the decline in infected parent flocks from 1997 to 2002 is based on numerically modest statistics.

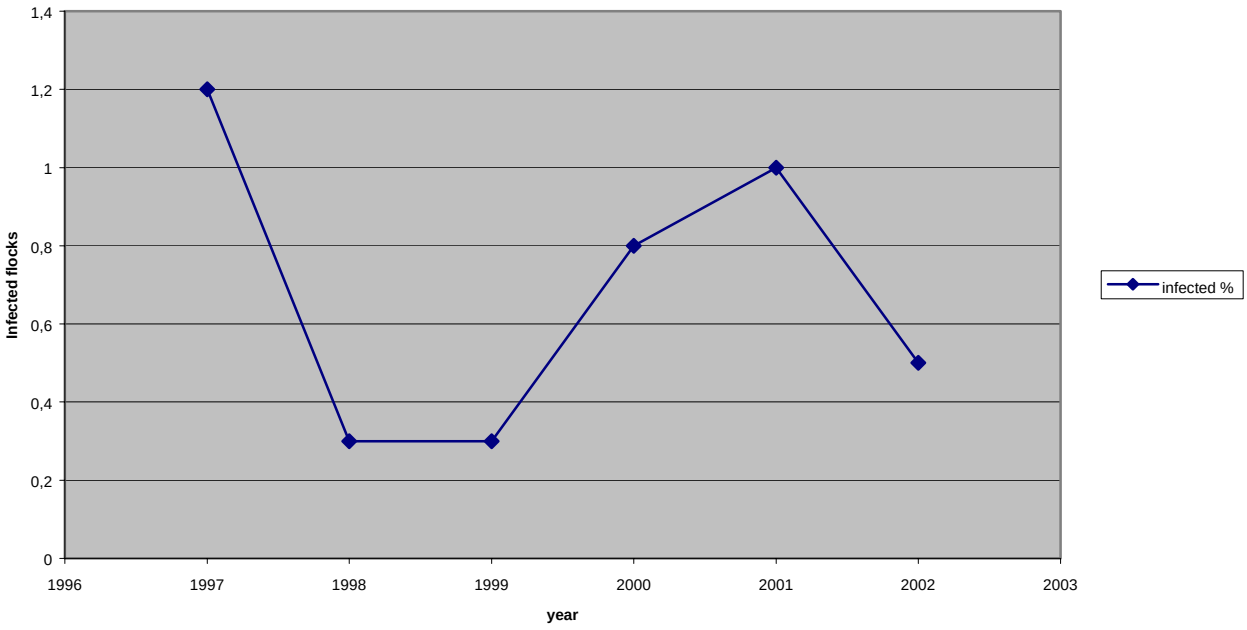


Figure 4: Percentage of infected examined flocks in the breeding, central rearing and multiplier segments (broiler sector).

No infected flocks have been found in the breeding segment for central rearing flocks (élite birds).

Only two infected flocks have been found since 1996 in the breeding segment for multiplier flocks (élite birds), one in 2000 and the other in 2002, both at the same holding. The flocks were declared infected with respectively *S.* Enteritidis FT 6 and *S.* Typhimurium DT 12.

One infected central rearing flock was found in 2000. The flock was declared infected with *S.* Tennessee.

The multiplier segment has had 24 infected flocks (including 1 flock in December 1996). The average prevalence of salmonella has been 1.2% of the flocks studied, mostly with serotypes *S.* Typhimurium and *S.* Enteritidis (Table 10).

Table 10: Number of infected multiplier flocks classified by serotypes and phage types, 1996-2002.

S. Typhimurium	Phage type:	DT 41	DT 12	DT 110	DT 66
	No.:	6	3	3	1
S. Enteritidis	Phage type:	FT 8	FT 1B	Non-typeable	-
	No.:	4	2	2	-
Exotic serotypes	Phage type:	Abony	Infantis	4.12:b:-	-
	No.:	1	1	1	-

In general the most frequently detected strains in flocks appear to be *S.* Typhimurium and *S.* Enteritidis. From 1996 to 2002, *S.* Typhimurium and *S.* Enteritidis were respectively detected in 54% and 33% of the infected multiplier flocks. The phage types DT41 and DT12 are the most frequently occurring *S.* Typhimurium isolates, whereas FT8 is the predominant phage type of *S.* Enteritidis.

Exotic strains of salmonella have tended to dominate the finds in routine sample-taking over the last three years. From 1997 to 2002, for instance, the number of flocks in which exotic strains have been suspected or detected through routine sample-taking has increased. It should be emphasised, however, that the exotic strains of salmonella rarely become firmly entrenched in the flocks, which is reflected in the fact that only a few of the suspected flocks are subsequently declared infected (Table 11).

Table 11: Exotic strains of salmonella in suspected multiplier flocks in the broiler sector, 2000-2002.

No. of suspected flocks	Salmonella serotype in the routine sample	Subsequently declared infected
25	Positive dust samples from hatchery	1 flock with *S.* Typhimurium DT41
7*	*S. Senftenberg*	
2	*S.* Hull	1 flock with *S.* Abony
2	*S.* Lambehurst	
3	*S.* Gbadago	
1	*S.* Carmel	
1	*S.* Virkow	
1	*S.* Cubana	
1	*S.* Bergen	
1	*S.* Mbandaka	
1	*S.* India	
1	*S.* Gnesta	
1	*S.* Derby	
5	Unspecific, non-typeable	

* Four of the suspect samples were taken from the same flock over a five-month period.

Of the 22 flocks suspected due to finds of an exotic strain, only 1 was declared infected. This flock was suspected on the basis of a find of *S.* Hull in routine samples and was subsequently found to be infected with *S.* Abony. In the other suspected flocks, the infection could not be confirmed in the suspect samples. During the same period, 12 flocks were suspected on the basis of either *S.* Enteritidis or *S.* Typhimurium, and 11 of these flocks were declared infected, i.e., the suspicion of infection was rejected in only 1 flock. Similarly, 25 flocks were suspected on the basis of positive dust samples from a hatchery, but only 1 of the flocks was subsequently declared positive (Table 11).

Infection has been repeatedly detected at several of the holdings during the course of the National Salmonella Control Programme. The house at one holding, for instance, has been declared infected four times during this period. Although two re-infections have also occurred in another house at the holding, the serotypes involved were not the same. Four of the holdings have houses that have been infected more than once. Eighteen percent (18%) of the infected flocks have been infected more than once in the same house.

Slaughtered poultry, including broiler flocks, are examined three weeks before slaughter by means of bacteriological analysis. The prevalence of salmonella in broiler flocks found at AM control declined from 12.9% in 1997 to 1.5% in 2002 (Fig. 5). A new and more sensitive method for detecting salmonella in broiler flocks was put into use in December 1994, resulting in the detection of considerably more infected flocks than before. At the start of AM control, the analysis method only detected salmonella if more than 20% of the birds were infected, whereas the new method can detect salmonella if 1% of the birds are infected.

16.1.2 Fresh broiler meat

In order to pursue the effort of controlling salmonella in primary production, salmonella monitoring was initiated at poultry slaughterhouses involving fresh meat from broiler flocks. After slaughter, each broiler flock was analysed by taking 5 pooled samples comprising 10 neck-skin samples each. Since the start of the monitoring programme in 1992, the prevalence of salmonella in fresh broiler meat at the slaughterhouses has declined from an average of 62% positively-tested flocks after slaughter in 1993 to some 3% positively-tested flocks in 2000 (Fig. 5).

The sample-taking procedure was changed in November 2000 so that each batch of broiler meat was subsequently tested for salmonella just before the fresh meat was packaged. One batch is defined as the production of broiler meat between two cleaning and disinfection processes. The purpose of the change was twofold: to improve the measurement of the prevalence of salmonella detected in the fresh broiler meat sold to consumers and to get a better idea of the extent of cross-contamination at the slaughterhouse. The sample-taking programme is designed so that several samples are taken from batches that include flocks which tested positive before slaughter. Twelve pooled samples consisting of 5 samples each were taken from batches that include positive flocks, and 4 pooled samples consisting of 10 samples each were taken from batches that included negative flocks. After the introduction of the new sample-taking model, the prevalence of salmonella has been about 5% of fresh meat from broiler flocks (Fig. 5).

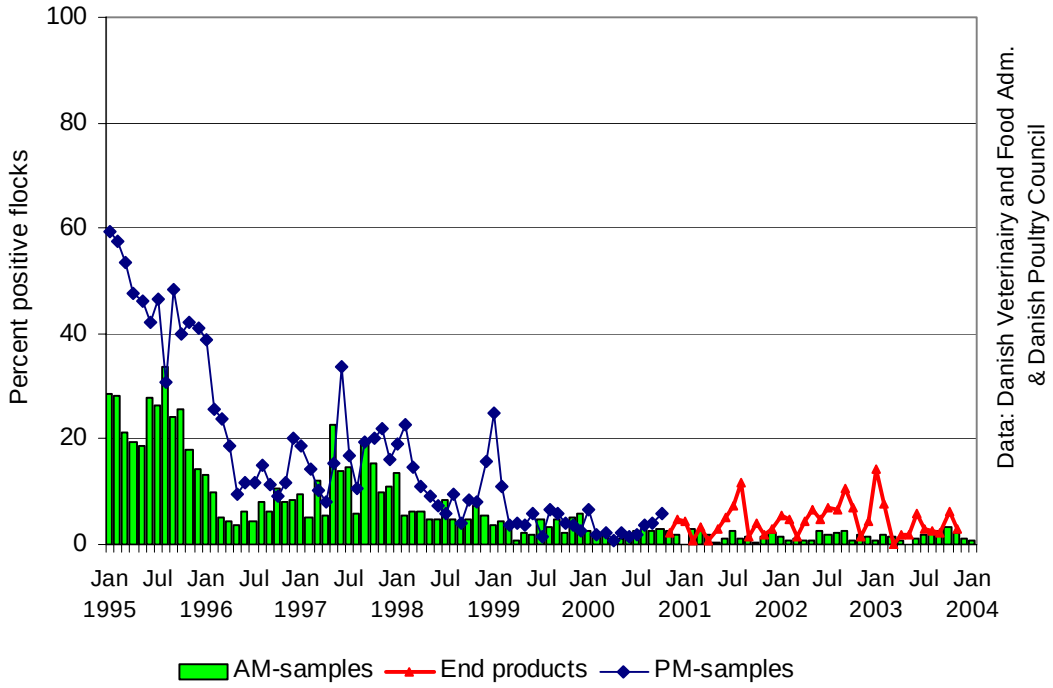


Figure 5: Salmonella in broilers before (AM) and after (PM) slaughter, 1995-2004.

16.2 Prevalence of Salmonella in the Table Egg Sector, 1997-2002

16.2.1 Live birds

Parent birds in the table egg sector were routinely analysed in accordance with the Zoonosis Directive from early 1994 till late 1996. No infected flocks were found during this period. But as Table 12 shows, *Salmonella* Enteritidis FT4 was detected in January 1997 in two central rearing flocks imported from abroad. Later that year, *S.* Enteritidis FT8 was found in two large multiplier flocks. The offspring from the two parent flocks were tracked down, and a total of 27 pullet-rearing flocks and 33 table egg flocks were declared infected. Although the parent-flock facilities were cleaned and disinfected, the same salmonella serotype and phage type was detected in the flocks again in October and November 1997. In December, one more house at one of the two mentioned parent-flock holdings tested positive for salmonella. During the course of 1997, 40% of the birds in the hatching egg production for the table egg sector tested positive and were destroyed. This led to an acute need to revise the original National Salmonella Control Programme, as all of the infected pullet-rearing and table egg flocks were associated with the same hatchery enterprise, which at the time produced approximately 55% of the table egg layer hens in Denmark. The prospects of an

acute shortage of Danish eggs and of rapidly depleting the indemnity funds resulted in the suspension of parts of the National Salmonella Control Programme in September 1997.

Table 12: Number of examined and infected flocks in the table egg sector, 1997-2002.

	1997		1998		1999		2000		2001		2002	
	Examined**	Infected*	Examined	Infected	Examined	Infected	Examined	Infected	Examined	Infected	Examined	Infected
Central rearing flocks*	14	2	13	0	12	0	13	0	13	0	15	0
Multiplier flocks*	24	5	27	0	27	1	31	0	23	0	22	0
Pullet-rearing flocks	-	27	349	11	375	10	218	8	342	4	330	9
Table egg flocks	-	33	781	105	727	37	667	31	614	35	619	16
Farm-gate	-	-	236	15	278	12	769	56	582	5	562	10
Barnyard ***	-	-	-	-	-	-	1679	31	303	-	185	-

** In figures involving infected flocks, a flock is defined as one epidemiological unit. This means that the infection of some flocks involves more than one house.
** The database is structured so that if the production type of one house is changed, and this is entered in the database, all previously entered data concerning the production type in the house concerned will also be changed.
*** As of 1 July 2000, two new sub-groups were formed, i.e., 'barnyard flocks' and 'farm-gate flocks'. After this, suspect samples were no longer taken from barnyard flocks and they were not declared infected, but a case is considered closed immediately after the regional veterinary and food control authority has notified the owner in writing of the risk of eating the eggs.
- No figures are available for the number of pullet-rearing, table egg, farm-gate and barnyard flocks in 1997.

Of the 12 suspected multiplier flocks since 1998, only one has been declared infected (Table 12). This flock was suspected on the basis of dust samples from the hatchery and was found to be infected with *Salmonella* 4,5,12:i.

The most frequently isolated serotype and phage type in pullet-rearing flocks has been *Salmonella* Enteritidis FT8. This serotype has caused re-infections at two sites (Fig. 6).

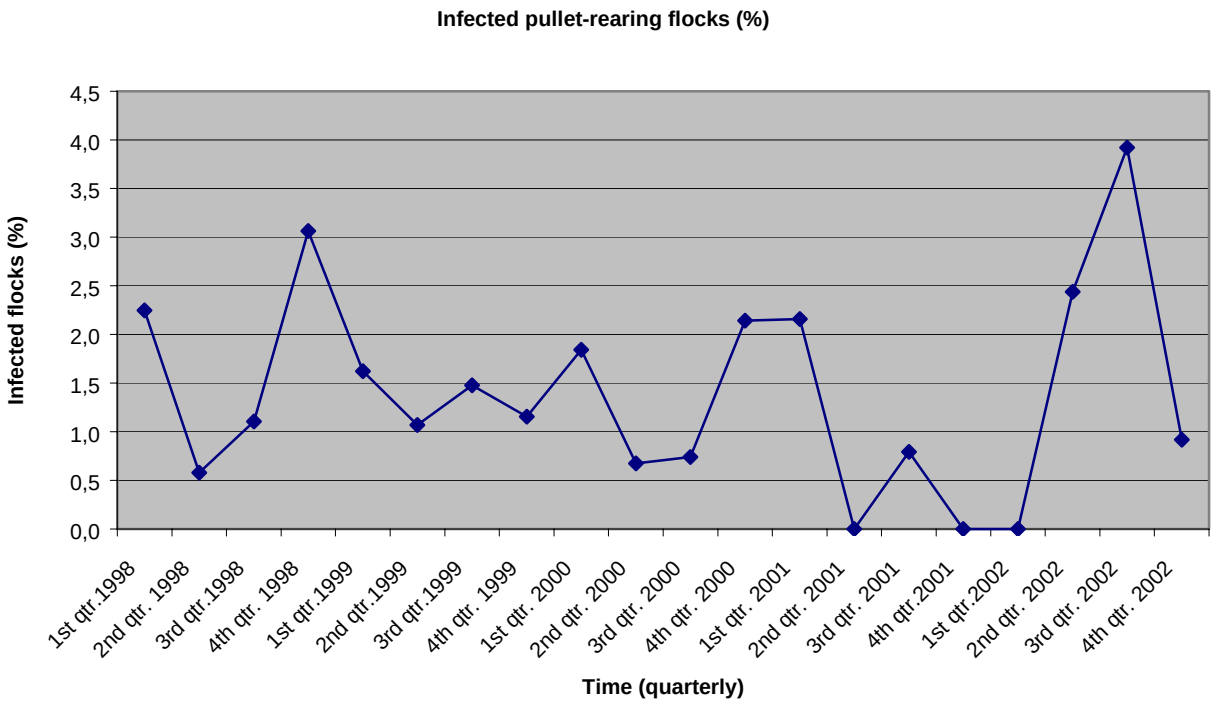


Figure 6: Infected pullet-rearing flocks, 1998-2002.

Most of the isolated serotypes in table egg flocks involve *Salmonella* Enteritidis. *S.* Enteritidis FT8 was found in 58% and 40% of the infected flocks in 1997 and 2000 respectively. In addition to this, *S.* Enteritidis FT4 and FT6 and *S.* Infantis were isolated in many of the infected flocks. The number of infected flocks found in 1998 was three times greater than in any other year of the National Salmonella Control Programme. This is related to the previously mentioned situation in which infection was detected in two large parent flocks with ensuing consequences for the production segment. Infection was found in 13.4% of the table egg layer flocks analysed in 1998, and compulsory heat treatment was imposed on them. The goal at the start of the National Salmonella Control Programme was to reduce the infection level to 5% by the end of the National Salmonella Control Programme. There were far more infected flocks after the first year than originally estimated, however, which resulted in an amendment of the National Salmonella Control Programme. By the end of 2002 the prevalence of salmonella in table egg flocks, had been reduced to 0.7%. A total of 2.6% table egg flocks tested positive in 2002 (Fig. 7).

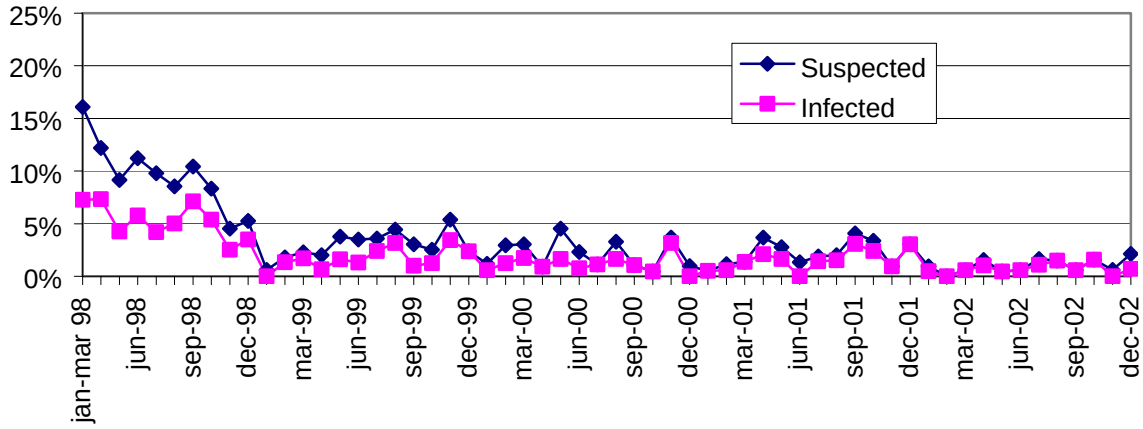


Figure 7: The percentage of suspected and infected table egg flocks compared to analysed flocks per month, 1998-2002.

The number of analysed farm-gate flocks and barnyard flocks rose sharply in 2000 (Table 12). This is presumably due to an incident involving the deaths of two persons after eating a cake made with unpasteurised eggs. This caused many producers – who either used their own eggs in household cooking or who had unregistered farm-gate sales – to register their flocks under the National Salmonella Control Programme. The number of analysed farm-gate and barnyard flocks has sharply declined since then, particularly the number of barnyard flocks (Fig. 8).

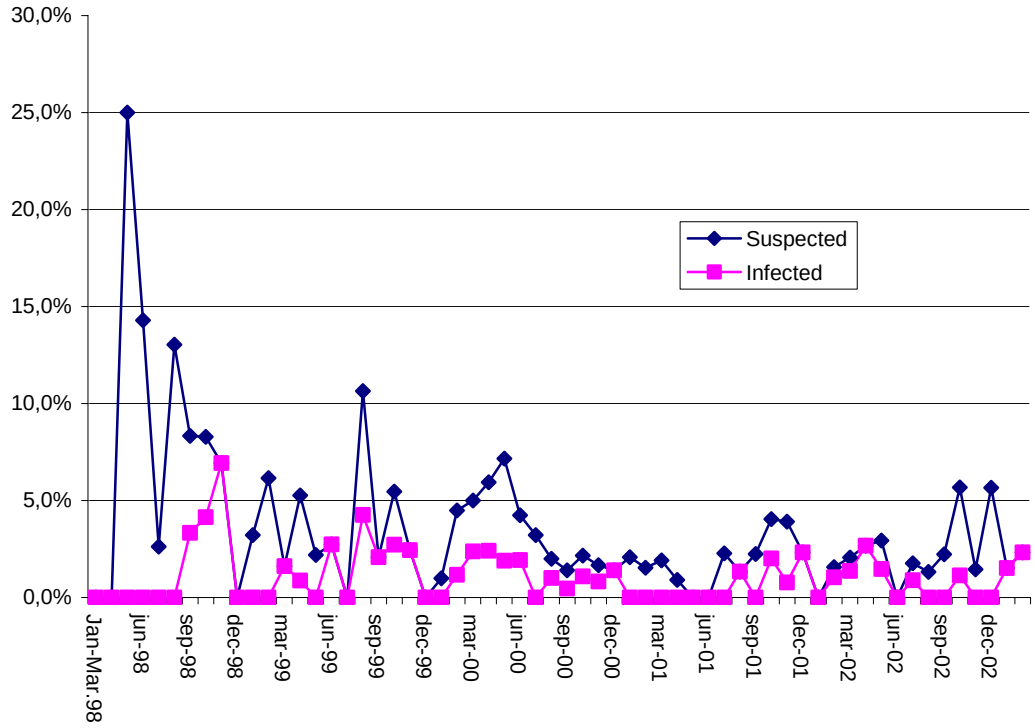


Figure 8: Percentage of suspected and infected farm-gate flocks of the flocks analysed per month, 1998-2002.

16.2.2 Shell eggs

An analysis of salmonella in Danish and imported shell eggs was carried out in 2002. The study involved 10,180 Danish eggs and 4,900 imported eggs. All Danish eggs were grade A, whereas the imported eggs were grades A and B. The analysis detected salmonella infection on the shell of five of the analysed Danish eggs (0.05%) and in the contents (yolk/white) of two (0.02%). By comparison, the analysis of imported eggs detected salmonella infection on the shell of 27 eggs (0.55%) and in the contents (yolk/white) of 12 (0.25%) (Report, 2004). A similar study in 1995 detected salmonella infection in an average of 0.1% of Danish shell eggs (Report, 1996).

16.3 Human Salmonellosis Trends, 1996-2002

Over the last two decades, there have been three periods during which the incidence of human salmonellosis in Denmark has risen. Most of the infections in the late 1980s were attributed to broiler meat, whereas pork and eggs were the sources in the early 1990s, and eggs dominated the late 1990s. In all three periods, control programmes were implemented to control salmonella at the specific source of infection, and the National Salmonella Control Programme was also initiated in 1996 to eradicate salmonella in table egg production. The incidence of human salmonellosis from 1996 to 2002 is shown in Figure 9.

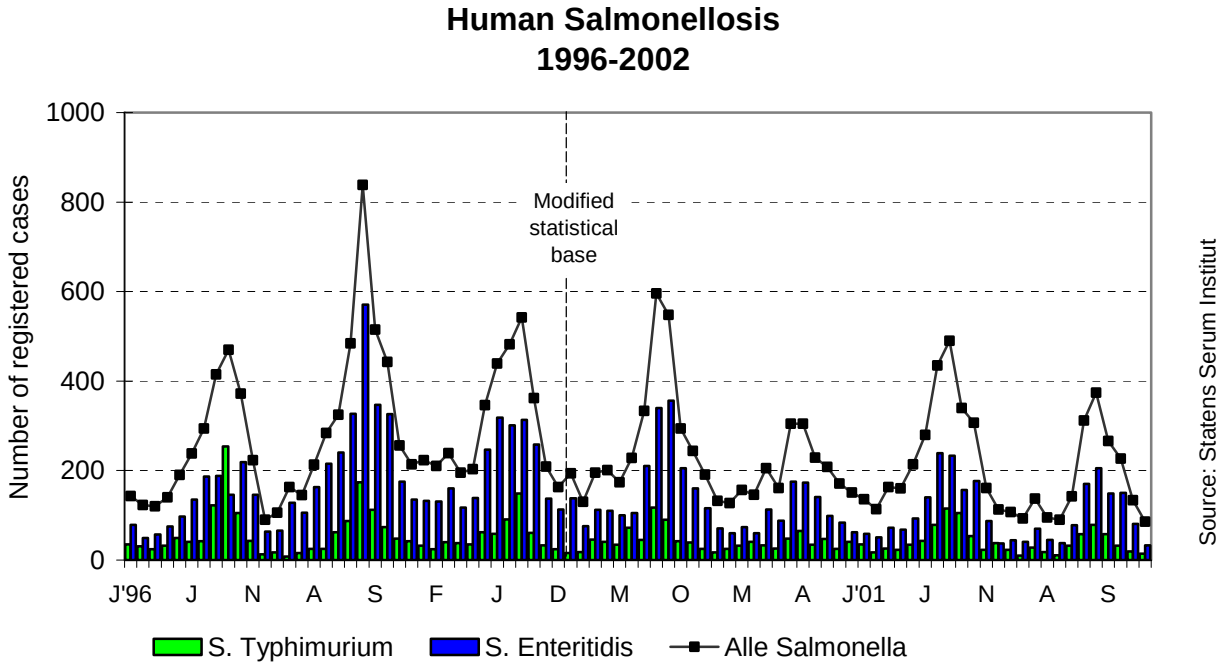


Figure 9: Registered monthly incidence of human salmonellosis, 1996-2002.

Of the table egg flocks in which salmonella infection was detected, 95% were infected with *S.* Enteritidis, whereas this serotype is only sporadically detected in other production livestock. In 1999, it was estimated that 60% of all cases of *S.* Enteritidis were caused by Danish eggs and that these infections constituted roughly 40% of all registered salmonella infections in Denmark. For this reason, *S.* Enteritidis will be the centre of attention in the following. Shortly after the start of the National Salmonella Control Programme in 1996, *S.* Enteritidis FT8 was detected at Denmark's largest table egg hatchery. The hatchery produced 55% of Denmark's egg-laying hens, and the infected parent animals resulted in the detection of far more infected production flocks during the course of 1997 than anticipated.

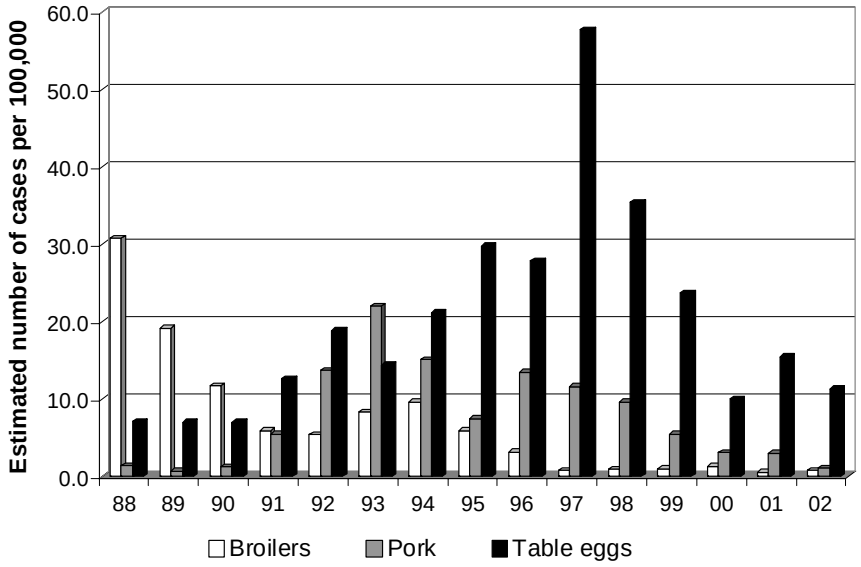
The situation was reflected in the number of human *S.* Enteritidis infections which almost skyrocketed during the summer of 1997, showing an overall increase of 54% compared to the year before. As a result, 1997 saw the greatest incidence (5,015 cases) of detected and registered human salmonellosis in Denmark of any year to date. The dominant *S.* Enteritidis phage types in table egg production and in humans were FT8, FT4 and FT6. 1998 and 1999, however, saw a striking reduction in the human infection rate caused by egg-related salmonella types, and 2000 saw the lowest number of human infection cases since the start of the National Salmonella Control Programme. The total of 2,208 cases in year 2000 was almost at the same level as in 1991 (2,203 cases). The optimistic trend was observed in table egg production in which the percentage of positive flocks declined from 13.4% in 1998 to 5% in 1999. 2001, on the other hand, saw a 25% increase in the incidence of human salmonellosis (2,918 total cases). This aroused concern that the trend was again headed in the wrong direction. As it turned out, however, this did not only involve a rise in infection caused by egg-related types of salmonella but also infections caused by many other dominating types. The same was also true of other bacterial zoonoses. The summer of 2000 was

colder and rainier than normal and experience shows that such climatic conditions lead to fewer human infections, presumably due to poorer growth conditions for bacteria such as salmonella. Home-made dishes containing raw eggs, such as ice cream, were also presumed to be less popular during periods of cold, wet weather. These conditions were reflected in the fact that the culmination of human cases ('the summer peak') in 2000 was considerably lower than the year before and the year after. As the number of infections in 2001 was still lower than the years 1992-1999, it was deemed more likely that the 2000 level had been the lowest, rather than 2001, which represented an increase.

The total number of registered cases of human salmonellosis in 2002 (2,071 cases) declined by 29% compared to year 2001 and 10% compared to year 2000 (Annual Reports on Zoonoses, 2000-2002). The incidence of *S.* Enteritidis and *S.* Typhimurium infections show the same trends, i.e., declines of 22% and 36% respectively from 2001 to 2002. In 2002 it was estimated that 74% of the human salmonellosis cases were domestic. It is estimated that roughly 25% of the human *S.* Enteritidis cases in 2002 are attributable to travel abroad.

In general, the results of the zoonotic monitoring show that the total number of human salmonella infections (59% reduction) and the number of infected table egg flocks fell considerably from 1997 to 2002.

Figure 10: Estimated incidence of human salmonellosis caused by broiler meat, pork and table eggs, 1988-2002.



Source: The Danish Zoonosis Centre

The comparative infection for 1997-2002 shows that the incidence of human salmonellosis attributable to eggs has sharply declined by roughly 80% (Figure 10). In contrast with 1997 when 60% of all salmonellosis cases (3009) in Denmark were estimated to be egg-related, only 31% (636) were egg-related in 2002.

The incidence of human salmonellosis caused by broiler meat has been reduced by 59% from 1997 to 2002 (100 to 41 estimated cases). The incidence of human salmonellosis estimated to be related to the consumption of Danish produced broilers has stabilised at 2%.

17. The National Audit Office of Denmark

In 1999-2000, the National Audit Office of Denmark reviewed the national salmonella control effort. The review of the National Audit Office showed that the internal accounts of the institutions under the Ministry of Food, Agriculture and Fisheries provided an expedient basis for financial follow-up of the appropriations for the National Salmonella Control Programme.

The National Salmonella Control Programme was based on preceding analyses of the prevalence of salmonella in the flock types on which it was targeted. The programme was also based on the latest research results. The National Audit Office was of the opinion that when the National Salmonella Control Programme was implemented, the Ministry of Food, Agriculture and Fisheries had assessed and considered any knowledge gained and methods applied in Sweden that were relevant to Danish circumstances. In the assessment of the National Audit Office, therefore, the National Salmonella Control Programme was carried into effect on a satisfactory foundation. The National Audit Office noted that the Ministry of Food, Agriculture and Fisheries had set distinct, quantitative goals for the National Salmonella Control Programme and pursued them. The effectuated National Salmonella Control Programme was based solely on the assessments of the status reports. The National Audit Office found that the Salmonella Database, around which the National Salmonella Control Programme was organised, provided a suitable instrument for the Ministry of Food, Agriculture and Fisheries in controlling the collection of samples and sample results and for processing statistics on salmonella in poultry flocks. The National Audit Office therefore assessed that the supervision of the salmonella control efforts as exerted by the Ministry of Food, Agriculture and Fisheries ensured that the control procedures were followed.

The review by the National Audit Office showed that the implementation of the National Salmonella Control Programme was responsible for significantly reducing in the incidence of human illness.

In the view of the National Audit Office, the joint efforts of the institutions of the Ministry of Food, Agriculture and Fisheries and of the Ministry of Health in working to control salmonella were satisfactory. The National Audit Office urged the Ministry of Food, Agriculture and Fisheries and the Ministry of Health to consider jointly expanding the overall strategy to include common objectives for monitoring, tracking down the source of and controlling salmonella in livestock and humans.

Therefore, it is the general assessment of the National Audit Office that the overall effort and results of the salmonella control programme are satisfactory – also compared to the results obtained in other countries.

18. The National Salmonella Control Programme After 2002

18.1 Transferring Responsibility to the Industry

From 1 January 2003, the industry has assumed much of the financial responsibility for the National Salmonella Control Programme, as the appropriations for the project organisation ceased at the end of 2002. For various reasons the new government orders did not take effect till 1 February 2003, when the last aspect of the financial responsibility was transferred to the industry, represented by the Danish Poultry Council. The Danish Poultry Council has also taken over the administration of the National Salmonella Control Programme. The Danish Poultry Council is now responsible for continuously updating the registration of all poultry producers (including farm-gate sellers). The Danish Poultry Council is also responsible for informing and guiding the producers so they are fully capable of complying with the National Salmonella Control Programme and for investigating any irregularities. The Danish Veterinary and Food Administration is still responsible for registering new cases of suspected infection in the poultry database, as the updating of suspected cases is a prerequisite for control. The Danish Veterinary and Food Administration will continuously monitor the Danish Poultry Council's administration and continuation of the National Salmonella Control Programme.

18.2 Supervision of the National Salmonella Control Programme After 2002

The Danish Poultry Council is responsible for the continuation of the National Salmonella Control Programme. The public sector is still involved in monitoring the compliance with the National Salmonella Control Programme. The project organisation's spheres of activity have been discontinued and the functions of the organisation have been allocated to the central offices of the Danish Veterinary and Food Administration in Mørkhøj, to the regional veterinary and food control authorities and to the Danish Poultry Council. The administration of investigating positive salmonella results has been delegated to the regional veterinary and food control authorities. Laboratory results are sent direct to the producer, the regional veterinary and food control authority and the Division of Zoonoses, Veterinary Department, VA2, where suspected cases are registered in the poultry database. To ensure a reaction to all positive laboratory results, a security procedure has been introduced whereby the file number of the case at the regional veterinary and food control authority is sent to VA2 to confirm the receipt of laboratory results. The file number is used when entering the suspected infection in the Salmonella Database. A regional categorisation of search options within the database enables each regional veterinary and food control authority to follow the progression of a suspected infection in its own area. One element on which the future supervision of the National Salmonella Control Programme will be based is the internal control exercised by each enterprise. Egg packing stations are under an obligation to discard the eggs of any producers who are placed under suspicion or who have not taken scheduled samples as planned. From 1 January 2003 the regional veterinary and food control authority will only order the destruction or slaughter

of parent flocks if they are infected with *S.* Enteritidis or *S.* Typhimurium. Parent flocks infected with exotic strains of salmonella will merely be placed under public supervision. The destruction of infected pullet-rearing flocks is no longer compulsory, but said flocks will be placed under public supervision. Indemnity will only apply to flocks that are compulsorily destroyed pursuant to the 'Order on expenditures and indemnity for controlling and preventing livestock disease'. From 1 February 2003 minor adjustments have been made to the sample-taking programme, whereby future 12th-week samples from pullet-rearing flocks will not be routinely taken by the regional veterinary and food control authority, but can also be taken by the holding's veterinarian. The complete funding of all samples under the salmonella programme has been transferred to the industry.

To be eligible for continued support for the National Salmonella Control Programme from the European Commission, the Danish Veterinary and Food Administration must still be responsible for the official reporting to the EU. In addition, the Danish Veterinary and Food Administration contributes information to 'The Annual Report on Zoonoses in Denmark' and the monthly update 'Zoonose-Nyt'. The Danish Veterinary and Food Administration will still be responsible for publishing the progress of the salmonella control in the poultry sector and for serving as an official complaints board.

19. Attainment of Goals

Original goals of the National Salmonella Control Programme (1996-2002):

● As the incidence of infection has declined from 13.4% in 1998 to 2.6% in 2002, the goal to reduce the percentage of infected table egg layer flocks to less than 5% by the end of the National Salmonella Control Programme has been achieved.

> ► In addition to this, the subsidiary goal to reduce the incidence of salmonella infection in the table egg flocks by one-third has been achieved, except for the year-end results from 1999 and 2000.

● As the incidence of infection has declined from 12.9% in 1997 to 1.5% in 2002, the goal to reduce the percentage of infected broiler flocks to less than 5% by the end of the National Salmonella Control Programme has been achieved.

> ► The subsidiary goal to reduce the incidence of salmonella infection in the broiler flocks by one-third has been achieved, except for the year-end results from 2000 and 2001.

● As the incidence of human salmonellosis has declined by 78%, from 3,109 estimated cases in 1997 to 677 cases in 2002, the goal to reduce the incidence of human salmonellosis related to Danish-produced poultry products has been achieved to the full.

Additional goals (1998-2002) of the National Salmonella Control Programme (1998-2002):

■ The goal to reduce the percentage of broiler flocks infected with *Salmonella* Typhimurium to less than 1% has been achieved (0.23% in 2002).

■ The goal to reduce the percentage of broiler flocks infected with *S.* Enteritidis to less than 1% has been achieved (0.02% in 2002).

■ The goal to reduce the percentage of broiler flocks infected with exotic strains of salmonella to less than 2% has been achieved (1.25% in 2002).

20. Discussion

The National Salmonella Control Programme has been effective because the goals set for the programme have been achieved. This has occurred even though more Danish table egg layers were infected with salmonella than originally anticipated. The production of hatching eggs in Denmark revolves around a few large enterprises. So when salmonella was detected at one of these enterprises in 1997, this had far-reaching consequences. The infection quickly spread to quite a few pullet-rearing and table egg holdings. On the other hand, the enterprise was fortunately able to eradicate the infection and no problems related to the spreading of infection have occurred since then. One of the reasons that the spread of infection could be contained within the production pyramid is that salmonella samples are taken at every stage of production, thereby optimising the potential to detect salmonella infection early on in the process. A poultry sector comprising a few, large enterprises also has the advantage of being easier to control and often has the requisite resources for implementing hygiene initiatives, installing new production equipment, etc.

The percentage of infected flocks in table egg production has sharply declined from 13.4% in 1998 to 2.6% in 2002. The producers currently burdening the statistics are primarily flocks that are repeatedly infected despite cleaning and disinfection between flocks. These holdings have difficulty getting rid of the infection because their production facilities consist of a large battery cage system. In order to come to grips with these persistently infected holdings, the technical task groups have participated in drawing up infection containment strategies, and some holdings have been granted a transitional exemption from aspects of the sample-taking programme under the National Salmonella Control Programme, allowing the eggs from these holdings to be sent direct to heat treatment beyond one flock rotation. On the condition, however, that the holding has a distinct, fixed schedule for converting a possible production of table eggs to normal terms of delivery. This goal must be achieved through eradication, replacement of production equipment and/or hygiene measures.

The most important prerequisite for being able to keep poultry houses salmonella-free is the ‘all in – all out’ principle, which some large-scale producers are unable to effectuate throughout the holding at the same time. The possibility of splitting up the houses into a few smaller units with separate egg conveyors, feeding systems, etc., could also be considered, as this would make it possible to implement the above-mentioned principle.

The percentage of infected broiler flocks declined from 12.9% in 1997 to 1.5% in 2002. When the National Salmonella Control Programme was initially implemented, voluntary initiatives had

already been introduced to reduce the occurrence of salmonella. The most significant decline in the number of infected broiler flocks is largely explained by the target-oriented effort made at the top of the breeding pyramid that made it initially possible to deliver salmonella-free day-old chicks and enabled the subsequent production segments to concentrate on preventing the introduction of infection through a series of initiatives in primary production involving the physical framework, hygiene barriers, personal behaviour and feedingstuffs (i.e., biosecurity). Re-infection also occurred in the broiler sector, in that 18% of the parent flocks infected were re-infections.

Poultry feedingstuffs are the presumed source of the exotic strains of salmonella found in breeding and parent flocks. The industry has tightened the requirements for heat-treatment of feed for poultry production and other initiatives, to reduce the possibility of transmitting salmonella bacteria from the feed mills. As a result, salmonella has not been found in the poultry feed samples taken by the Plant Directorate for routine inspection. Strict hygiene requirements and rules have been imposed on access to poultry houses, and as a result the houses are ideally viewed as being virtually isolated environments. Feedingstuffs and litter are potential sources of external infection, however.

Combining bacteriological and serological analyses – as in the Denmark's National Salmonella Control Programme – seems to be the most reliable approach to tracking down positive flocks. The advantage of bacteriological detection is that the bacterium is actually present in the sample, which enables supplementary analyses, such as phage-typing and analyses of antibiotic resistance. The advantage of serological analysis is that it is much more sensitive than bacteriological analysis.

It is interesting to note that the National Salmonella Control Programme has also had the desired effect on the incidence of human salmonellosis, which has clearly declined since 1997. The decline in the number of persons infected with *S.* Enteritidis and the number of infected table egg flocks have thus coincided chronologically. Most of this reduction is probably a direct effect of the National Salmonella Control Programme. The comparison of infection sources also shows that human illness caused by infected eggs has declined drastically.

Sweden and Finland have a lower occurrence of salmonella. Sweden has been officially controlling this bacterium ever since 1961. In 2001, salmonella was found in 0.58% of table egg flocks and 0.10% of broiler flocks, whereas no breeding flocks or parent flocks tested positive for salmonella. In terms of human infection, 4,508 cases of human salmonellosis were reported in 2001, yet only 668 of these cases (15%) involved persons who were infected in Sweden. The rest are presumably attributable to travel abroad, because the persons infected had been out of the country during the incubation period. Most cases of human salmonellosis in Denmark are still domestic (74% in 2002).

The social costs of human salmonellosis are considerable, primarily in the form of lost earnings and hospitalisation expenditures. The precise savings for society resulting from the National Salmonella Control Programme are difficult to estimate. This is related to the fact that estimating the rise in the incidence of human salmonellosis due to poultry-related salmonella types in the event that the National Salmonella Control Programme had not been implemented is naturally a highly uncertain process. Assuming that the incidence of human salmonellosis had remained at the same level as in 1997, and that a general price increase of 2% had occurred, then 40,000-150,000 persons have avoided salmonella infection from 1998-2001, thereby saving Danish society roughly DKK 250-650 million (Wegener & Borck, 2003).

Even though it is gratifying to note that the occurrence of salmonella in table egg and broiler production has declined as much as it has, it is important to emphasise that eating raw eggs is still a somewhat risky venture. The National Salmonella Control Programme is no guarantee that eggs are salmonella-free. In conclusion, consumers should remember that Denmark imports eggs from other countries which do not control salmonella with comparable intensity. Depending on the monitoring programme of the country in question, these eggs are a potential source of infection for Danish consumers. Therefore, raw eggs should be used with care, and it is recommended to use pasteurised eggs for dishes that cannot be heated, such as homemade ice cream and egg-nog.

21. Future Prospects

Consumers prefer, and have the right to expect, that the poultry products (eggs and broiler meat) available for purchase in the retail trade are not infected. Considering the current occurrence of salmonella at the holdings, this expectation cannot be fully met at present. It is worth mentioning in this context, however, that the salmonella bacterium cannot be eradicated. Accordingly, a future goal should be to uphold – and possibly further reduce – the occurrence of salmonella among poultry and with that the incidence of human illness caused by poultry products produced in Denmark. Fulfilling this ambition should always be motivated by the great cost of human salmonellosis, both personal and economic. In addition, this will also heighten the industry's potential to sell safer food products – in domestic and export markets alike.

During the National Salmonella Control Programme, much of the discussion has focused on the significance of the exotic strains of salmonella. Some experts have advocated that only *Salmonella* Enteritidis and *S.* Typhimurium should be included in the National Salmonella Control Programme, in accordance with the Zoonosis Directive, but in the light of the serotype dispersion in human salmonellosis around the world, all serotypes are clearly potential pathogens. In several countries, the serotypes that we view as exotic are the most frequent causes of human salmonellosis. Furthermore, *S.* Typhimurium and *S.* Enteritidis are not as invasive as certain other types and are therefore not as potentially hazardous. Even so, they are still the most frequently occurring types in Denmark, both in livestock production and as a cause of human illness. As long as all serotypes are included under the National Salmonella Control Programme, it will also be possible to detect the occurrence of other rapidly-spreading strains that demand special attention. These considerations are crucial to any consideration of the salmonella serotypes that should be included in the National Salmonella Control Programme in the future.

Compared to other European countries, Denmark has managed to go from a high occurrence of salmonella to a strikingly lower level within a relatively short period of time. When the common EU rules on the control of salmonella – as stipulated in the new Zoonosis Directive and the new Regulation – have been implemented, the country of origin will be required to have an approved control and management programme if said country wishes to export products to Denmark. This will also lead to fewer imports of products infected with salmonella in the years to come.

The DKK 188.1 million that were earmarked for the implementation of the National Salmonella Control Programme have paid for themselves, considering the reduction in human salmonellosis

and the resulting savings. It is important to emphasise, however, that Ministry of Food, Agriculture and Fisheries has not wavered from its goal: to reduce the occurrence of salmonella in the poultry population and to reduce the incidence of human salmonellosis. At the same time, consumers must be constantly kept informed on food safety through information campaigns, etc.

The National Salmonella Control Programme has been a satisfying, constructive team effort involving the public and private sectors, without which the programme would not have achieved such successful results. A continuation of this satisfying teamwork – involving the industry, public authorities and the research sector – will also be useful for endeavours to achieve even better results in the future. The prerequisites for doing so are present as this goal has the undivided interest of all the parties.

22. Conclusion

The National Salmonella Control Programme from 1996 to 2002 was an overwhelming success as the stipulated goals for reducing the occurrence of salmonella in humans and poultry have been achieved.

23. Bibliography

Anonymous (1992). Council Directive 92/117/EEC of 17 December 1992 concerning measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications.

Anonymous (1996). Memorandum on a 'Three-year programme for expanding the control of salmonella in the production of broilers and table eggs in Denmark'. File no. 2a 57-12-0-62/96.

Anonymous (1997). Annual Report on Zoonoses in Denmark 1996. Danish Zoonosis Centre. Ministry of Food, Agriculture and Fisheries.

Anonymous (1998). Annual Report on Zoonoses in Denmark 1997. Danish Zoonosis Centre. Ministry of Food, Agriculture and Fisheries.

Anonymous (1999). Annual Report on Zoonoses in Denmark 1998. Danish Zoonosis Centre. Ministry of Food, Agriculture and Fisheries.

Anonymous (2000). Annual Report on Zoonoses in Denmark 1999. Danish Zoonosis Centre. Ministry of Food, Agriculture and Fisheries.

Anonymous (2000). Report for the National Auditors on national efforts to control salmonella. The National Audit Office of Denmark: 08/99 - April 2000.

Anonymous (2001). Annual Report on Zoonoses in Denmark 2000. Danish Zoonosis Centre. Ministry of Food, Agriculture and Fisheries.

Anonymous (2001). Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in Finland in 2000. Ministry of Agriculture and Forestry. Publication by Department of Food and Health 7/2001.

Anonymous (2002). Annual Report on Zoonoses in Denmark 2001. Danish Zoonosis Centre. Ministry of Food, Agriculture and Fisheries.

Anonymous (2002). Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in the European Union and Norway in 2001. European Commission SANCO/56/2003, part 1.

Anonymous (2002). Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in the European Union and Norway in 2001. European Commission SANCO/56/2003, part 2a.

Anonymous (2003). Annual Report on Zoonoses in Denmark 2002. Danish Zoonosis Centre. Ministry of Food, Agriculture and Fisheries.

Anonymous (2003). Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents.

Anonymous (2003). Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents.

Bisgaard, M. (1992). A voluntary salmonella control programme for the broiler industry implemented by the Danish Poultry Council. *International Journal of Food Microbiology* 15, 219-224.

Danish Poultry Council (1989). God produktionspraksis for fremstilling af fjerkræfoder [Sound production practice for manufacturing poultry feed].

Danish Veterinary and Food Administration (1999). Order No. 696 of 3 September 1999 on the control of salmonella in table egg flocks and young stock for them.

Danish Veterinary and Food Administration (2000). Order No. 367 of 23 May 2000 on the control of salmonella in table egg flocks and pullets reared for them.

Danish Veterinary and Food Administration (2000). Order No. 368 of 23 May 2000 on the control of salmonella in hatching egg layer hens and pullets reared for them.

Danish Veterinary and Food Administration (2001). Order No. 862 of 1 October 2001 on the control of salmonella in hatching egg layer hens and pullets reared for them.

Danish Veterinary and Food Administration (2001). Order No. 863 of 1 October 2001 on the control of salmonella in table egg flocks and pullets reared for them.

Danish Veterinary and Food Administration (2002). Order No. 806 of 26 September 2002 amending the order on the control of salmonella in hatching egg layer hens and pullets reared for them.

Danish Veterinary Services (1993). Order No. 1022 of 15 December 1993 on the investigation of salmonella bacteria and the control and prevention of *Salmonella* Enteritidis and *S.* Typhimurium in poultry.

Danish Veterinary Services (1994). Order No. 874 of 18 October 1994 on the investigation of the occurrence of salmonella infection among hatching egg layer hens.

Danish Veterinary Services (1996). Order No. 1059 of 9 December 1996 on the control of salmonella in hatching egg layer hens.

Danish Veterinary Services (1996). Order No. 1060 of 9 December 1996 on the control of *Salmonella* Typhimurium and *Salmonella* Enteritidis in table egg flocks and pullets reared for them.

Enøe, C., Flensburg, M. F., Thomsen, L. & Bertelsen, P. (2002). Monitoring salmonella in table egg flocks. Memorandum.

Feld, N. C., Ekeroth, L., Gradel, K. O., Kabell, S. & Madsen, M. (2000). Evaluation of a serological *Salmonella* Mix-ELISA for poultry used in a national surveillance programme. *Epidemiology and Infection* 125, 263-268.

Finance Committee of the Danish Parliament. Act No. 325 of 29 May 1996.

Finance Committee of the Danish Parliament. Act No. 441 of 10 September 1996.

Finance Committee of the Danish Parliament. Act No. 329 of 9 September 1997.

Finance Committee of the Danish Parliament. Act No. 154 of 25 February 1998.

Finance Committee of the Danish Parliament. Act No. 229 of 10 May 2000.

Finance Committee of the Danish Parliament. Act No. 83 of 2 December 2003.

Flensburg, J. (1996). Programme for accelerating the control of salmonella bacteria in Denmark's production of broiler meat and table eggs. File no. 2a 57-12-0-1/96 of the Danish Veterinary Services.

Gradel, K. O. (2000a). Varme og Salmonella [Heat and Salmonella]. *Dansk Erhvervsfjerkræ* 4, 110-112.

Gradel, K. O. (2000b). Bakteriefund efter varmebehandling af en burstald [Bacteria finds after heat treatment of a battery cage house]. *Dansk Erhvervsfjerkræ* 6, 183-184.

Gradel, K. O. (2001). Salmonellafund før og efter damp- og formalinbehandling af en burstald [Salmonella finds before and after treatment of a battery cage house using steam and formalin]. *Dansk Erhvervsfjerkræ* 4, 114-116.

Gradel, K. O. (2002a). Varmedesinfektionsprojektet: Resultater fra laboratorieforsøg [Thermal Disinfection Project: laboratory results].
Dansk Erhvervsfjerkræ 7, 208-211.

Gradel, K. O. (2002b). Heat treatment of persistently *Salmonella* infected poultry houses.
In *Salmonella and Salmonellosis - Proceedings May 29-31*, Eds. Colin, P. and Clement, G., p. 595. Saint-Brieuc, France.

Gradel, K. O. (2003). Desinfektionsmiddeltolerance og stationære salmonellainfektioner i fjerkræstalde: Er der en sammenhæng? [Tolerance to disinfection agent and stationary salmonella infections in poultry houses: a possible correlation?], *Dansk Erhvervsfjerkræ* 6, 181-183.

Gradel, K. O. & Randall, L. P. (2003). Minimum inhibitory concentrations of and adaptation to five disinfectants commonly used towards *Salmonella* in the poultry industry. In *XIth International Congress in Animal Hygiene*, 23-27 February, Ed. Saltijeral, J., pp. 339-344. International Society for Animal Hygiene, Mexico City, Mexico.

Gradel, K. O. & Rattenborg, E. (2003). A questionnaire-based, retrospective field study of persistence of *Salmonella* Enteritidis and *Salmonella* Typhimurium in Danish broiler houses. *Preventive Veterinary Medicine* 56, 267-284.

Gradel, K. O., Feld, N. C. & Andersen, J. (2001). Serologic reaction against Salmonella in samples from broiler parent stock with and without preceding colibacillosis: A case-control study. *Avian Diseases* 45, 486-491.

Gradel, K. O., Jørgensen, J. C., Andersen, J. S. & Corry, J. E. L. (2003a). Laboratory heating studies with *Salmonella* spp. and *Escherichia coli* in organic matter, with a view to decontamination of poultry houses. *Journal of Applied Microbiology* 94, 919-928.

Gradel, K. O., Jørgensen, J. C., Andersen, J. S. & Corry, J. E. L. (2003b). Monitoring the efficacy of steam and formaldehyde treatment of naturally *Salmonella*-infected layer houses. *Submitted for publication.*

Gradel, K. O., Nielsen, B. L., Jessen, B. & Knudsen, V. (2002). Varmedesinfektionsprojektet: Resultater fra feltforsøg [Thermal Disinfection Project: field trial results]. *Dansk Erhvervsfjerkræ* 8, 238-244.

Hald, T. & Ekeroth, L. (2001). Assessment of serological and bacteriological methods for detecting salmonella infection in table egg production. Memorandum, Ministry of Food, Agriculture and Fisheries, Danish Veterinary Laboratory.

Hearing on salmonella (1998). The Danish Board of Technology. The Committee of the Danish Parliament for Food, Agriculture and Fisheries.

Jordan F. T. W. & Pattison, M. (2001). *Poultry Diseases*. 4th Edition. Baillière Tindall, London.

Kristiansen, B. (2003). Statistics regarding the occurrence of salmonella in poultry production.

Mølbak, K. & Neimann, J. (2002). Risk Factors for Sporadic Infection with Salmonella Enteritidis, Denmark, 1997-1999. *American Journal of Epidemiology*, Vol. 156, no. 7.

National Salmonella Control Programme (1996). File no. 2a 57-12-0/96 of the Danish Veterinary Services.

Olsen, C. B., Gradel, K. O. & Olsen, T. B. (2000). Undersøgelse af drabseffekten på salmonella ved opvarmning af en burhønsestald [Study of the eradication effect on salmonella from heating a battery cage house]. *Dansk Erhvervsfjerkræ* 11, 16-23.

Pedersen, K. B. (1996). What can be done in Denmark? Hearing of the Ministry of Agriculture and Fisheries, 11 January 1996.

Project Manual (1996). The National Salmonella Control Programme. Danish Veterinary Services.

Report (1996) from the Danish Veterinary Services and the Danish Veterinary Laboratory on the study of the occurrence of salmonella in table eggs in 1995.

Report (2004) from the Danish Veterinary and Food Administration and the Danish Institute for Food and Veterinary Research on the study of the occurrence of salmonella in table eggs in 2002.

Skov, M. N., Carstensen, C., Tornøe, N. & Madsen, M. (1999). Evaluation of sampling methods for detection of salmonella in broiler flocks. *Journal of Applied Microbiology* 86, 695-700.

Skov, M. N., Feld, N. C., Carstensen, B. & Madsen, M. (2002). The serologic response to Salmonella Enteritidis and Salmonella Typhimurium in experimentally infected chickens, followed by an indirect lipopolysaccharide enzyme-linked immunosorbent assay and bacteriologic examinations through a one-year period. *Avian Diseases* 46, 265-273.

Skovgaard, N. (1989). Suggestions for the manufacture of salmonella-free poultry feedingstuffs.

Veterinary Services and Food Administration (1997). Order No. 703 of 8 September 1997 on the control of *Salmonella* Typhimurium and *Salmonella* Enteritidis in table egg flocks and pullets reared for them.

Veterinary Services and Food Administration (1998). Order No. 148 of 02.03.98 on the control of salmonella in table egg flocks and pullets reared for them.

Veterinary Services and Food Administration (1998). Order No. 149 of 2 March 1998 on the control of salmonella in hatching egg layer hens and pullets reared for them.

Wegener, H. C. (1996). Current salmonella situation in Denmark. Hearing of the Ministry of Agriculture and Fisheries, 11 January 1996.

Wegener, H. C. & Borck, B. (2003). Estimates of social savings in conjunction with the National Salmonella Control Programme for poultry. Memorandum of 30 January 2003.

Wegener, H. C., Hald, T., Wong, D. L., Madsen, M., Korsgaard, H., Bager, F., Gerner-Smidt, P. & Mølbak, K. (2003). Salmonella control programmes in Denmark. *Emerging Infectious Diseases* 9, 774-780.