A new *Salmonella* surveillance and control programme in Danish pig herds and slaughterhouses

Ein neues Überwachungs- und Kontrollprogramm für *Salmonella* in dänischen Schweineherden und Schlachthöfen

B. Nielsen\(^1\), L. Alban\(^1\), Stege Helle\(^2\), L. L. Sørensen\(^1\), V. Mogelmose\(^1\), J. Bagger\(^1\), J. Dahl\(^1\), D. L. Baggesen\(^2\)

\(^1\)Veterinary and Food Advisory Service, Danish Bacon and Meat Council, Axeltorv 3, DK-1609 Copenhagen, Denmark, Email: ben@danskeslagerier.dk; \(^2\)Danish Veterinary Laboratory, Bülowvej 27, DK-1790 Copenhagen, Denmark

Summary:
The Danish Salmonella Surveillance and Control Programme for pigs operates at all stages of the production chain and has been applied nationally since 1995. Due to the program the level of *Salmonella* in Danish pork has declined from 3.5% in 1993 to 0.7% in the year 2000. Simultaneously, the number of human cases with salmonellosis due to pork has declined from approximately 1,144 in 1993 to 166 in 2000.

In year 2001, the programme has been improved at a number of stages. A new classification scheme for the serological surveillance of finisher herds has been developed. The individual test cut-off in the mix-ELISA has been reduced to 20 OD%. Only herds producing more than 200 finishers/year are sampled. Based on the serological result from the last 3 months a new weighted salmonella index is calculated.

The Danish Bacon and Meat Council has agreed on a new stricter penalty system. Level 2 and 3 herds get a penalty of 2% and 4% of the value per slaughter carcass, respectively.

A new method of *Salmonella* testing on carcasses has been introduced; 5 carcasses per slaughter day are swabbed at 3 defined areas at 100 cm\(^2\) for each sample. This method is more sensitive than the one used previously.

Herd infected with multiresistant *Salmonella* Typhimurium DT104 have to follow special restrictions. These include a requirement for a herd intervention plan, restriction on livestock trade, and a requirement for special slurry handling.
Carcasses from DT 104 herds must be heat-treated or decontaminated with hot water.

Keywords: *Salmonella* serotypes, pork, serology, classification scheme, DT 104

Introduction
In 1993, the Ministry of Food, Agriculture and Fisheries of Denmark and the Danish Bacon and Meat Council initiated an ambitious programme to eliminate pork as an important source of human salmonellosis. In the beginning of the 1990’s pork had become recognized as an increasing important source of human salmonellosis in Denmark (Wegener and Baggesen, 1996).

Estimates from the Danish Zoonosis Centre show that the total number of cases of salmonella poisoning has fallen by 50 % since 1997. The number of incidents peaked in 1997 when 5,000 people were taken ill compared to around 2,500 cases in the year 2000. The main sources of salmonella poisoning are food related, both home produced and imported, as well as travel abroad.

Eggs used for fresh consumption have accounted for the majority of the cases during the past few years.

The cases associated with pork peaked in 1993 after which a series of initiatives to reduce salmonella in the pig industry were implemented. Consequently, pork has steadily declined as a source of human salmonellosis since 1996. Results from year 2000 show an incidence of only 3.1 cases per 100,000 inhabitants in Denmark equal to a total number of 160 human cases related to pork (Figure 1).

The Danish *Salmonella* Control Programme in pigs operates at all stages of the production chain and has applied nationally since 1995 (Mousing et al., 1997; Nielsen and Wegener, 1997).

By August 1st 2001 a new *Salmonella* Surveillance and Control Program have been implemented where a number of parameters in the programme have been adjusted.

Basically, the level of *Salmonella* is controlled at various stages:

- Feedstuffs
- Breeder- and multiplier herds
- Weaner producers
- Finisher herds with a production of more than 200 animals per year
- At the slaughterhouse, including special hygienic slaughter of highly infected herds
Feedstuffs
The control with feedstuff continues unchanged. Compounded feedstuffs are heat treated at 81 °C to eliminate *Salmonella* bacteria. The national programme requires mandatory *Salmonella* testing in all plants producing animal feed. The test involves microbiological analysis of compounded feedstuffs, as well as the collection of samples from critical control points during production. The level of *Salmonella* spp. in final products is low; in year 2000 only 0.3 % of the examined samples were test positive.

Breeding and multiplying herds
The surveillance of breeder and multiplier herds’ continues unchanged. Each month, all herds are blood sampled and examined for *Salmonella* antibodies. Based on the level of antibodies a *Salmonella* index is calculated. If the index exceeds 5, pen faecal samples must be taken and examined for the presence of *Salmonella* spp. When the index exceeds 15, as sales ban on breeding pigs is imposed until the index has declined below 15 again.

Weaner producers
If a sow herd sells weaners to a *Salmonella* level 2 or 3 finishing herd, pen faecal samples must be taken and examined for the presence of *Salmonella* spp.

New classification scheme in finishing herds
Since 1995, all finishing herds producing >100 finishers per year have been tested for Salmonella antibodies. The testing is mandatory and paid for by the Danish state. Each month the herds have been divided into 3 levels with respect to the proportion of seropositive samples during the last 3 months. Level 1 herds are herds with no or few seropositive samples, level 2 herds are herds with moderate number of seropositive samples, while level 3 herds are herds with a high proportion of seropositive samples.

After 5 years it was of interest to improve the classification scheme by examining the following questions:

1) Which sample size is appropriate for different herd sizes?
2) What is the effect of leaving out the smallest herds of the surveillance?
3) Which individual cut-off OD % for the serological test is mostly appropriate?
4) How should the results from the previous three months be weighed?
5) How many *Salmonella* herd levels should be applied and what should be the inclusion criteria for each level?
In order to answer these questions, results from 2 screenings carried out in 1,902 Danish slaughter pig herds were used (Anonymus, 1998; Sørensen et al., 2000). Additionally, data from all Danish slaughter pig herds that had delivered finishers for slaughter between June 1st and August 31st 2000 were used. The statistical software program SAS was used, in particular the following procedures: PROC GENMOD, PROC CORR, and PROC FREQ (SAS, 1989ab). Sample size calculations were carried out to estimate how large a sample should be to ensure that an all-negative sample would come from a population with maximum 5 % sero-prevalence. Next, it was taken into account that the serological test was imperfect, since sensitivity and the specificity are not 100 %. (Cameron and Baldock, 1998).

Results and discussion of the new classification scheme

Which sample size is appropriate for different herd sizes?
For small herds (annual kill ≤ 2000 finishers) 60 animals should be sampled per year, for medium sized herds (annual kill 2001-5000) 75 animals per year, and for large herds (annual kill >5000) 100 animals per year. This will ensure a detection level < 5% in all herds, and in the largest herds down to < 3%. Compared to the previous classification scheme, there is an increase in the number of samples taken in the small herds, an almost equal number of samples taken in the medium sized herds and a reduction in the number of samples taken in the large herds.

What is the effect of leaving out the smallest herds of the surveillance?
In the previous classification scheme, herds with an annual kill of ≤ 100 finishers were not included, since too many animals would need to be sampled in order to estimate the herd prevalence with sufficient precision. This implied that around 124,000 animals (0.6 %) were already outside surveillance. The limit has been increased to 200, which means that 193,000 animals (1.0 %) extra are left out, since it was judged that this did not jeopardize food safety.

Which individual cut-off value for the serological test is appropriate?
Since 1995, a screening cut-off at 40 OD % in the serological test has been used. However, data showed that the best association was observed when using individual cut-off OD % 11, and the higher the cut-off, the poorer the association. It was of interest to introduce a Level 0 for herds, which react seronegative in all samples. However, presence of false-positives would constitute a problem in particular for a Level 0 herd, and the lower the cut-off OD%, the higher the likelihood of false positive reactions. Therefore, it was decided to apply individual
cut-off OD % 20. By use of individual cut-off OD % 40, 4.0 % of the samples taken were judged positive, while by use of cut-off OD % 20 almost twice as many (7.7 %) were judged positive.

**How should the results from the previous three months be weighed?**

In the previous system, the serological results of the previous three months were averaged. However, a weighing may improve the association between serology and bacteriology. For cut-off OD % 20, a relative weighing of 1:1:3 (absolute: 0.2:0.2:0.6) was suggested. The combination of applying individual cut-off OD% 20 and the weighing was called the serological *Salmonella* index for slaughter pig herds.

The weighing implies that in case of an increase in sero-prevalence in a herd, the herd may be assigned a higher level one month earlier than in the previous system. Likewise, as *Salmonella* reducing procedures are implemented in such a herd, the herd will leave the high level sooner than in the previous system. Furthermore, there would be a better timely overlay between the possible shedding of bacteria and the special measures taken at the slaughterhouse. This would reduce the possible *Salmonella* spp. contamination of the meat.

**How many levels should be included and what should be the cut-off point for each level?**

In the previous classification scheme, three official *Salmonella* levels were applicable. The association between the serological *Salmonella* index and the likelihood of finding *Salmonella* in the ten samples of caecum content was estimated by use of data from the 2 screenings carried out in 1,902 herds (Figure 2).

The figure shows that among herds that were seronegative during the entire three-month period, no *Salmonella* was found in the caecum samples in 94.4 % of the herds. The figure also shows that there was an almost linear association ($r^2 = 85$ %) between the index and the proportion of herds in which *Salmonella* was found in the caecum content.

Considering that the number of herds assigned the highest *Salmonella*-level should not exceed the capacity on the abattoirs to slaughter possibly *Salmonella* contaminated carcasses safely, index 40 and 70 was chosen. If index 40 and index 70 were used as limits for Level 2 and Level 3 respectively, around 3.3 % of the herds would be assigned to Level 2 and 1.6 % to Level 3.
Summary for the new classification scheme
The classification scheme for slaughter pig herds in The Danish Surveillance and Control Program for Salmonella were adjusted on the following points by August 2001:

- The sampling will be simplified into 60, 75, or 100 samples per year depending on the herd size.
- Herds with an annual kill < 200 slaughter pigs will not be a part of the surveillance, hereby 1.6% of the slaughter pigs are outside the surveillance scheme.
- The cut-off for evaluating individual meat juice samples will be reduced from OD % 40 to OD % 20, hereby doubling the number of positive samples.
- The previous three months serological samples will be weighed 0.2:0.2:0.6, and the weighed average is called the serological Salmonella index for slaughter pig herds.
- A herd will be assigned to one of three levels monthly. The limit between Level 1 and Level 2 will be set to index 40, and the limit between Level 2 and Level 3 will be index 70.

New financial penalties
The Danish Bacon and Meat has agreed on a new stricter penalty system. The purpose is to improve the Salmonella control as much as possible in herds with positive samples. In practice, an intervention plan in order to reduce the prevalence of Salmonella carries this out. The producer and his advisers work out the plan in detail.
The level of financial penalty corresponds to the level to which the herd is assigned:

<table>
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<tr>
<th>Penalty (% of the slaughter value):</th>
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<tbody>
<tr>
<td>Level 1</td>
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<tr>
<td>0%</td>
</tr>
<tr>
<td>Level 2</td>
</tr>
<tr>
<td>2%</td>
</tr>
<tr>
<td>Level 3</td>
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<tr>
<td>4%</td>
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Level 2 and 3 Herds
The veterinary authorities require that faecal samples are taken in order to identify the Salmonella serotype. Herds assigned to level 3 have to be slaughtered under special hygienic precautions. This is done at specially designated slaughterhouses at the end of the day to prevent cross-contamination with other carcasses. Carcasses from level 3 herds also have to be heat-treated or subject to other special
treatment. Slaughterhouses may also randomly test carcasses on the basis of guidelines issued by the Danish Veterinary and Food Services.

**New surveillance method for *Salmonella* in fresh pork**
Since 1993, fresh pork has been surveyed for *Salmonella* spp. at the slaughterhouses every month. The prevalence has declined during the last two years (Table 1).
In general, 10 - 15 different serotypes are isolated from Danish pork. However, *S. Typhimurium* constitute approximately 60 % of the isolates (Table 2).
Multiresistant *Salmonella* Typhimurium DT104 has only been detected very rarely (0.002 %) in Danish pork.

A new method of Salmonella testing on carcasses was introduced by January 1st 2001; 5 carcasses per slaughter day are swabbed at three defined areas (the sternum, the hind leg near the tail and the jowl) at 100 cm$^2$ for each sample. The swabbing areas were originally defined by USDA, USA, and are currently used in the USA as the national *Salmonella* monitoring method on swine carcasses. This method is more sensitive than the one used previously, and the number of positive samples recorded is expected to increase. Preliminary results for 2001 show a prevalence of 1.4 - 1.8 %. This should be regarded as an effect of the improved test sensitivity and not increased *Salmonella* prevalence as such.

**DT104 Herds**
Herds infected with multiresistant *Salmonella* Typhimurium DT104 have to follow additional restrictions. The herd is given a Zoonotic Restriction Order. This includes a requirement for a herd intervention plan, restriction on livestock trade, and a requirement for special slurry handling. The herd intervention plan is made to ensure that salmonella reducing measures are implemented in the herd for at least 12 months, and the restriction on livestock trade is to prevent the spread of DT104 infection to other herds.

**Hot Water Decontamination**
Finishing pigs infected with multiresistant *Salmonella* Typhimurium DT104 may either be slaughtered under special hygienic conditions as with Level 3 herds with subsequent heat-treatment or may be decontaminated with hot water. Decontamination is applied to carcasses after removal of organs. The carcass is showered with 80 °C hot water for 14 - 16 seconds, which produces a significant reduction in the bacterial count on the surface. Five carcasses from each batch are tested to ensure that the process is effective. If *Salmonella* spp. is not detected, the whole batch may be used for fresh consumption.
References


Table 1
The prevalence of *Salmonella* spp. in Danish pork, 1996-2000

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<tr>
<td>% Positive samples</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>0.9</td>
<td>0.7</td>
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Table 2
Distribution of serotypes in Danish pork year 2000

<table>
<thead>
<tr>
<th>Serotype</th>
<th>%</th>
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<tbody>
<tr>
<td><em>S. Typhimurium</em></td>
<td>58.8%</td>
</tr>
<tr>
<td><em>S. Infantis</em></td>
<td>7.6%</td>
</tr>
<tr>
<td><em>S. Derby</em></td>
<td>4.2%</td>
</tr>
<tr>
<td>Exotic serotypes, n=7</td>
<td>5.9%</td>
</tr>
<tr>
<td>Rough isolates</td>
<td>14.3%</td>
</tr>
<tr>
<td>Non typeable</td>
<td>5.9%</td>
</tr>
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Figure 1. Sources of human salmonellosis in Denmark, 1988-2000.
Ministry of Food, Agriculture and Fisheries
The serological salmonella index for slaughter pig at individual cut-off OD% 20

Figure 2
The association between serological *Salmonella* index (a weighted average of the previous three months serological results) and bacteriology among 1,902 Danish finisher herds ($r^2=85\%$)