Assessment of the effect of proposed changes to the management of multi-resistant Salmonella Typhimurium DT104 in primary food animal production in Denmark

Danish Zoonosis Centre

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i Background and Terms of reference

In the spring of 2002, Danske Slagterier contacted the Veterinary and Food Administration with a request for changes to the existing regulations for handling of pig herds infected with multi-resistant *Salmonella* Typhimurium DT104. Following deliberations with stakeholders in the primary industries and the Danish Veterinary Institute, the Veterinary and Food Administration at a meeting on June 6 2002 requested that the Danish Zoonosis Centre conduct a risk assessment to determine the effects of the proposed changes. A draft 'Terms of reference' (see below) was circulated to the stakeholders and agreed on June 19 2002.

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Kommissorium for arbejdsgruppe vedr. analyse af forekomst og spredning af DT104 i primærproduktionen i Danmark

Efter anmodning fra Fødevaredirektoratet udarbejder Dansk Zoonosecenter en analyse af forekomst og spredning af Salmonella Typhimurium DT104 i primærproduktionen i Danmark.

Analysen har som særligt fokus at søge at estimere konsekvenserne af en ændret håndtering af DT104-smittede svinebesætninger som foreslået af Danske Slagterier ved møde med repræsentanter fra Fødevaredirektoratet og DVI den 6. juni 2002. Analysen omfatter tillige risiko for spredning til miljøet og herigennem til andre husdyrarter end svin. Dansk Zoonosecenter kan til brug for analysearbejdet inddrage supplerende data og ekspertise hos Fødevaredirektoratet og husdyrbrancherne.

De eksisterende hygiejneprocedurer på slagterierne og deres evne til at håndtere et eventuelt ændret smittepres med DT104 vil ikke indgå i analysen.

Analyseresultaterne beskrives i et notat til Fødevaredirektoratet. Før notatet fremsendes i endelig form gives Fødevaredirektoratet, Danske Slagterier, Det Danske Fjerkræråd, Dansk Kvæg og Kødbranchens Fællesråd mulighed for at kommentere analyserne.

Resultaterne af analysearbejdet vil foreligge i endelig form senest den 1. november 2002.

FLB

The assessment should specifically attempt to estimate the effects of the changes to the handling of pig herds as proposed by Danske Slagterier, but should also address the risk of spread of DT104 to the environment and to other production animal species. The consequences to hygiene procedures in slaughterhouses were not part of the assessment. The assessment should be ready no later than November 1 2002.

In accordance with the ‘Terms of reference’, the following subjects will be included in the report:

1. General description of the occurrence and spread of DT104 in the primary production.
2. Probabilistic model estimating the true number of multi-resistant DT104 infected pig herds.
3. Probabilistic model estimation the consequences of lifting trade restrictions
4. General description of the spread of DT104 to the environment and to other production animal species.

ii Proposed changes to management strategy

At present, a herd infected with DT104 is put under a Zoonosis restriction order (ZT). Herds under ZT are required to implement *Salmonella* reducing intervention plans as well as high-level rodent control and restricted use of slurry and manure. DS suggest that DT104 herds be handled according to the Danish Salmonella surveillance and control program III, and the use of Zoonosis restriction orders abolished. Trace back of contact herds selling to and buying from the infected herds, and the requirement to inform all persons/businesses with contact to the MRDT104 herd is to be maintained. Bacteriological examination of contact herds producing finishers will stop, as all herds are to be assumed infected. At present, contact herds can be tested free of MRDT104 by bacteriological examination.

Animals from herds under ZT are hot water decontaminated (VVS) after slaughter. For slaughter pig herds, a four months period with a salmonella-index below 20.0 is presently required before ZT is lifted. Farrow-to-finish herds require two negative bacteriological examinations prior to a one-month period with an index below 20.0. Sow herds as well as breeder and multiplier herds test free after two negative bacteriological examinations. DS suggest that the VVS slaughtering be cancelled when the salmonella-index has been 20.0 or below for a two months period. If the index within the following six months increase to more than 20.0 the finishers will be VVS slaughtered again. As noted in the ‘terms of reference’, the consequences to hygiene procedures in slaughterhouses is not part of this assessment.

Table ii: Proposed changes to management strategy

Existing intervention measure	Proposed change	Comment
Zoonosis restriction order (ZT) for MRDT104 positive herds	Stop use of ZT for present and future MRDT104 infected herds	
Herd intervention measures	No DT104 specific intervention measures. Measures according to current general *Salmonella* control program apply	Bacteriological follow-up in breeder and multiplier herds with serological index >=5; in farrow to grower herds selling piglets to finisher herds in Levels 2 or 3; and in finisher herds in Levels 2 or 3
Specific high-intensity rodent control program	Normal rodent control	
Requirement that slurry and manure must be ploughed in after disposal on fields	No restrictions on slurry and manure disposal	The Danish Bacon and Meat Council collaborate with the DVI and the RVAU in a study of the survival of Salmonella in slurry deposited on fields. The preliminary results indicate that Salmonella levels are reduced quickly. Salmonella was detected on day 0 and in one sample on day 7 after depositing. The samples from week 2 and 3 after spreading on fields were negative
Restrictions on sharing of agricultural implements and machinery between farms	Restrictions abolished	
Restrictions on selling live pigs in Denmark	Restrictions on selling live pigs in Denmark abolished	
Ban on live export of pigs	Normal exports conditions apply	
Requirement that herd owner must inform all persons with contact to the herd about the DT104 status	No change proposed	
Trace-back to contact herds. Herds selling to or buying from the infected herd within the last 6 months have pen faecal samples collected twice for bacteriological examination		
Breeder and multiplier herds	Unchanged	
Farrow-to-grower	Unchanged	
Finisher	Abolished	
Hot water decontamination of carcasses after slaughter (VVS) or special DT104 slaughter of pigs from MRDT104 infected herds		
Sows and boars	Unchanged	
Finishers	Extended to include finishers from herds, which has brought in growers from a MRDT104 positive farrow-to -grower herd.	Finisher herds with contact to a DT104 positive farrow to grower herd, even when negative in trace-back will be subjected to VVS slaughter as long as their *Salmonella* index is greater than 20.0. The VVS requirement is cancelled when the index is 20.0 or lower for 2 consecutive months. Should the index subsequently increase to more than 20.0, VVS slaughter will be re-instated.
Testing herds ”free” of DT104		
Farrow to grower herds	Unchanged	
Breeder and multiplier herds	Unchanged	
Finisher herds	The VVS requirement is cancelled when the index is 20.0 or lower for 2 consecutive months. Should the index subsequently increase to more than 20.0, VVS slaughter will be re-instated	

iii Executive summary

Introduction (Section 1.1)

Multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) is known as an important food borne pathogen in many countries. MRDT104 has been reported to spread rapidly between animals within herds, between herds and to other species. The combination of the ability to spread rapidly and the multi-resistance towards antibiotics used frequently in animals and humans implies that MRDT104 can pose a serious health problem for both animals and humans. Even though other countries with significant occurrence of MRDT104 have no general programs to control *Salmonella*, the risk management practices adopted in Denmark was based on the worst case scenario that the general control measures were not able to contain MRDT104.

General *Salmonella* surveillance programs in Denmark (Section 1.2)

In Denmark, detection of MRDT104 relies on the general *Salmonella* surveillance programs, parts of which are based on detection of *Salmonella* antibodies (serological methods) while other parts are based on the detection of the *Salmonella* bacterium itself in samples collected in slaughter-houses or on farms. The serological test results produced by the surveillance programs can only be interpreted at herd-level and the requirement for bacteriological follow-up depends on the number of reactors required for the herd or flock to be defined as infected with *Salmonella*. It is only when bacteriological samples are collected that MRDT104 can be identified. In general, the tests are not 100 percent sensitive, which means that in some of the herds, *Salmonella* infection may be present, but remain undetected.

Recognising the difficulty of complete eradication of *Salmonella*, the programs have been designed to have as much impact on the incidence of human salmonellosis as possible, and only major, commercial flocks or herds have been included. We estimated that approximately 18-21% of the pig herds producing 1.6 % of the total number of slaughter pigs, was not under surveillance in 2001. We were not able to estimate the number of poultry flocks not under surveillance, but in general most broiler- and turkey flocks are included, while a greater part of flocks of geese and ducks are outside the surveillance. In cattle herds, microbial sampling does not follow up the serological surveillance of bulk-milk, due to the low predictive value of a seropositive test. Therefore, detection of MRDT104 infection in cattle herds as well as in herds not under surveillance depends on examination of clinical samples or trace-back due to contact with other known MRDT104 herds.

Salmonella Typhimurium phage type DT104 (Section 1.3)

MRDT104 is commonly thought of as being resistant to five antibiotics: ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACStSuT). Some of the penta-resistant clones have also acquired resistance to flouroquinolones. The resistance pattern of DT104 in pig herds found via the general salmonella surveillance, showed that from 1998 until 1st August 2002, 50-60% of the isolates were penta-resistant, and less than 1% of the herds were infected with a fluoroquinolone-resistant DT104. Not all DT104 found in pig herds are multi-resistant, and 20-30% of the DT104 isolates were fully susceptible to the antimicrobials in the test panel. Seemingly, the proportion of fully susceptible DT104 from pig herds has been increasing, rising from 27% in 2000 to 45% of the isolates in 2002.

The first isolates of multi-resistant MRDT104 were found in a Danish slaughter pig herd in 1996, and later that year a farm with cattle was also found infected. Retrospective analysis of isolates stored in the DVI strain collection showed that a Danish sow herd in 1991 had been infected with MRDT104. After 1996, the number of MRDT104 herds increased, and in 2000 the highest number of infected herds (57) until now was found. In December 2000, the first Danish poultry flocks were found infected with MRDT104. By the end of August 2002, MRDT104 has been detected in 90 pig herds, 46 farms with both pigs and cattle, 23 with cattle alone, two turkey flocks, two broiler flocks and in a herd of farmed foxes. In total, 164 herds have been reported as infected with MRDT104.

In October 1997, the Danish Veterinary and Food Administration issued the first DT104-order, making detection of MRDT104 in food animals notifiable. Infected herds were put under official veterinary supervision imposing in a number of restrictions, including on the sale of live animals, requirements for special hygiene at slaughter, as well as a mandatory epidemiological investigation of the herd and its trade contacts. An attempt to eradicate MRDT104 from infected pig herds was initiated in the beginning of 1997, but the increasing number of infected pig herds made this approach economically unfeasible. The pig industry's strategy of stamping out infected pig herds was terminated in June 2000, and in the latest revision of the DT104-order, a new kind of official veterinary supervision was introduced – the Zoonosis Restriction order (Bek. nr. 435 af 31. maj 2001).

Distribution of MRDT104 in Danish pig herds compared to other *Salmonella* Typhimurium phage types (Section 1.4)

During the period 1997-2001, 800-1000 pig herds were found infected with *S.* Typhimurium (ST) each year. In 2000, the fraction of ST pig herds reported infected with multi-resistant MRDT104 doubled, rising from 1-2% to almost 5%. Thus, the high number of MRDT104 pig herds observed in 2000 and 2001 was not due to a general increase in the prevalence of *Salmonella* Typhimurium infection.

The number of *Salmonella* isolations with outbreaks of clinical disease in pig and cattle herds reported in the Annual report on Zoonoses in Denmark until year 2001 included all *Salmonella* infected herds under official veterinary supervision. Therefore, herds infected with MRDT104 have been over represented in the list. On average 6.6% of all MRDT104 positive herds with pigs only have been detected due to examination of diagnostic samples, compared to 51% of the MRDT104 infected herds with cattle only. This, however, most likely reflects the absence of general *Salmonella* surveillance in cattle, so that clinical salmonellosis and trace-back in contacts to known DT104 herds represents the only means of detection in this species.

It has been argued that MRDT104 relatively more often result in clinical disease in pigs compared to other *Salmonella* Typhimurium phage types. When including all DT104 infections, 2.4% of the infected herds report diarrhoea to the DVI database, whereas 3.2% of the pig herds infected with MRDT104 report diarrhoea. Compared to other abundant phage types, MRDT104 does not appear to lead to relatively more clinical cases.

Trace back to other herds is mandatory only in the case of MRDT104. In all other cases, sampling only occurs at the herd identified as having a *Salmonella* problem – not in the contact herds. When comparing the prevalence of MRDT104 with other *S.* Typhimurium phage types, it is more appropriate to compare the relative distribution found during follow-up of serological surveillance, thus avoiding the bias resulting from trace back procedures. Modelling the number of infected herds found per month during follow-up of serological surveillance using a second order local regression model shows that the number of herds infected with DT104 (incl. susceptible DT104 strains) increased within the same range as other phage types such as DT120, DT170 and DT193. From mid-2000, the model shows that the rate at which herds infected with DT104 were found decreased,

reaching a stable level during 2002. However, there is no indication that the incidence of DT104 is decreasing.

Factors affecting the spread of MRDT104 (Section 1.5)

In 2000, Danske Slagterier conducted an interview study of pig herds infected with or exposed to MRDT104. The results of the analysis were not conclusive.

It would seem reasonable to assume that usage of antimicrobials, in particular the ones to which MRDT104 is resistant, might predispose a herd to colonisation by MRDT104, given that it is exposed. However, a simple comparison of usage of ampicillin, streptomycin, sulphonamides and tetracycline, based on information available in the VetStat database, does not suggest that use of these antimicrobials constituted a risk factor for MRDT104 infection or colonisation of herds. It appears that the usage of antibiotics during a 30-day period prior to detection of MRDT104 in the pig herds was lower than the usage in herds infected with DT17, but not significantly. The herds, which had trade contact with MRDT104 herds but were not infected, had a significantly higher total usage of these four antibiotics. We caution that definite conclusions regarding the role of antimicrobials as a risk factor for colonisation of pig herds with MRDT104 must await formal studies that control for possible confounding effects of, for example herd size, length of exposure window and other factors.

Estimated true occurrence of MRDT104 in pig herds (Section 2)

During the 1-year period from August 1st 2001 to 31st July 2002, 32 pig herds were found infected with MRDT104. Some of them were detected as a result of the general *Salmonella* surveillance program, while others were found during the cause of compulsory trace-back from known positive herds.

Detection of MRDT104 infection depends on the sensitivity of the serological and bacteriological tests, and as tests are less than 100 % sensitive a proportion of herds infected with MRDT104 may go undetected. In order to estimate the number of Danish pig herds that are truly infected with MRDT104, compared with the number that were detected, a risk assessment model was developed.

The estimation was stratified on three different herd types: 1) Herds – some of them farrow-to-finish herds - producing slaughter pigs; 2) sow herds with production of weaner and grower pigs;

and 3) breeder and multiplier herds. The proportion of herds infected with S. Typhimurium and specifically MRDT104 was estimated from bacteriological examinations of pen faecal samples from level 2 and 3 herds, as well as from swab samples taken at the slaughterhouses.

In total, we estimate that 97 pig herds were truly infected with MRDT104 during the year between August 1st 2001 and July 31st, 2002 (83, if based on addition of individual model outputs after rounding). However, the true number could be as low as 62 herds or as high as 212 (90 % confidence interval). In comparison, 33 (34 %) were actually detected by the general *Salmonella* surveillance programme and the trace-back procedures applying in cases of MRDT104 infection.

Modelling the spread of MRDT104 in Danish pig herds through trade contacts (Section 3)
We used Monte Carlo simulation to estimate the number of pig herds infected with MRDT104 in each of three successive years if current practices of handling the infected herds are maintained and if the proposed changes are implemented. We modelled how the infection is transmitted from breeder and multiplier herds via sow herds to the slaughter pig herds. For each breeder or multiplier herd, and sow herds in contact with it, a number of trade contacts are assigned, and the resulting number of MRDT104 infections is simulated. Two versions of the model were run. The *restricted trade model* simulating the current situation and the *continued trade model* simulating the DS proposed changes.

The change in trade pattern and method for estimating undetected MRDT104 herds had no influence on number of infected breeder and multiplier herds, and the models predict one (90% CI: 0, 4) MRDT104 infected B/M herd will be detected per year. For the other herds types, the lower 50 percent of the distributions are relatively unaffected by the change in trade pattern. For the upper part of the distributions an important effect of continued trade is observed, as the possibility of having years with a very large number of MRDT104 infected herds increase.

If continued trade is allowed, the model predict that the number of detected MRDT104 sow herds will increase from 15 (90% CI: 8, 28) to 23(90% CI: 10, 68) herds per year. The number of detected slaughter pig producing herds in Level 1 were estimated to increase from 30 (90% CI: 15, 64) to 49 (90% CI: 20, 157), whereas the number of detected Level 2 and 3 herds only increased marginally. Comparison with the actual number of detected herds during the last two years indicate that the

models probably overestimates the number of detected sow herds and slaughter pig-producing herds by approximately 14%.

At the current strategy most of the sow herds are infected via other sources than contacts to breeder and multiplier herds, but the models predict that continued trade will increase the proportion of trade related infections to about 50%. Approximately 70% of the slaughter pig herds are currently infected via trade contacts, and if continued trade is allowed, this proportion increases to about 80%.

The models are run in two scenarios concerning the relationship between detected and undetected MRDT104 herds. In scenario I, we assume that the spread of MRDT104 between undetected herds is unknown. But we assume that the proportion of undetected MRDT104 herds, as estimated in section 2 are constant for all years in the simulations, and that the additional number of MRDT104 herds arising from the free trade have the same proportional number of undetected herds all other detected herds. In scenario II we assume that the rate at which the infection spreads between undetected herd is equal to the rate among detected herds, but that the risk of an undetected MRDT104 breeder and multiplier herd is relatively lower, as estimated in section 2. We also assume that the additional number of MRDT104 herds arising from the free trade leads to no more undetected herds.

The effects are most pronounced in scenario I, where the herds infected via their trade contacts during the period of continued trading, are assumed to result in more undetected herds. When trade is continued scenario I, predicts that the total number of MRDT104 sow herds will increase from 76 (90% CI: 32, 210) to 111 (90% CI: 41, 437) herds per year. The total number of slaughter pig producing herds in Level 1 were estimated to increase from 52 (90% CI: 19, 252) to 89 (90% CI: 89, 527).

Survival of MRDT104 in the environment and risk of horizontal transmission (Section 4)
Farm animal waste is widely recognised as an important vehicle for the transmission of *Salmonella*. Deposition of solid manure (a mixture of feces and straw as opposed to slurry which is a mixture of urine and faeces with minimal straw) seems to constitute less of a risk of infection with, for example *Salmonella,* as it undergoes a composting process with significant reduction of the

pathogen load. *Salmonella* bacteria can survive for extended periods following deposition on farm land. In a recent study involving pig slurry naturally contaminated with MRDT104 at a low concentration, sampling did not recover DT104 from soil after 1 week, using a method with limited sensitivity. Had the concentrations of *Salmonella* applied been higher, the time to final recovery would have been longer. Wildlife most often is *Salmonella* free but may become infected through contact with infected farm animals or their waste. It is likely that flies, rodents, birds or other animals can transport MRDT104 from slurry deposited on farm land into the farm environment. However, the risk has not been quantified.

An analysis combining data on the DNA types of MRDT104 from 166 Danish farm with information about their geographical location and the time of detection indicated that horizontal transmission may have played a role in the infection of some farms where epidemiological trace-back did not show trade contact as a likely cause. Among farms most likely infected horizontally are two broiler flocks.

iiii Conclusions

1. By the end of August 2002, MRDT104 has been detected in 90 pig herds, 46 farms with both pigs and cattle, 23 with cattle alone, two turkey flocks, two broiler flocks and in a herd of farmed foxes. In total, 164 herds have been reported as infected with MRDT104.

2. Since 1998, approximately 50-60% of the DT104 pig herds have been infected with a penta-resistant clone, whereas 20-30% of the pig herds were infected with a fully susceptible DT104 strain. Seemingly, the proportion of susceptible DT104 is increasing.

3. The increase in number of MRDT104 pig herds that occurred in 2000 and 2001 cannot be explained by a general increase in the number of herds infected with *S*. Typhimurium.

4. Model estimates show that the number of herds infected with DT104 (incl. non-MRDT104) found during follow-up of serological surveillance increased within the same range as other phage types such as DT120, DT170 and DT193. The rate at which herds infected with DT104 are detected is decreasing, and number of new infections reached a stable level during 2002. The model does not indicate that the number of DT104 herds found per month during follow-up of serological surveillance is decreasing.

5. According to the DVI database, 2.4% of the DT104 herds report diarrhoea, compared to 3.2% of the pig herds infected with MRDT104. Compared to other phage types found in pigs, MRDT104 does not appear to lead to relatively more clinical cases.

6. The investigation of risk factors for MRDT104 infection in pig herds did not led to any conclusive results.

7. While a detailed study will be necessary to establish the relationship, a preliminary analysis does not suggest that use of antimicrobials constituted a risk factor for MRDT104 infection or colonisation of pig herds.

8. In total, we estimate that 97 pig herds were truly infected with MRDT104 during the year between August 1st 2001 and July 31st, 2002. However, the true number could be as low as 62 herds or as high as 212 (90 % confidence interval).

9. The general *Salmonella* surveillance programme and the trace-back procedures applying in cases of MRDT104 infection detected in the same period 33 infected herds, corresponding to 34 %.
10. The change in trade pattern had no influence on number of MRDT104 breeder and multiplier herds, but there was an overall tendency towards having more MRDT104 infected sow herds and slaughter pig producing herds when trade restrictions was lifted. The effects of lifting the trade restrictions were relatively small when relatively low numbers of infected herds were simulated. When relatively high numbers were simulated, the possibility of having years with a very large number of MRDT104 infected herds increased.

11. If continued trade was allowed, the model predict that the number of detected MRDT104 sow herds will increase from 15 to 23 herds per year, and the number of detected slaughter pig producing herds in Level 1 will increase from 30 to 49 herds per year.

12. The models probably overestimates the number of detected sow herds and slaughter pig-producing herds by approximately 14%, and the output of these models is dependent on the assumptions concerning trade contacts and probability of infection.

13. At the current strategy most of the sow herds are infected via other sources than contacts to breeder and multiplier herds, but the models predict that continued trade will increase the proportion of trade related infections to about 50%. Approximately 70% of the slaughter pig herds are currently infected via trade contacts, and if continued trade is allowed, this proportion increases to about 80%.

14. The effects are most pronounced in scenario I, where the herds infected via their trade contacts during the period of continued trading, are assumed to result in more undetected herds. When trade is continued scenario I predicts that the total number of MRDT104 sow herds will increase from 76 (90% CI: 32, 210) to 111 (90% CI: 41, 437) herds per year. The total number of slaughter pig producing herds in Level 1 were estimated to increase from 52 (90% CI: 19, 252) to 89 (90% CI: 89, 527).

15. The total number of MRDT104 infected slaughter pig herds under the current strategy were estimated to be between 107 and 114 herds per year, depending on how undetected herds was estimated. When trade is continued, the total number increased to 139-196 new MRDT104 infections per year.

16. *Salmonella* bacteria can survive for extended periods following deposition on farm land. In a recent study involving pig slurry naturally contaminated with MRDT104 at a low concentration, sampling did not recover DT104 from soil after 1 week, using a method with limited sensitivity.

17. Wildlife most often is *Salmonella* free but may become infected through contact with infected farm animals or their waste. It is likely that flies, rodents, birds or other animals can transport MRDT104 from slurry deposited on farm land into the farm environment. However, the risk has not been quantified.

18. An analysis combining data on the DNA types of MRDT104 from 166 Danish farm with information about their geographical location and the time of detection indicated that horizontal transmission may have played a role in the infection of some farms where epidemiological trace-back did not show trade contact as a likely cause.

1 MULTI-RESISTANT *SALMONELLA* TYPHIMURIUM DT104 IN DANISH PRIMARY PRODUCTION 1996-2002

1.1 Introduction

Since the late1980's, it has been the policy of the Danish government that the increasing incidence of food borne salmonellosis must be controlled by exercising risk management options in primary production in particular, not excluding measures applied during harvest procedures. While risk management has focused on the farm level of production, surveillance has been implemented in several steps of the farm-to-fork chain. The efforts have resulted in three distinct *Salmonella* control programs, for broilers, pigs and layers implemented in 1989, 1993 and 1996, respectively. Additionally, a program to monitor *Salmonella* Dublin in cattle was implemented late 2002 with the objective of certifying freedom of infection.

Multi-resistant *Salmonella* Typhimurium DT104 (MRDT104) is known as an important food borne pathogen in many countries (Threlfall et al. 1994, Besser et al. 1997, Wall et al. 1997, Gay et al. 1998, Imberechts et. al 1998). MRDT104 has been reported to spread rapidly between animals within herds, between herds and to other species (Wall et al. 1997). The combination of the ability to spread rapidly and the multi-resistance towards antibiotics used frequently in animals and humans implies that MRDT104 can pose a serious health problem for both animals and humans.

Even though other countries with significant occurrence of MRDT104 (see references above) have no general programs to control *Salmonella*, the risk management practises adopted in Denmark were based on the worst case scenario that the general control measures were not able to contain MRDT104.

In Denmark, detection of MRDT104 relies on the general *Salmonella* surveillance programs, parts of which are based on detection of *Salmonella* antibodies in body fluids (serological methods) while other parts are based on the detection of the *Salmonella* bacterium itself in samples collected in slaughterhouses or on farms. Since 1998, multi-resistant *Salmonella* Typhimurium DT104 has officially been designated an unwanted pathogen in any food. Consequently, when MRDT104 is detected, a series of control measures are initiated. At present, trade with animals from herds registered as infected with MRDT104 will be restricted, a mandatory trace back procedure is

performed by use of serology and culture methods, and steps must be taken to reduce the *Salmonella* burden in infected herds.

1.2 General *Salmonella* surveillance programs in Denmark

1.2.1 *Salmonella* surveillance in primary production

While the objective for the broiler and the layer programs has been a zero prevalence, the pig program has a continued decreasing prevalence of infection as it's objective. Recognising the difficulty of complete eradication of *Salmonella*, the programs have been designed to have as much impact on the incidence of human salmonellosis as possible, in other words only major, commercial flocks or herds have been included. Therefore, a number of small farms are not monitored for *Salmonella* even though they may occasionally put meat on the market.

While the first major *Salmonella* control program relied on bacteriological culture for detection of infected broiler flocks, the more recent programs have used serological examination of samples of blood, meat juice or egg yolk for general surveillance, with bacteriological follow-up on farms where the serological test indicates a *Salmonella* infection may be present. Serological test results produced by the programs can only be interpreted at herd-level and the requirement for bacteriological follow-up depends on the number of reactors required for the herd or flock to be defined as infected with *Salmonella*. In general, as the tests are not 100 percent specific, one or more reactors may be accepted before follow-up is initiated. In the *Salmonella* Dublin programme in cattle, bacteriological follow-up is not compulsory.

Pigs. The serological surveillance programme for detection of *Salmonella* infection in slaughter pig herds was implemented in the beginning of 1995 (Table 1.1). The slaughter pig herds are assigned to one of three levels based on the proportion of samples with a positive serological reaction during the previous three months (Level 1: no action required; Level 2: herd intervention necessary; Level 3: herd invention and increased hygienic precautions during slaughter is implemented). Herds placed in Level 2 or Level 3 is required to collect pen-faecal samples in order to determine the distribution of *Salmonella* in the herd. At the end of 1995, 5.4% of the pig herds were placed in Level 2 or 3, compared with 3-4% of the herds since 1998.

In 2000, the cut-off value at which a serological test is considered positive was reduced, resulting in twice as many seropositive test results as before. Calculation of a *Salmonella* index based on the surveillance data was introduced, where the results from the most recent month are given relatively more weight.

There is no direct *Salmonella* surveillance in sow herds, but sow herds producing piglets for slaughter pig herds placed in Level 2 or 3 are also obliged to collect pen-faecal samples. Breeding and multiplying pig herds are monitored monthly by serological testing of blood samples. If a specific cut-off level in these samples is reached, the herd owner is obliged to collect pen-faecal samples.

The number of samples collected from a given herd during a 12-month period ensures – if all are *Salmonella* antibody negative – that the true *Salmonella* seroprevalence in the herd is less than 5% (Alban et al., 2002). In any given month, the weighting used for the calculation of *Salmonella* index based on results for the most recent months ensures that if all samples are negative, the true sero-prevalence is less than 11.2 percent in large herds, increasing to 18 percent in small herds where fewer samples are taken. This means that in some of the pig herds placed in Level 1, *Salmonella* infection may be present, but remain undetected.

Cattle. In 2001, serological screening of S. Dublin in dairy herds was initiated, where all dairy herds were tested by bulk-milk samples collected every 3 months. The *Salmonella* surveillance of the non-dairy cattle production is based on serological testing of blood samples collected for the BVD surveillance program. Based on the results from the pilot phase, the national surveillance program started 1 October 2002. In contrast to the program running in the pig production, the cattle program aims at identifying herds free of *S.* Dublin infection. The serological surveillance of bulk-milk is not followed up by microbial sampling, due to the low predictive value of a positive test (Anon., 2001b). Therefore, identification of MRDT104 infection in cattle herds depends on examination of clinical samples or trace-back due to contact with other known MRDT104 herds. *S.* Typhimurium was also included in the serological screening, but data analysis is not concluded.

Table 1.1 *Salmonella* surveillance in Denmark. Data from Annual Report 2001

Animal category		**Min. yearly Production**	**Approx. no. of samples in 2001**	**Testing method and type of sample**
Pigs	Slaughter pig herds	200	Ca. 700.000	Serological testing of meat juice
	Breeding/multiplying herds	All	Ca. 60.000	Serological testing of blood samples
	Sow herds			Monitored via the slaughter pig herds
	Carcasses		Ca. 35.000	Microbial testing of swab samples (previously meat samples)
	Retail		Ca. 1.700	Microbial testing of meat samples
Cattle	Dairy herds		Ca. 35.000	Serological testing of bulk-milk
	Beef cattle	Not implemented yet		Serological testing of blood samples
	Carcasses		Ca. 11.000	Microbial testing of swab samples (previously meat samples)
	Retail		Ca. 1.000	Microbial testing of meat samples
Broiler and egg production				
	Central rearing stations	All	Ca.13.000	Microbial testing of wet dust, "sock" samples and dead chickens
	Breeders	All	Ca. 350.000	Microbial testing of sock samples, dead chickens and crate material
	Hatcheries	All	Ca. 500	Serological testing of blood samples
	Rearing-table-egg prod.	All	Ca. 17.000	Microbial testing of sock samples, dead chickens and crate material
			Ca. 20.000	Serological testing of blood samples
	Table-egg production	All	Ca. 8.000	Microbial testing of "sock" samples
			Ca. 250.000	Serological testing of egg samples
	Broilers	20.000	Ca. 225.000	Microbial testing of sock samples (AM) and meat samples (PM - previously neck skin)

Broilers. Surveillance of commercial broiler flocks for *Salmonella* commenced in 1989 and although the sampling scheme has been changed since then, it still relies on bacteriological examination of each flock about two weeks prior to slaughter (ante mortem (AM) samples, Table 1.1). The program includes a combination of bacteriological and serological surveillance of parent birds, with intensive bacteriological follow-up when a parent flock is found to be seropositive or when offspring from a flock is found infected with *Salmonella* at ante mortem testing. Parent stock infected with any *Salmonella* serotype is culled. The current scheme provides a high degree of certainty that MRDT104 in broilers will be detected. Flocks of turkeys, ducks and geese are monitored for *Salmonella* by bacteriological examination of "sock samples" collected ante mortem, three weeks prior to slaughter.

Layers. In December 1996, a surveillance and control program for the eradication of *Salmonella* in the table-egg production was implemented. The program includes serological surveillance of blood samples from hatcheries and rearing flocks, as well as serological examination of the yolk from 60

eggs submitted from production flocks every 9 weeks. The serological samples are combined with routine bacteriological examination of several types of material (Table 1.1).

1.2.2 Herds not included in the general *Salmonella* surveillance

Until August 2000, all herds producing more than 100 pigs for slaughter per year were monitored by serological testing of meat juice. The actual number of pig herds not included in the serological surveillance plan is not known, but Table 1.2 shows an estimate, based on the herd sizes reported to "Danmarks Statistik" annually. If we assume that a herd can produce three or four batches of slaughter pigs per year, herds reporting less than 25 –33 slaughter pigs in the census can be assumed to produce less than a 100 slaughter pigs per year. Based on this we estimate that the proportion of herds not included in the surveillance plan was reduced from 18-21% in 1996 to 11-14% in 2001, due to the structural changes occurring in the pig production.

Table 1.2 Estimated numbers of pig herds and number/percentage of slaughter pigs not included in the *Salmonella* surveillance program. The estimate is based on the official livestock census figures from Danmarks Statistik. Assuming a production of three or four batches of slaughter pigs per year, herds reporting a census figure of less than 25 –33 animals may be assumed to produce less than a 100 slaughter pigs per year.

	Total no. Herds	Pig herds not under surveillance [1)]		Slaughter pigs not under surveillance (10^3)	
1996	19,821	3,667–4,215	(18.5–21.3%)	160–169	(1.5–1.6%)
1997	18,829	2,956–3,490	(15.7–18.5%)	134–147	(1.2–1.3%)
1998	17,688	2,385–3,018	(13.5–17.1%)	117–144	(1.0–1.2%)
1999	15,483	2,313–2,726	(14.9–17.6%)	108–118	(0.9–1.0%)
2000	13,231	1,660–1,854	(12.5–14.0%)	79–82	(0.6–0.7%)
Primo 2001	12,936	1,489–1,816	(11.5–14.0%)	71–82	(0.6–0.7%)
Ultimo 2001[2)]	12,936	2,349–2,684	(18,2–20,7%)	200–210	(1.6–2.0%)

1) This number includes herds supplying weaners and grower pigs to slaughter pig herds included in the *Salmonella* surveillance. With the current data it is not possible to estimate the number of sow herds under indirect *Salmonella* surveillance.
2) In august 2001, the minimum herd size was changed from 100 to 200 slaughter pigs per year, so that herds reporting less than 50-66 pigs are assumed too small to be included in the *Salmonella* surveillance.

This left approximately 160.000-169.000 slaughter pigs outside the *Salmonella* surveillance program in 1996, whereas in 2000, 79.000-82.000 slaughter pigs were not included. From August 2001, herds producing less than 200 pigs for slaughter per year were no longer included in the surveillance. With the new surveillance plan approximately 18-21% of the herds and 1.6 % of the total number of slaughter pigs, was not included (Table 1.2). In the year from August 1st 2001 to July 30th 2002 the number of pigs slaughtered outside the surveillance program was approximately 130,000 (data from the Zoonosis register, ZOOR and the DS slaughterhouse database).

Since 1996, the total number of pig herds has been reduced by 35%, and the number of herds estimated to produce less than 200 slaughter pigs per year has declined by 44% (yearly average of 14%) (Table 1.2). The number of small pig herds outside surveillance was reduced by 7% in 2001 compared to 2000. Assuming that this rate of decrease continues until 2005, the number of slaughter pigs coming from herds not included in the surveillance will decrease from approximately 200.000 to 150.000 in 2005.

The estimates presented above include sow herds, which are indirectly monitored via the receiving slaughter pig herds and breeding/multiplying pig herds also under serological surveillance. An alternative way to estimate the number of slaughter pig producing herds not under surveillance is count the number of herds with a slaughter pig production registered in the salmonella surveillance register (Zoor), excluding sow herds, breeder and multiplier herds. If a herd has less than seven pen places registered for slaughter pigs for each pen registered for sows in the CHR-register, we assumed that the herd was a sow herd primarily producing weaners and growers, thus being indirectly monitored via the receiving slaughter pig herds. All breeder and multiplier herds are registered as such, and can be excluded. During the period from 1st August 2001 to 1st August 2002, approximately 1900 slaughter pig producing herds were outside surveillance, producing in total 125.000 slaughter pigs.

Most of the organic and free-range pig herds will be included in the serological surveillance. According to Friland Food, 95% of free-range herds and approximately 80% of the organic-pig herds produce more than 200 pigs per year (Alban et al., 2001).

In the poultry production, farms producing less than 20.000 broilers, 15.000 ducks or geese or 10.000 turkeys per year are not included in the surveillance program. We have not been able to establish how many flocks this involves. In general, most broiler and turkey flocks are included, while a greater part of flocks of geese and ducks are outside the surveillance.

1.2.3 *Salmonella* surveillance of meat products

Post-harvest surveillance of *Salmonella* has been an integral part of the surveillance programs in slaughter poultry and in pigs. In poultry, until November 2000, surveillance consisted of bacteriological examination of 50 samples of neck skin collected on the slaughter floor and

examined in pools of five. In November 2000 this was changed to end-product control, based on examination of samples collected immediately prior to packing (Table 1.1).
A program for surveillance *Salmonella* in beef and pork at the slaughterhouses was initiated in July 1993, based on bacteriological testing of meat cuts. The number of samples, as well as the type of cuts to be sampled, was determined by the Veterinary and Food Administration in proportion to the throughput of each plant. From January 2001 the procedure for surveillance of fresh meats has changed and now consists of swab samples taken from three designated areas of chilled carcasses (Table 1.1). For these official programs, all sample results are entered into central, official databases. In other words, denominator information is available so that the data is suited for monitoring trends in *Salmonella* prevalence.

In addition to the official sampling schemes there is, in particular for pork and beef, extensive company-based bacteriological testing of products in association with export to certain countries. Information about serotype and phage types for positive samples is available centrally. Information about the number of export samples examined is not available, however, Danske Slagterier have provided some data on the issue, shown in Section 1.3.3 below.

1.3 *Salmonella* Typhimurium phage type DT104

The *Salmonella* Typhimurium serotype may be divided into subtypes using a variety of different typing systems. One of these is phage typing, where the susceptibility of an isolate to a panel consisting of 36 bacterial phages defines the phage type, also known as the definitive type (DT). Other typing methods include antibiogram typing, where susceptibility to a panel of antibiotics is determined, and pulsed gel electrophoresis (PFGE), where the composition of bacterial DNA is determined using certain enzymes.

Subtyping of DT104. A typing study of penta-resistant *Salmonella* Typhimurium DT104 isolated from Danish human cases using PFGE and plasmid profiling showed that the bacterium is highly clonal (Skov et al, 2002). The study included 227 isolates and found that over 90 percent of them belonged to one PFGE type. Within the most common PFGE type, two plasmid profiles predominated. These plasmid types were observed also in DT104 isolates from pigs and cattle, as well as from domestically produced and imported meat. In other words, the clonal structure of

Salmonella Typhimurium DT104 in Denmark makes it difficult to discriminate between sources of infection on the basis of typing methods.

Antibiotic resistance of DT104. MRDT104 is commonly thought of as being resistant to five antibiotics: ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACStSuT). It is also known as penta-resistant DT104. Some of the penta-resistant clones have also acquired resistance to trimethoprim, and during recent years isolates with decreased susceptibility to e.g. flouroquinolones have been detected as well (Mølbak et al., 2000).

Table 1.3 Antibiogram distribution (resistance profiles) of DT104 isolates found during the normal *Salmonella* surveillance in slaughter pig herds, sow herds and breeding and multiplying herds from 1998 until 1 August 2002. DT104 herds found via trace-back are not included. When data from more than one isolate per herd per year were available, the isolate with the lowest registration number was chosen, even though more than one resistant type was found during the investigations. Abbreviations: AM – ampicillin; CH – chloramphenicol; NA – nalidixic acid; NE – neomycin; ST - streptomycin; SU – sulphonamide; TE – tetracycline; TR – trimethoprim.

Resistance profile of DT104 in Slaughter pig herds	1998	1999	2000	2001	2002	Total	Percentage
AM,CH,ST,SU	0	1	0	0	0	1	1%
AM,CH,ST,SU,NA	0	1	0	0	0	1	1%
AM,CH,ST,SU,TE	4	1	8	15	3	31	46%
AM,CH,ST,SU,TE,NA	0	0	0	0	1	1	1%
AM,CH,ST,SU,TE,NE	0	0	0	1	0	1	1%
AM,CH,ST,SU,TR	0	1	0	0	0	1	1%
AM,ST,SU	0	0	1	1	0	2	3%
AM,ST,SU,TE	1	0	0	0	0	1	1%
AM,SU	0	0	0	1	0	1	1%
ST,SU	1	0	1	0	0	2	3%
SU	1	0	0	0	0	1	1%
TE	0	0	0	0	1	1	1%
TR	0	0	1	1	1	3	4%
Fully susceptible	1	0	4	10	5	20	30%
Total	8	4	15	29	11	67	100%

Resistance profile of DT104 in sow herds	1998	1999	2000	2001	2002	Total	Percentage
AM,CH,ST,SU,TE			3	4	1	8	53%
AM,CH,ST,TE,NE			0	1	0	1	7%
NE,TR			1	0	0	1	7%
ST,SU,TE,NE			0	0	1	1	7%
Fully susceptible			1	2	1	4	27%
Total			5	7	3	15	100%

Resistance profile of DT104 in multiplying and breeding herds	1998	1999	2000	2001	2002	Total	Percentage
AM,CH,ST,SU	0	1	0	0	0	1	20%
AM,CH,ST,SU,TE	0	0	1	0	2	3	60%
Fully susceptible		0	0	1	0	1	20%
Total	0	1	1	1	2	5	100%

We have examined resistance patterns of DT104 isolated from Danish pig herds between 1998 and August 1 2002. We have included only one isolate per herd per year and we have excluded isolates from all herds detected in the course of trace-back procedures. In cases where more than one isolate was available, we selected the first one only, irrespective of its resistance profile. For comparison, using the same selection criteria we have examined the resistance profiles of *Salmonella* Typhimurium DT17, another phage type recently introduced in Danish pig production. The results are shown in Tables 1.3 and 1.4.

Table 1.4 Antibiogram distribution (resistance profiles) of DT17 isolates found during the normal *Salmonella* surveillance in slaughter pig herds, sow herds and breeding and multiplying herds from 1998 until 1 August 2002. When data from more than one isolate per herd per year were available, the isolate with the lowest registration number was chosen, even though more than one resistant type was found during the investigations. For abbreviations, please refer to Table 1.3 above.

Resistance profile of DT17 in Slaughter pig herds	1998	1999	2000	2001	2002	Total	Percentage
AM	1	0	0	0	0	1	0%
AM,ST,SU,TE	1	0	0	0	0	1	0%
AM,ST,SU,TE,NE	0	0	1	1	0	2	1%
AM,ST,SU,TE,NE,TR	0	0	0	1	0	1	0%
CH	1	0	0	0	0	1	0%
CH,SU	0	2	0	0	0	2	1%
ST,SU	0	0	1	0	0	1	0%
ST,SU,TE,NE,TR	0	0	0	1	0	1	0%
ST,SU,TE,TR	0	1	0	0	0	1	0%
ST,SU,TR	0	3	0	0	0	3	1%
Fully susceptible	42	57	55	49	10	212	94%
Total	45	63	57	52	10	226	100%

Resistance profile of DT17 in sow herds	1998	1999	2000	2001	2002	Total	Percentage
AM,ST,SU,TE,NE,TR	0	0	0	1	0	1	2%
ST,SU	0	0	0	0	1	1	2%
ST,SU,TR	0	1	0	0	0	1	2%
ST,TE	0	0	0	1	0	1	2%
SU	0	1	0	0	0	1	2%
SU,TR	0	0	2	1	0	3	7%
TE,TR	0	0	1	0	0	1	2%
Fully susceptible	0	10	11	14	2	37	80%
Total	0	12	14	17	3	46	100%

Resistance profile of DT17 in multiplying and breeding herds	1998	1999	2000	2001	2002	Total	Percentage
ST,SU,TE,NE,TR	0	0	0	0	1	1	20%
ST,SU,TR	0	1	0	0	0	1	20%
Fully susceptible	0	1	1	1	0	3	60%
Total	0	2	1	1	1	5	100%

It is clear from Table 1.3 that only about half of DT104 from pigs are penta-resistant. This trend seems not to have changed over the years included in the study. A few are multi-resistant without being penta-resistant and about one third of all isolates is fully susceptible to the antimicrobials tested. Seemingly the proportion of fully susceptible DT104 from pig herds has been increasing, going from 13% in 1998, to 27% in 2000, whereas 45% of the isolates selected from 2002 were non-resistant. This proportion is much higher than in humans (see Section 1.3.4). In comparison, very few DT17 isolates are penta-resistant or multi-resistant. Indeed, 80-90% of them are fully susceptible to antimicrobials.

1.3.1 Occurrence of MRDT104 in the primary production

Following extension of the panel of phages used for phage typing, the first isolates of multi-resistant DT104 were found in a Danish slaughter pig herd in 1996 (Baggesen et al., 1998). Retrospective analysis of isolates stored in the DVI strain collection showed that a Danish sow herd had been infected with MRDT104 in 1991. Until 1998, less than ten herds in total were found infected with MRDT104. In 1998, an increase in the number of infected pig herds was observed (Figure 1.1). A nation-wide screening for MRDT104 in the period from June 1998 to February 1999 included 2633 pig herds and 265 cattle herds. It was conducted to provide a basis for decision about the appropriate control strategy to be adopted. The screening found a low apparent prevalence, with MRDT104 detected in one slaughter pig herd, whereas MRDT104 was not found in pig multiplier and breeding herds, sow herds producing growers only or in any of the cattle herds. In 1999, the number of MRDT104 infected pig herds declined, while an increase in the number of MRDT104 infected cattle herds occurred. Most of the MRDT104 cattle herds came from one specific area in Aarhus County (the Brædstrup area, Figure 1.2), and in most cases trade relations could explain the route of infection. A more than four-fold increase in the number of identified MRDT104 infected herds with pigs occurred during year 2000 and approximately half of them had cattle. In 2001, the total number of identified MRDT104 infected herds decreased by 25%, whereas the number of infected herds with pig production only increased by more than 50%.

By the end of 2000, the first Danish poultry flocks were found infected with MRDT104. First in a turkey flock originating from Germany, and later in a broiler flock raised near two MRDT104 infected pig herds. In 2002, MRDT104 has been found in one additional turkey flock and also in a broiler flock located closely to an infected pig farm.

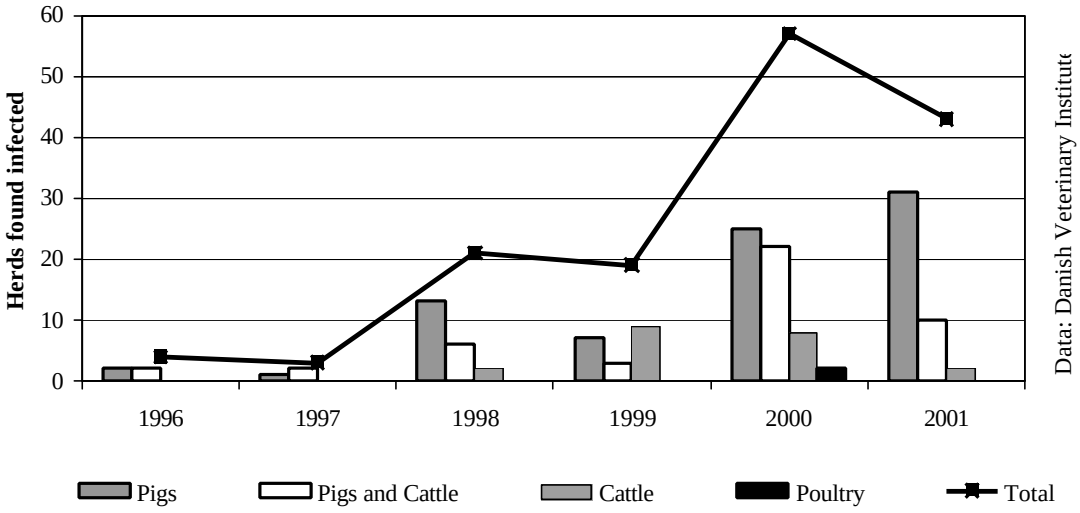

Figure 1.1 Herds found infected with MRDT104 in Denmark from Jan. 1996 until the end of 2001. The data for 2000 include an infected broiler flock where the diagnosis was not confirmed until January 2001 and two pig herds where pen sampling could not confirm the finding of MRDT104 at the slaughterhouse. The data for 2001 include one slaughter pig herd rearing MRDT104 piglets.

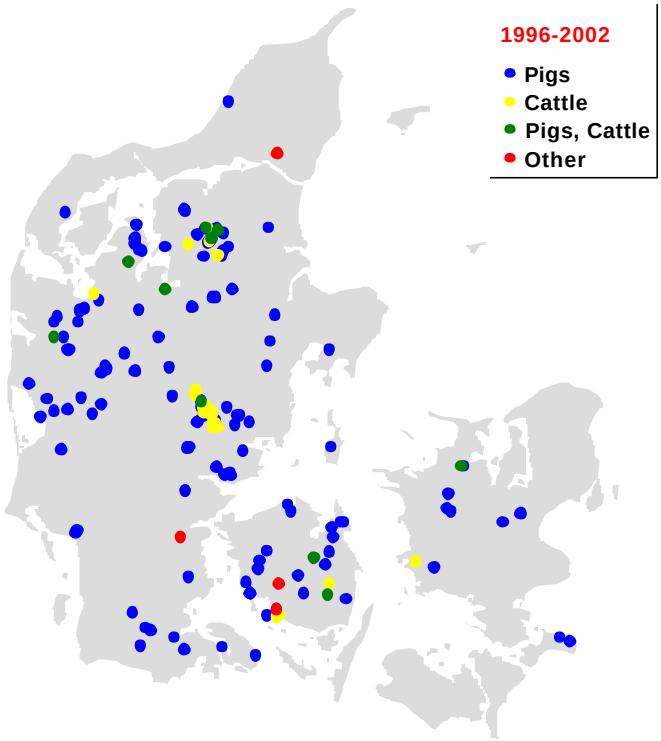

Figure 1.2 The regional distribution of MRDT1 04 herds in Denmark during the period 1996 – 2002. The group 'Other' includes poultry and farmed foxes.

By the end of August 2002, MRDT104 has been detected in 90 pig herds, 46 farms with both pigs and cattle, 23 with cattle alone, two turkey flocks, two broiler flocks and in a herd of farmed foxes. In total, 164 herds have been reported as infected with MRDT104 (Figure 1.1).

Figure 1.2 shows the regional distribution of the MRDT104 herds from 1996 to 2002. The first cases were found in Jutland in 1996, and in 1998 the first cases occurred on Funen and on Zealand.

The apparently uneven distribution of infected farms reflects to some extent the uneven distribution of farm animals in Denmark. However, as for other *Salmonella* Typhimurium phage types the main mode of transmission for MRDT104 under Danish herd management conditions are likely to be the transfer of animals between farms. However, transfer between farms has been shown to play a role in some instances (Rattenborg, personal communication). For example, analysis of the Brædstrup cluster of infected dairy herds showed that for some farms common ownership or communal use of farm machinery constituted a likely risk factor for transmission.

Until August 2002, only two commercial broiler flocks have been found infected with MRDT104. Although vertical spread of *S.* Typhimurium is possible in poultry, the absence of MRDT104 positive samples from parent stock and from surveillance in hatcheries makes it most likely that the infection occurs as a result of horizontal transmission. The first of these positive flocks was located close to two MRDT104 positive pig herds (within a distance of 500 and 400 meters, respectively). The second one was located 750 meters as the crow flies from a pig herd found positive 6 months previously and still under zoonosis restriction order when the neighbouring broiler flock was found infected. In the first case, however, the MRDT104 isolate carried a small plasmid not found in the isolates from the pig herds near by and these therefore may not be the origin of infection.

1.3.2 Control strategy in primary production

In October 1997, an order (the DT104-order) was issued by the Danish Veterinary and Food Administration to make the detection of MRDT104 in food animals notifiable. Infected herds were put under official veterinary supervision resulting in a number of restrictions, including requirements for special hygiene at slaughter, on trade in live animals as well as a mandatory epidemiological investigation of the herd and its trade contacts. Two negative herd examinations (based on at least 20 pen faecal samples collected each time) at 45 days interval were required to lift the restrictions. Restrictions could also be lifted if the herd was depopulated followed by very

thorough cleaning and sanitation of the premises. The order was issued to prevent spread of MRDT104 between herds as well as from animals to humans. In August 1999, the order was replaced with a new order extending the authorities' powers to investigate the spread of MRDT104. According to this order, all animal species on an infected farm and herds associated with the infected herd by e.g. trade of live animals or by location, may be ordered examined.

Accordingly, when MRDT104 is detected on a farm, a mandatory trace back procedure is initiated. The epidemiological investigation of the MRDT104 herd (primary case) and its trade contacts, has often lead to the finding of other MRDT104 herds (secondary cases). In some cases, the herd that apparently is the source of infection (the index case) can be identified. Clusters containing a primary and one or more secondary cases are defined as an outbreak.

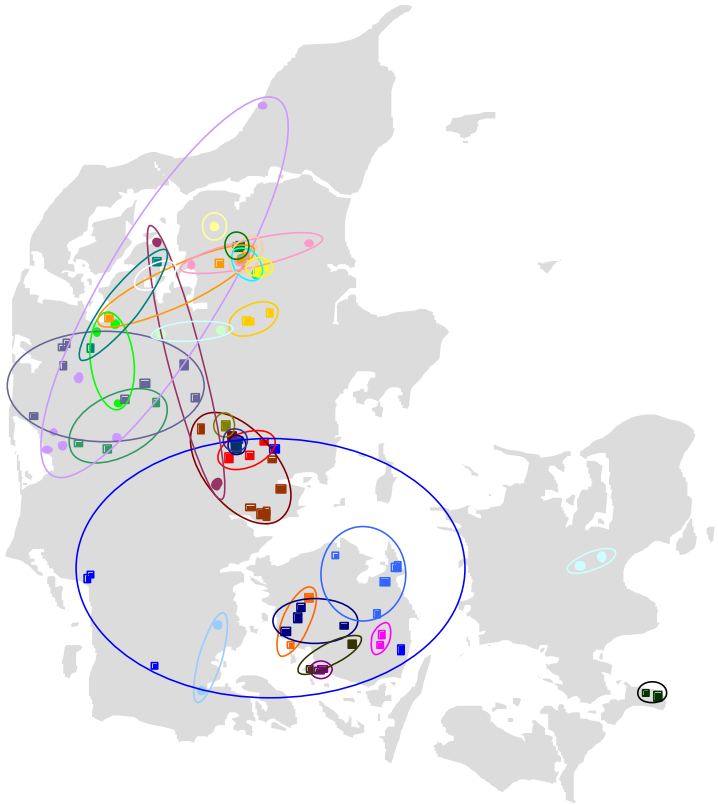

Figure 1.3 Map showing known clusters of herds infected with MRDT104 as determined by trace back procedures. Circles and squares having the same colour identify clusters.

From the 164 known MRDT104 herds, 78 outbreaks have been identified (Figure 1.3 and 1.4), including the incidences in 2000, where the sampling in two pig herds could not confirm the finding of MRDT104 at the slaughterhouse.

An attempt to eradicate MRDT104 from infected pig herds was initiated in the beginning of 1997 by Danske Slagterier in co-operation with the Danish Veterinary Institute and at industry expense. This program was based on stamping out the infected herd, including a thorough cleaning and sanitation of the buildings before introducing new pigs at the farm. After reestablishment of the production, the herd was monitored intensively in order to ascertain the success of the eradication. In 7 of about 50 herds, sampling showed that the herd remained infected, despite the efforts.

The pig industry's strategy of stamping out infected pig herds was terminated in June 2000 as an increasing number of infected pig herds made this approach economically unfeasible. In the latest revision of the DT104-order, a new kind of official veterinary supervision was introduced – the Zoonosis Restriction order (Bek. nr. 435 af 31. maj 2001). Owners of herds placed under zoonosis restriction order are required to prepare a strategy for reduction of the level of *Salmonella* in the herd to below the level of detection for MRDT104. The strategy must be sanctioned by the district veterinary officer and must run for a minimum of 12 months. To lift the sanctions, the serological *Salmonella* index for slaughter pig herds must be below 20.0 for a 4-month period. Farrow-to-grower herds require two sets of MRDT104 negative pen faecal samples examined at 30 days interval and farrow-to-finisher herds also need a one-month period with an index below 20.0 before the herds are assumed free of MRDT104. Sanctions may also be lifted if the herd is depopulated.

Detection of MRDT104 in broiler and turkey flocks leads to slaughter and heat treatment or, alternatively, destruction of the flock. Should MRDT104 be found in the Danish table-egg production, the flock and the eggs will be destroyed.

1.3.3 MRDT104 in meat products

In 2001, the routine *Salmonella* surveillance of fresh meat in slaughter plants detected two instances of contamination with MRDT104. The registered isolates at the DVI database show an additional number of findings in samples analysed by meat companies in association with export or in samples collected as part of a plant's own control.

Table 1.8 shows a summary of the finding of MRDT104 in Danish export plants when testing batches of products prior to shipment to Sweden and Finland. The samples have been examined in pools of five and the results shown are approximate means, based on the years 1998, 1999 and 2000. The results are in agreement with the finding of MRDT104 by the official surveillance program.

Table 1.8 Results of examining export shipments of pork for *Salmonella*. The maximum possible DT104 prevalence is an estimate, based on the assumption that all individual samples in positive pools were positive for MRDT104. Data provided by Danske Slagterier.

Number of pools analysed per year	Number of individual samples	*Salmonella* prevalence in pools, all serotypes	MRDT104 prevalence in pools	Maximum DT104 prevalence, individual samples
37,000	185,000	1 %	0.002 %	0.01 %

Since July 1998, MRDT104 has been monitored in fresh meat imported from the EU and third countries (Figure 1.7). In 1998, the initial screening program found a prevalence of MRDT104 in imported fresh meats of 0,8% (19 of 2.466 samples), whereas the overall prevalence of MRDT104 in imported meat in 2001 was reduced to 0.15% (5 of 3,247 samples, Annual Reports, 1998-2001). During the period from 1998 to 2001, the apparent prevalence of MRDT104 has consistently been higher in the imported pork than in pork of domestic origin. It seems reasonable to conclude that the prevalence of MRDT104 in Danish meat products on average is lower than in the imported meat.

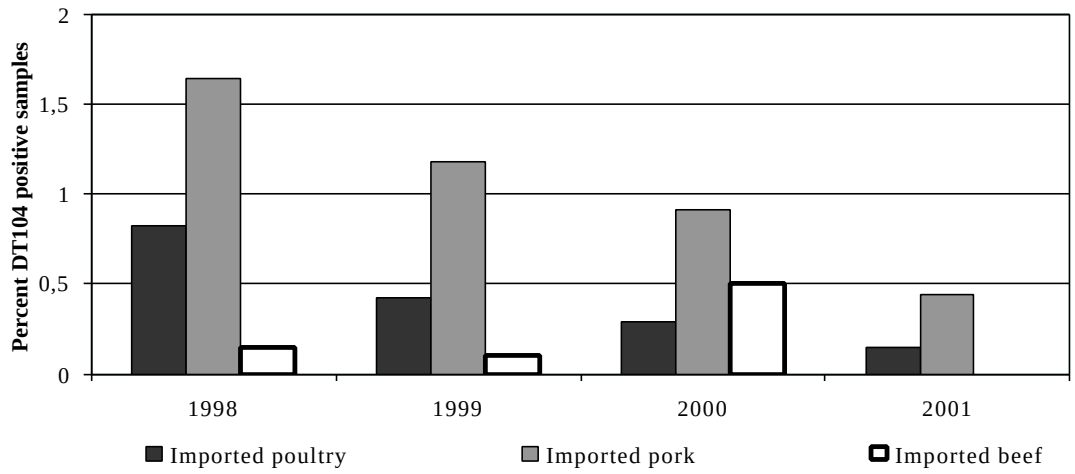

Figure 1.7 Percentage MRDT104 positive samples of fresh poultry, pork and beef meat imported from the EU and third countries. Data from 1998 is from the screening (July –December), where as data from the following years are from the surveillance programme.

1.3.4 MRDT104 in humans

Until 1996, *Salmonella* Typhimurium isolates from cases of human disease were not routinely tested for antibiotic resistance and only a subset of the isolates was phage typed. However, following a nosocomial outbreak of MRDT104 infection in a hospital and the detection of MRDT104 in domestic food animal herds, Statens Serum Institut initiated antibiogram and phage typing of all human *S.* Typhimurium isolates in 1997. In addition, each individual case is followed up by a telephone interview to determine the possible source of infection and to determine whether the exposure could have occurred abroad. The phage and antibiogram typing also includes strains where the primary isolation has been carried out by the clinical microbiology departments in counties not submitting samples to the SSI, in other words national coverage of this passive surveillance is complete.

An analysis of DT104 isolated from human cases of disease between 1997 and August 2002 shows that 3% of the isolates were resistant to 4 antimicrobials, 65.8% were penta-resistant while 22.5% were resistant to more than 5 antimicrobials. Only 3,5% of isolates were fully susceptible to antimicrobials (Ethelberg, personal communication).

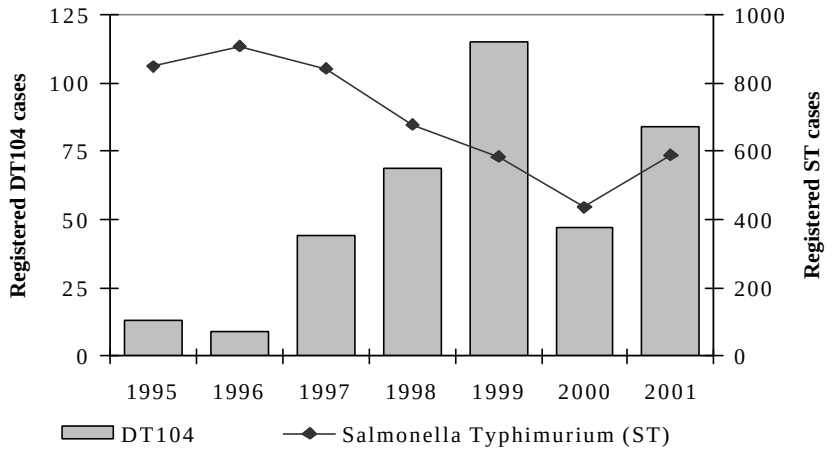

Figure 1.8 The total number of human cases with *Salmonella* Typhimurium, and the number of cases with the phage type DT104 during the period January 1995 until December 2001. Data from Statens Serum Institut

During the period from 1996 to 1999 the number of human DT104 cases increased, whereas the total number of *S.* Typhimurium (ST) cases declined (Figure 1.8). In 1999, 25% of the ST cases were due to DT104, thus making DT104 the most common human *S.* Typhimurium phage type. The number of outbreak associated MRDT104 cases in particular increased from 1996 to 1999. During the summer of 1998, the first community outbreak of MRDT104 was registered in Denmark, and

the source of the 25 cases was traced back to pork of Danish origin (Annual Report, 1998). In 1999, there were two general outbreaks of MRDT104, where contaminated food from a restaurant and a caterer resulted in 58 human MRDT104 cases (Annual Report, 1999). No community outbreak has been reported since 1999, and most of the domestically acquired human cases have since then been sporadic (40-50 cases per year, Annual Report, 2001).

The number of sporadic domestic human cases of MRDT104 increased by approximately 25 % from 2000 to 2001. There was no increase in the occurrence of MRDT104 in domestic or imported meat that could explain this. However, the increased number of infected herds with pig production only, suggests that domestically produced pork is likely to be a source, even though the prevalence of MRDT104 on pork carcasses does not reflect this (Annual Report, 2001).

1.4 Distribution of MRDT104 in Danish pig herds compared to other *Salmonella* Typhimurium phage types

During the period 1997-2001, 800-1000 pig herds annually were found infected with *S.* Typhimurium (Figure 1.5). In 2000, the fraction of *Salmonella* Typhimurium pig herds reported infected with multi-resistant MRDT104 doubled, rising from 1-2% up to almost 5% (Figure 1.5a). Thus, the high number of MRDT104 herds observed in 2000 and 2001 was not due to a general increase in the prevalence of *Salmonella* Typhimurium infection. The number of MRDT104 pig herds found in 2000 through the normal *Salmonella* surveillance (primary cases) increased four-fold compared to the 13% increase in the total number of *S.* Typhimurium infected pig herds. The number of secondary cases ranges from 0 to 8 per primary case, but on average (from 1996-2002) one primary case has lead to 1.1 secondary cases. In 2000 a five-fold increase was observed in the number of secondary cases, possibly reflecting the more extended trace back procedures implemented in August 1999 (Figure 1.5b). The proportion of MRDT104 remained the same in 2001, even though the industry stopped depopulating MRDT104 pig herds, with 26 infected pig herds before, and 30 after July 1st 2000.

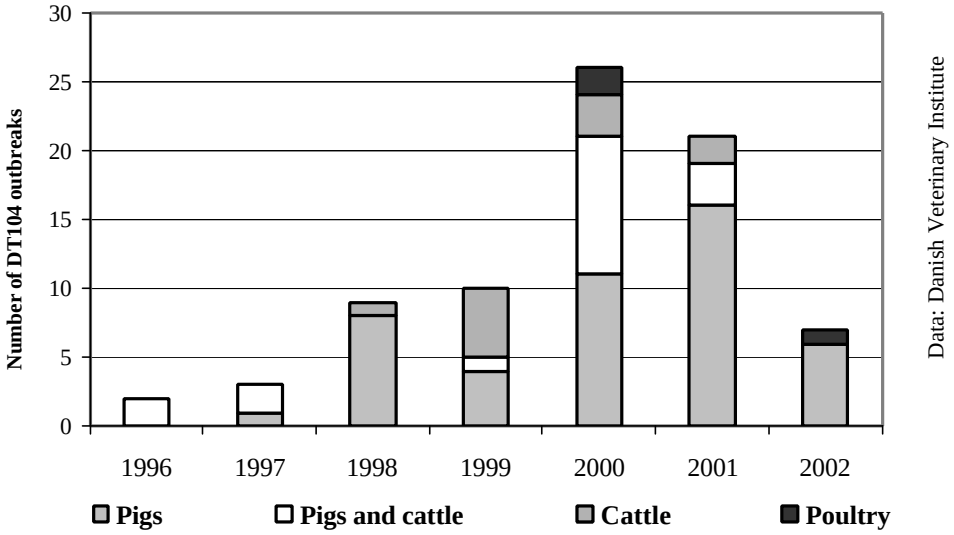

Figure 1.4 The number of MRDT104 outbreaks in Denmark from January 1996 until June 2002. Clusters containing a primary and one or more secondarily infected herds are defined as an outbreak. Grouping by herd type of the primary case.

Among pig herds infected with MRDT104, 48 were identified through the general *Salmonella* surveillance program because they were placed in Level 2 or 3 and 88 through follow-up in contact herds. These numbers include 7 herds found positive (one herds found positive on two occasions) in control samples following intervention or follow-up because DT104 was found in *Salmonella* surveillance of meat. In 6 herds with only pigs (6.6% of all MRDT104 positive herds with pigs only), the primary diagnosis was made through examination of diagnostic samples (Table 1.5).

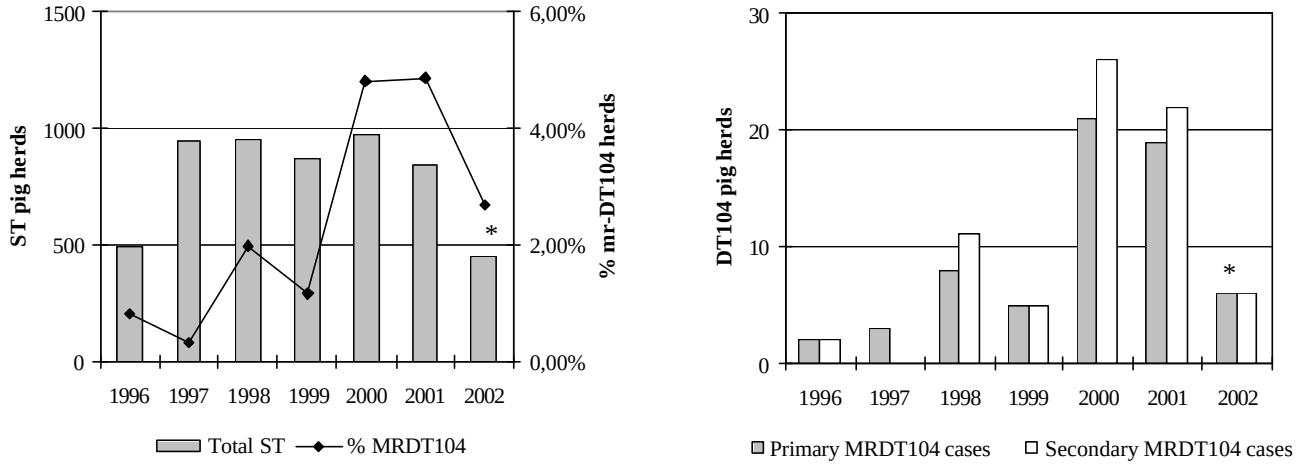

Figure 1.5 A: Total number of pig herds infected with *Salmonella* Typhimurium and the percentage of herds infected with MRDT104. Infected herds are included only once per year. * Until end July 2002

B: Number of MRDT104 pig herds found through routine surveillance (primary) and by trace back (secondary). * Until end July 2002.

Table 1.5 Number of MRDT104 herds with primary diagnosis due to clinical symptoms.

Clinical symptoms	Herds with pigs only	Herds with cattle only	Herds with pigs and cattle	Farmed fox	Poultry	Total herds
1996	0	0	1	0	0	1
1997	0	0	0	0	0	0
1998	0	2	0	0	0	2
1999	0	6	1	0	0	7
2000	5	2	4	1	0	12
2001	0	0	0	0	0	0
2002 [1)]	1	2	0	0	0	3
Sum: clinical symptoms	6	12	6	1	0	25
Sum: all MRDT104 herds	90	23	46	1	4	164
% herds: clinical symptoms	6.6%	51%	13%	100%	0 %	15.2%

1) Data until 1 august 2002.

In comparison, clinical symptoms appear to be more common in MRDT104 infected cattle herds, where 51% (12 of 23 herds with cattle only) have reported clinical symptoms. The DT104 screening (1998-1998) indicates that sub-clinical infection of MRDT104 is uncommon in cattle herds. However, this also reflects the absence of general *Salmonella* surveillance in cattle, so that clinical salmonellosis and trace-back in contacts to known DT104 herds represents the only means of detection in this species.

The number of *Salmonella* isolations with outbreaks of clinical disease in pig and cattle herds reported in the Annual report on Zoonoses in Denmark until year 2001 included all *Salmonella* infected herds under official veterinary supervision. Therefore, herds infected with MRDT104 have been over represented in the list, even though a minority of these infections resulted in overt disease.

It has been argued that MRDT104 relatively more often result in clinical disease in pigs compared to other *Salmonella* Typhimurium phage types. Clinical symptoms are only recorded as such in the DVI database when this information is available from the sample submission form. There is only limited information regarding clinical symptoms in the DVI database, and only four of the 12 MRDT104 herds found by examination of diagnostic specimens are noted as having diarrhoea. Consequently, the information available about whether MRDT104 is associated with clinical disease or not, may be unreliable and the results shown in Table 1.6 treated with caution.

Table 1.6 The total number of pig herds and the number recorded as having reporting diarrhoea by *Salmonella* Typhimurium phage types during the period 1st January 1998 to 1st August 2002. Data include all DT104 infections (sensitive as well as resistant isolates), and only isolates where diarrhoea has been entered in the database has been included. The number of clinical MRDT104 pig herds is presented in Table 1.5.

Phage type	Total number infected pig herds	Pig herds reporting diarrhoea	% diarrhoea
DT12	1726	59	3,4%
DT17	303	17	5,6%
DT66	350	12	3,4%
DT170	249	9	3,6%
DT193	203	9	4,4%
DTU288	76	7	9,2%
DT10	92	5	5,4%
DT104 (MRDT104)	212 (129)	5 (4)	2.4% (3.2%)
DT120	90	5	5,6%
DT208	54	4	7,4%
DT110	76	3	3,9%
DTU302	40	2	5,0%
DTU312	35	2	5,7%
DT107	56	1	1,8%
DT108	2	1	50,0%
DT15A	80	1	1,3%
DT46A	7	1	14,3%
No phage type	231	28	12,1%
Not typable	498	12	9,5%
Total	4631	183	4%

Table 1.6 shows the total number of pig herds infected with specific *S.* Typhimurium phage types and the number/percentage of herds reporting diarrhoea. This data set from the DVI database includes all DT104 infections (antibiotic susceptible as well as resistant isolates). When including all DT104 infections, 2.4% of the infected herds report diarrhoea, whereas 3.2% of the pig herds infected with MRDT104 report diarrhoea (Table 1.6). Compared to other abundant phage types, MRDT104 does not appear to lead to relatively more clinical cases, and the proportion of herds recorded as having reported diarrhoea is actually in the lower end of the scale.

Trace back to other herds is mandatory only in the case of MRDT104. In all other cases, sampling only occurs at the herd identified as having a *Salmonella* problem and in the associated sow herd – not in other herds in contact with the associated sow herd. Therefore, the total number of MRDT104 is not directly comparable to the total numbers of other phage types. When comparing the prevalence of MRDT104 with other *S.* Typhimurium phage types, it is more appropriate to compare

the relative distribution found during follow-up of serological surveillance, thus avoiding the bias resulting from trace back procedures.

The relative distribution of *S.* Typhimurium phage types in the slaughter pig herds found during follow-up of serological surveillance in the primary production appears to have changed during the years (Table 1.7). DT12 is the predominant phage-type, but during the last four years the proportion of DT12 infections has decreased from almost 50% to 37% in 2001. This decrease is highly significant. During this period, the relative abundance of phage types such as DT104, DT120, DT170 and DT193 has increased.

The number of infected herds found per month during follow-up of serological surveillance was modelled using a second order local regression model (S-PLUS 6.1, Insightful Corp.). The model shows that the number of herds infected with DT104 (incl. non-MRDT104) increased within the same range as other phage types such as DT120, DT170 and DT193 (Figure 1.6). From mid-2000, the model shows that the rate at which herds infected with DT104 were found decreased, reaching a stable level during 2002. The model does not indicate that the number of DT104 herds found per month during follow-up of serological surveillance is decreasing.

Table 1.7 Distribution (percentage and number) of *S.* Typhimurium phage-types in slaughter pig herds found during follow-up from serological surveillance. The data include all DT104 infections (susceptible as well as resistant isolates), whereas the MRDT104 infections are presented at the bottom of the table.

Phage type	1998	1999	2000	2001	2002[1)]
DT12	49,6% (405)	42,5% (317)	41,4% (316)	36,6% (244)	39,0% (78)
DT17	7,1% (58)	9,7% (72)	8,2% (63)	8,1% (54)	5,0% (10)
DT66	6,5% (53)	10,2% (76)	9,4% (72)	7,2% (48)	4,0% (8)
DT170	1,1% (9)	6,2% (46)	6,7% (51)	9,5% (63)	7,5% (15)
DT193	2,9% (24)	3,4% (25)	4,2% (32)	5,0% (33)	6,5% (13)
DT135	2,6% (21)	2,5% (19)	2,1% (16)	1,7% (11)	2,0% (4)
DT104	1,0% (8)	0,5% (4)	2,0% (15)	4,4% (29)	5,5% (11)
DT15A	2,3% (19)	1,9% (14)	2,4% (18)	1,8% (12)	1,0% (2)
DT10	1,5% (12)	1,7% (13)	2,4% (18)	2,7% (18)	1,0% (2)
DT120	1,1% (9)	1,2% (9)	1,8% (14)	2,4% (16)	7,0% (14)
DT110	2,2% (18)	2,0% (15)	1,2% (9)	1,4% (9)	1,5% (3)
DTU288	2,2% (18)	1,2% (9)	1,7% (13)	0,6% (4)	2,0% (4)
DT107	1,6% (13)	2,5% (19)	0,9% (7)	1,2% (8)	0,5% (1)
Others incl. non typable	18,4% (150)	14,5% (108)	15,7% (120)	17,6% (117)	17,5% (35)
Total slaughter pig herds	100,0% (817)	100,0% (746)	100,0% (764)	100,0% (666)	100,0% (200)
MRDT104	0,6% (5)	0,5% (4)	1,0% (8)	2,4% (16)	2,0% (4)

1) Until 1st August 2002

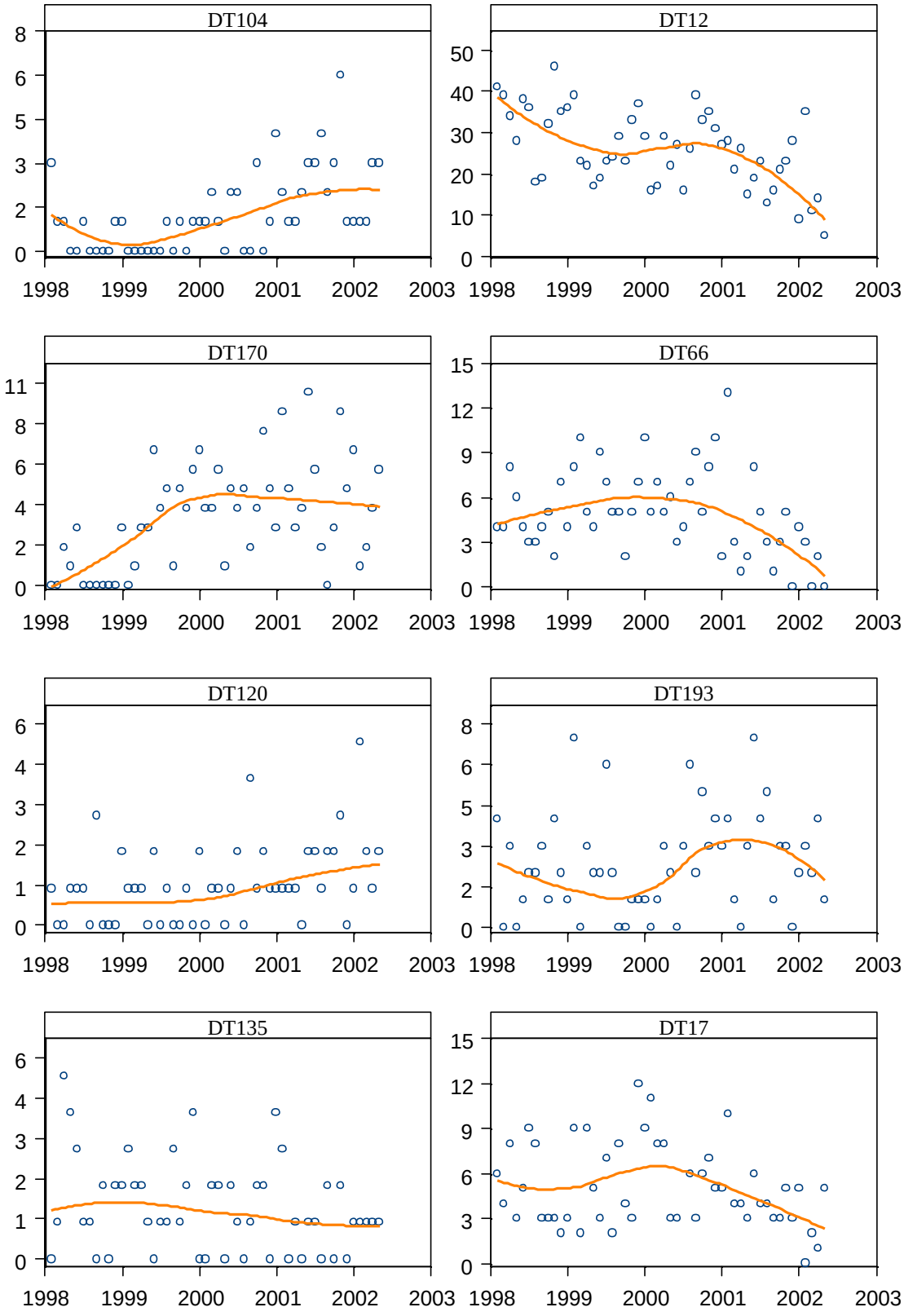

Figure 1.6 Best-fit model of the number of infected herds found per month during follow-up of serological surveillance using a second order local regression model (S-PLUS 6.1, Insightful Corp., span=75%). The circles show the number of level 2 and 3 herds found with the specific phage type each month.

1.5 Factors affecting the spread of MRDT104.

Specific risk factors for the transmission of MRDT104 between Danish herds and for the colonisation of the herds are not well known. However, it may be assumed that the risk factors applying to *Salmonella* Typhimurium in general also apply to MRDT104, even though additional factors may be of particular importance to the latter.

1.5.1 MRDT104 risk factor study

In 2000, Danske Slagterier conducted an interview study of pig herds infected with or exposed to MRDT104.

Table 1.9 Results of the univariate analysis. All factors were included in the multivariate model before reduction by backwards elimination.

Variable	Level	OR	P-value
Type of feed used for finishers	Ready-mix Prepared on farm	4.00 1	0.1420
Purchase of piglets from several sow herds	No Yes	5.62 1	0.04338
Purchase of pigs from 'pig pool'	No Yes	None of controls purchased piglets from pig pool	0.0259
Dogs present on farm	No Yes	5.00 1	0.0183
Management form	Alt in - all out Continuous	7.64 1	0.0026
Ante room available	No Yes	0.16 1	0.0156
Family members access to pigs	No Yes	2.60 1	0.1246
Lorry driver's access to pigs	No Yes	Drivers did not have access in any of the case herds	0.0118
Slurry from the herd deposited on 3rd party fields	No Yes	0.15 1	0.0052
Slurry from 3rd party farms on your land	No Yes	4.15 1	0.1126
Measures taken against vermin	No Yes	0.51 1	0.2793
Cleaning of tools	No Yes	2.14 1	0.2206
Salmonella or diarrhoea among family members (1999-2000)	No Yes	4.50 1	0.1262
Travel abroad	No Yes	0.20 1	0.0916

Included as cases were 29 herds from which MRDT104 had been isolated and 17 controls, consisting of herds with contact to a MRDT104 infected farm, but where MRDT104 had not been recovered. Case interviews took place during the second half of 2000, while interview with control farms were conducted during November 2000. The herd owners, who had received the questionnaire prior to the interview, were asked about management practices and exposures during the 12-month period prior to the first MRDT104 positive sample from their herd. The questionnaire data have been analysed in a logistic regression model with herd status (case or control) as the outcome variable. The regression used back-wards elimination from a full model that included all variables that were statistically associated with the outcome in the initial univariate analysis.

The final model included feeding as a protective factor (preparation of feed on the farm was protective) as was the presence of dogs on the farm, while all in – all out production and spreading of slurry on fields belonging to other farms were risk factors. So considering these results with those of the univariate analysis shown in Table 1.9 and relating the findings to existing knowledge about risk factors for other *Salmonella* serotypes, the study may be regarded as inconclusive.

1.5.2 Antibiotic usage and DT104

On a national level, the DANMAP surveillance has shown a correlation between the usage of specific antibiotics and the prevalence of bacterial resistance to these antibiotics in pigs. It would seem reasonable to assume that usage of antimicrobials, in particular the ones to which MRDT104 is resistant, might predispose a herd to colonisation by MRDT104, given that it is exposed. There appears to be no published scientific studies on the role of antimicrobial usage in the epidemiology of MRDT104. While the time available for this risk assessment has not permitted us to carry out a formal study, we have done a simple comparison of usage, based on information available in the VetStat database.

In order to examine the role of antibiotic usage in MRDT104 pig herds, the usage in these herds was compared to the usage in herds where trade contacts with MRDT104 herds did not result in MRDT104 infections (negative contacts), and in pig herds infected with *Salmonella* Typhimurium phage type DT17, which is rarely multi-resistant. The farms included in the comparison are all MRDT104 pig herds (n=36) infected after January 1st 2001 and before August 2nd 2002 with their associated "negative trade contacts" (n=50), as well as all pig herds found infected with DT17 during this period (n=70).

We extracted information from VetStat on the amount (in terms of mg active substance) of ampicillin, streptomycin, sulphonamide and tetracycline prescribed for each herd during a 30 and a 60-day period prior to detection of MRDT104 or DT17 (or collection of samples in the case of negative contact herds). Chloramphenicol is not used in production animals. Based on information about the herd size available in the "CHR register", the average usage per pig was estimated (Table 1.10). Due to missing data in CHR on the number of weaner pigs on the farms, the average usage of antibiotics only included antimicrobials prescribed for use in slaughter pigs and sows, even though the most intensive use of antimicrobials is in weaners.

In seven cases, the pig herds on record as having been investigated for MRDT104 or DT17 had no information about the number of pigs available in the CHR.

In 60 of the 156 herds, where animals were registered on the farm, antibiotics had been administered during the 30 days prior to detection of the infection (Table 1.10). Only 20-30% of the herds with *Salmonella* (MRDT104 or DT17) had used antibiotics, whereas 60% of herds, which had trade contact with MRDT104 herds but were not infected, had used antibiotics during the 30 days period. The mean usage in DT104 herds receiving antibiotics was lower (538 mg active substance during 30 days) than in DT17 herds (1478 mg) and herds not infected with *Salmonella* (2351 mg). This difference was not significant.

Table 1.10 Distribution of herds according to whether they had been administered antibiotics or not 30 days prior to detection of MRDT104 or DT17.

	No antibiotics	Antibiotics administered	Total
DT104	28	8	36
DT17	48	22	70
Neg. contact	20	30	50
All categories	96	60	156

Including herds, which had not received antibiotics, the average usage of antibiotics 30 days prior to detection of MRDT104 in the pig herds, was lower than the usage in herds infected with DT17, but not significantly (Table 1.11). The herds, which had trade contact with MRDT104 herds but were not infected, had a significantly higher total usage of these four antibiotics. Analysis of usage data for the 60-day period prior to collection of samples gave a similar result.

Table 1.11 Average usage in herds infected with DT104, DT17 or not infected with salmonella, including herds that had not received antibiotics during this period. Mg active substance per pig administered 30 days prior to detection of MRDT104 or DT17.

	Herds	Ampicillin	Streptomycin	Sulphonamides	Tetracycline	Total*
DT104	36	18	5	3	3	29[a]
DT17	70	45	1	3	13	62[b]
Neg. Contact	50	119	5	3	33	160[c]

* The usage per pig was significantly higher in the negative contact herds than in the herds infected with either MRDT104 or DT17. Kruscal-Wallis one-way anova on Ranks, Multiple comparisons (Dunn's method). Different superscripts indicate that the difference was statistically significant at the 5 percent level.

So, even though this simple analysis does not provide a basis for definite conclusions regarding the role of antimicrobials as a risk factor for colonisation of pig herds with MRDT104 must await formal studies that control for possible confounding effects of, for example herd size, length of exposure window and other factors, the results do not suggest that use of antimicrobials constituted a risk factor for MRDT104 infection or colonisation of these herds.

1.6 Conclusions

1. By the end of August 2002, MRDT104 has been detected in 90 pig herds, 46 farms with both pigs and cattle, 23 with cattle alone, two turkey flocks, two broiler flocks and in a herd of farmed foxes. In total, 164 herds had been reported as infected with MRDT104.
2. Since 1998, approximately 50-60% of the DT104 pig herds have been infected with a penta-resistant clone, whereas 20-30% of the pig herds were infected with a fully susceptible DT104 strain. Seemingly, the proportion of susceptible DT104 is increasing.
3. The increase in number of MRDT104 pig herds that occurred in 2000 and 2001 cannot be explained by a general increase in the number of herds infected with *S*. Typhimurium.
4. Model estimates show that the number of herds infected with DT104 (incl. non-MRDT104) found during follow-up of serological surveillance increased within the same range as other phage types such as DT120, DT170 and DT193. The rate at which herds infected with DT104 are detected is decreasing, and number of new infections reached a stable level during 2002. The model does not indicate that the number of DT104 herds found per month during follow-up of serological surveillance is decreasing.
5. According to the DVI database, 2.4% of the DT104 herds report diarrhoea, compared to 3.2% of the pig herds infected with MRDT104. Compared to other phage types found in pigs, MRDT104 does not appear to lead to relatively more clinical cases.

6. The investigation of risk factors for MRDT104 infection in pig herds did not led to any conclusive results.
7. Preliminary analysis does not suggest that use of antimicrobials constituted a risk factor for MRDT104 infection or colonisation of pig herds.

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2 ESTIMATING THE TRUE NUMBER OF PIG HERDS INFECTED WITH MULTI-RESISTANT *SALMONELLA* TYPHIMURIUM DT104

2.1 Introduction

During the 1-year period from August 1st 2001 to 31st July 2002, 33 pig herds (CHR numbers) were found infected with MRDT104. Some of them were detected as a result of the general *Salmonella* surveillance program, while others were found during the cause of compulsory trace-back from known positive herds.

Primary detection of MRDT104 infection depends on the sensitivity of the serological test – which is dependant on serotype; on the sensitivity of the serology based *Salmonella* surveillance program: a certain number of reactors are required before a herd is placed in Level 2 or 3 where bacteriological follow-up is carried out; on the sensitivity of bacteriological follow-up based on examination of pen faecal samples which is less than 100 %. The consequence, therefore, is that a proportion of herds infected with MRDT104 may go undetected.

The objective of this section is to estimate the number of Danish pig herds that are truly infected with MRDT104, compared with the number that were detected.

2.2 Estimation method used

We have used probabilistic risk analysis models as described by Vose (1996) to estimate the true number of herds infected with MRDT104. Probability distributions were used to model uncertainty and variability about the included parameters and the Monte Carlo simulation models were set up in @RISK (@Risk 4.5, Palisade Corp.). The sampling method was Latin Hypercube and 10.000 iterations per simulation were run.

The estimation was stratified on three different herd types: 1) Herds – some of them farrow-to-finish herds - producing slaughter pigs; 2) sow herds with production of weaner and grower pigs including farrow-to-finish herds with less than 7 place units for slaughter pigs per sow; and 3) breeder and multiplier herds. Data from the general *Salmonella* surveillance of pig herds in the 1-year period between August 1st, 2001 and July 31st, 2002, available at the Danish Zoonosis register (ZOOR) was used as input to the models. For slaughter pig herds, we used data available from the

ZOOR tables CS2 and CP2 (bck 0902). According to these, 926 herds had been placed in either Level 2 or 3 for at least one month during the 1-year period. Additionally, 5,912 herds were in Level 1.

The total number of herds with both sows and slaughter pigs was 5,964. These consisted of 4,257 herds likely to sell weaner or grower pigs because they had less than 7 pen places for slaughter pigs per sow, and these were included in the model as sow herds. The remaining 1,707 herds were not likely to sell piglets as they had 7 or more pen places for slaughter pigs per sow and they were included in the model as slaughter pig herds. The total number of herds with sows only was found from ZOOR (table CB2) to be 1,528 herds (sow herds were defined as sows only, artbrugs=11, 13, 17). We included among sow herds 112 herds with specialised production of grower pigs in the 7-30 kg weight range, purchased from sow herds. The sow herd group therefore consists of a total of 5,897 herds, including the 4,257 farrow-to finish herds with less than 7 pen places for slaughter pigs per sow.

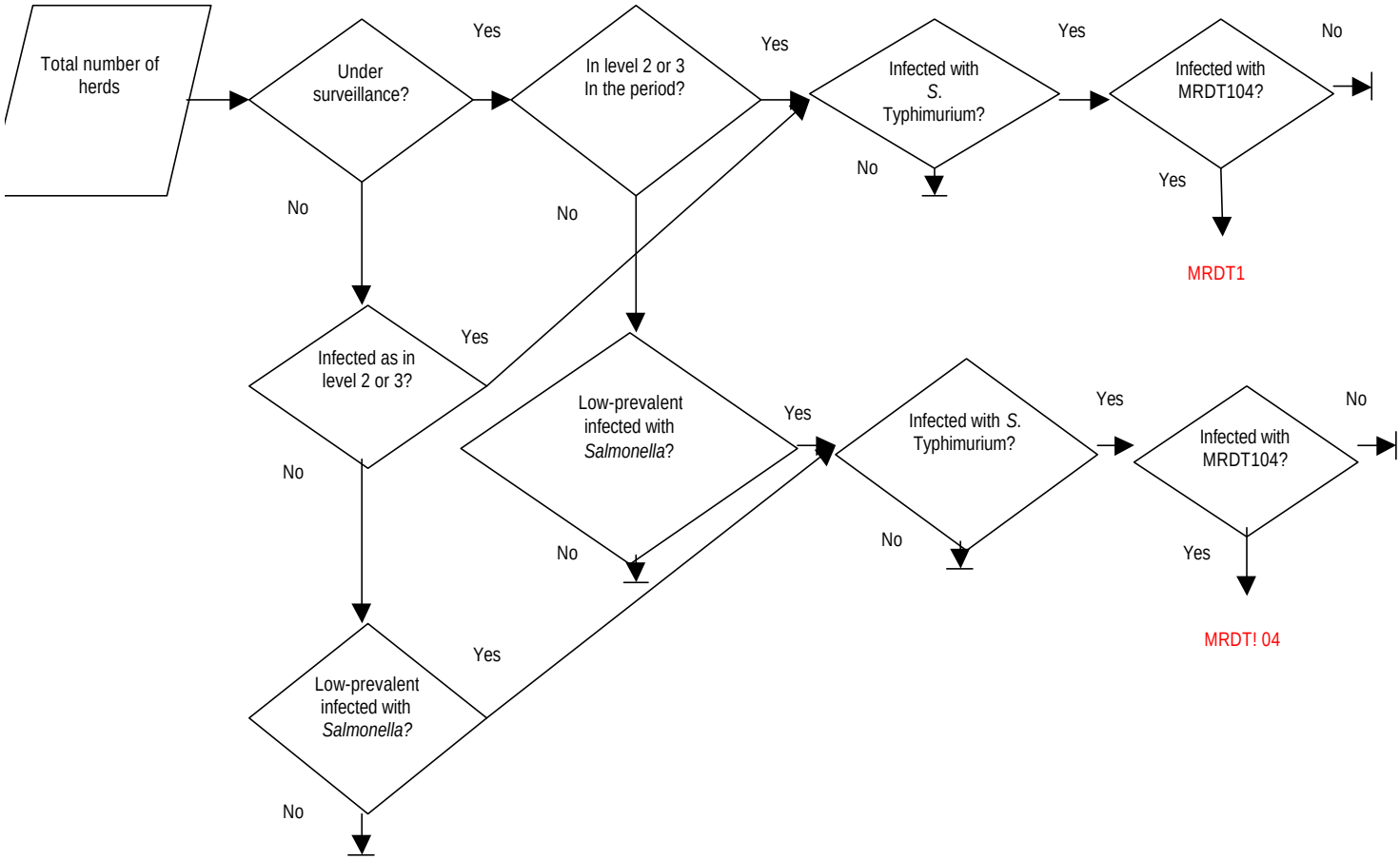
The DANAVL pig breeding company had 42 breeder and 144 multiplier herds, i.e. a total of 186 herds, distributed on 248 Central Husband Registry (CHR) numbers, while the Pig Improvement Company (PIC) had 37 breeder and multiplier herds. This gave a total of 285 CHR numbers with breeder and multiplier herds in Denmark during the 1-year period.

In all models, the unit of calculation has been herds (CHR numbers) rather than owners.

2.3 Estimation of MRDT104 infection among herds producing pigs for slaughter

The true total number of MRDT104 infected slaughter pig herds includes those placed in Levels 2 and 3 whether they have been detected as MRDT104 positive or not, and herds in Level 1 that remained undetected by the serology-based surveillance. We have also calculated estimates for the group of herds too small to be included in the surveillance programme, as these may also be infected with MRDT104. A scenario tree illustrating the construction of the model is shown in Figure 2.1.

Figure 2.1 Scenario tree illustrating the risk of detected or undetected herds with multiresistant *Salmonella* DT104 among slaughter pig herds.



2.3.1 Herds in Levels 2 and 3

Bacteriological follow-up in 926 herds in this category resulted in recovery of *Salmonella* from pen faecal samples in 598. In 512 herds, the serotype was *S.* Typhimurium and eight of the herds were positive for MRDT104. The proportion of S. Typhimurium infections among the bacteriologically positive herds was 86%. However, as the *Salmonella* Mix Elisa is more sensitive towards *S.* Typhimurium than other serotypes, this proportion would be underestimated among the bacteriologically negative herds in Levels 2 and 3. The proportion of *S.* Typhimurium could be as high as 100% and as low as among the bacteriologically positive. This was modelled using a simple uniform distribution resulting in 88% (90% CI 86%, 91%) *S.* Typhimurium positive among the bacteriologically negative herds. The proportion of *S.* Typhimurium positive herds infected with MRDT104 was

1.37% (Table 2.1.a). The model estimated that there were 4 (90% CI 1,7) undetected herds with MRDT104 among the bacteriologically negative herds.

In total, the model estimated that 11 (90% CI 8, 14) slaughter pig herds in Levels 2 and 3 were infected with MRDT104 during the 1-year period, compared with the 7 that were detected (Table 2.1.a).

2.3.2 Herds in Level 1

A proportion of herds in Level 1 only during a 1-year period are likely to be infected with *Salmonella*, even though the herd prevalence during a year is not high enough to warrant intervention measures as required in Level 2 or 3. Therefore, results of bacteriological follow-up are not available for Level 1 herds.

We defined a herd in Level 1 as infected with *Salmonella* at a low prevalence if at least one of the serological samples collected in the 1-year period had a *Salmonella* value (OD% minus 10) higher than or equal to 5 and, in addition to that, at least one serological sample with a *Salmonella* value higher than 1. We chose to base this categorisation on all individual test results for each herd, as we considered this to give a better estimate of the herd prevalence than would the *proportion* of individual samples above 5 or 1 in each herd.

We recognise that as large herds have more samples collected due to the classification scheme used (Alban et al., 2002), this procedure may underestimate the herd prevalence among small herds. However, small herds, where only one serological sample had been taken during the period, were defined as positive if the *Salmonella* value of this single sample was higher than or equal to 5. In total, 5,156 of Level 1 herds had one or more serological reactions higher than or equal to 5 as well as one or more higher than or equal to 1.

Table 2.1.a Estimating the actual number of MRDT104 positive herds producing slaughter pigs in Denmark between August 1st 2001 and July 31st 2002. Estimation concerning herds under surveillance.

Level 2 or 3 herds	Formula	Label	Number	5th perc.	50th perc.	95th perc.
Number of herds tested		a1	926			
Number of herds with *Salmonella* positive samples		b1	598			
Number of herds with *S*. Typhimurium positive samples		c1	512			
Number of MRDT104 positive samples		d1	7			
Proportion of BU positive herds	a1/b1	e1	65%			
Proportion of *S*. Typhimurium in BU positive herds	c1/b1	f1	86%			
Maximum number of possible *S*. Typhimurium infected	a1-(b1-c1)	g1	840			
Estimated maximum proportion of *S*. Typhimurium in level 2 or 3 herds	beta(g1+1,(a1-g1)+1)	h1		89%	91%	92%
Estimated proportion of *S*.Typhimurium in BU negative herds	if(h1<=f1;h1; uniform(h1,f1))	j1		86%	88%	91%
Estimated number of BU negative herds with *S*. Typhimurium*	(a1-b1)*j1	k1		282	289	298
Proportion of *S*. Typhimurium positive herds with MRDT104	d1/c1	l1	1.37%			
Estimated number of undetected MRDT104 herds among BU negative herds*	binomial(k1,l1)	m1		1	4	7
Estimated total number MRDT104 herd in level 2 or 3*	**d1+m1**	**n1**		**8**	**11**	**14**
Level 1 herds						
Number of herds in level 1 only		a2	6888			
Number of herds with low grade *Salmonella* infection, 1 *Salm.* value >=5+1 *Salm.* Value >1		b2	5156			
Number of *Salmonella* positive samples found in swab samples among level 1 herds		c2	120			
Number of *S*. Typhimurium positive samples found in swab samples among level 1 herds		d2	36			
Number of MRDT104 positive samples found in swab samples among level 1 herds		e2	0			
Number of MRDT104 positive herds found due to trace-back		f2	15			
Estimated proportion of *Salmonella* positive herds with *S*. Typhimurium	beta(d2+1,(c2-d2)+1)	g2		24%	30%	37%
Estimated number of *S*. Typhimurium positive herds*	g2*b2	h2		1222	1558	1925
Estimated proportion of *S*. Typhimurium positive herds with MRDT104	beta(e2+1,(d2-e2)+1)	i2		0.1%	1.9%	7.8%
Estimated number of undetected MRDT104 herds in level 1*	if((h2*i2)-f2<0;0; (h2*i2)-f2)	j2		0	14	106
Estimated total number of MRDT104 herds in level 1*	**f2+j2**	**k2**		**15**	**29**	**121**

* Rounded estimated number of herds.

From Screening (1998): Proportion bacteriologically positive among herds with 1 salm>=5+1 salm>1

Table 2.1.b Estimating the actual number of MRDT104 positive herds producing slaughter pigs in Denmark between August 1st 2001 and July 31st 2002. Estimation concerning herds outside surveillance.

Herds not under surveillance	**Formula**	**Label**	**Number**	**5th perc.**	**50th perc.**	**95th perc.**
Number of herds not under surveillance		a3	1881			
Number of MRDT104 positive herds found due to trace-back		b3	1			
Number of herds delivering 200-1000 pigs (small herds under surveillance)		c3	2531			
Number of *Salmonella* infected small herds under surveillance, 1 S*alm.* value>=5+1 *Salm.* value>1		d3	1591			
Number of herds in level 2 or 3 among small herds under surveillance		e3	165			
Proportion of *Salmonella* among small herds under surveillance	d3/c3	f3	63%			
Estimated number of *Salmonella* infected herds not under surveillance*	binomial(a3,f3)	g3		1148	1182	1217
Proportion of herds in level 2 or 3 among small herds under surveillance	e3/c3	h3	7%			
Estimated number of herds not under surveillance infected as in level 2 or 3*	binomial(a3,h3)	i3		105	123	140
Estimated number BU positive among herds infected as in level 2 or 3*	i3*e1	j3		68	79	90
Estimated number BU negative among herds infected as in level 2 or 3*	i3-j3	k3		37	44	50
Estimated number *S*. Typhimurium positive herds infected as in level 2 or 3*	(j3*f1)+(k3*j1)	l3		91	106	122
Estimated number MRDT104 herds infected as in level 2 or 3*	l3*l1	m3		1	2	2
Estimated number of herds not under surveillance infected as in level 1*	g3-i3	n3		1021	1060	1098
Estimated number *S*. Typhimurium positive herds infected as in level 1*	n3*g2	o3		250	320	396
Estimated number MRDT104 herds infected as in level 1*	o3*i2	p3		0	6	25
Estimated number of undetected MRDT104 herds not under surveillance*	if(m3+(p3-b3)<0;0; m3+(p3-b3))	q3		1	7	26
Estimated total number of MRDT104 herds not under surveillance*	**b3+q3**	**r3**		**2**	**8**	**27**
*Estimated number of slaughter pig herds infected with MRDT104**	*n1+k2+r3*			*27*	*47*	*162*

* Rounded estimated number of herds

The proportion of these infected with *S.* Typhimurium was estimated using data from the surveillance of *Salmonella* in pork in slaughterhouses (swab sampling of carcasses) and the uncertainty around the estimate modelled using a beta distribution (Table 2.1.a). However, as swab samples are taken from carcasses from Level 2 and some Level 3 herds as well as from level 1 herds, the proportion of samples from Level 1 carcasses was estimated using the figures from the 1998 screening of Danish pig herds. In total, 39% of *Salmonella* positive swab samples and 28% of *S.* Typhimurium positive swab samples was estimated to originate from herds infected at a low prevalence as defined by the criteria above. The corrected number of positive swab samples estimate that 30% (90% CI 24%, 37%) of the low prevalent infected herds was infected with *S.* Typhimurium, corresponding to a figure of 1,558 (90% CI 1,222, 1,925) herds in Level 1 truly infected with *S.* Typhimurium (Table 2.1.a).

Among the 36 *S.* Typhimurium positive swab samples from Level 1 herds, no MRDT104 positive samples were found during the observation period. However, modelling the proportion of *S.*

Typhimurium positive infected with MRDT104 from these figures using a beta distribution we found the most likely estimate to be 1.9% (90% CI 0.1%, 7.8%), reflecting the uncertainty caused by the small number of samples taken. The model estimates that a total of 29 Level 1 herds (90% CI 15, 121) were infected with MRDT104 (Table 2.1.a). In comparison, 14 Level 1 herds were found due to the mandatory MRDT104 trace-back procedure and one following detection of MRDT104 in a diagnostic submission.

2.3.3 Herds not under surveillance

The pig herds not under surveillance were defined as herds producing less than 200 slaughter pigs per year. 1,881 herds met this criterion and therefore have no samples taken. However, there are likely to be additional herds without surveillance and we estimate the total in this category to be around 2000 herds.

We assumed that the prevalence of *Salmonella* in the herds not under surveillance is the same as the prevalence in the small herds producing between 200 and 1000 slaughter pigs per year. The latter group included 2,531 herds and 1,591 or 63% of these were *Salmonella* seropositive by the criteria used above. In total, the number of herds not under surveillance but infected with *Salmonella* was then estimated to be 1,182 (90% CI 1,148, 1,217; Table 2.1.b).

For the estimation of the proportion of *S.* Typhimurium infected among *Salmonella* positive herds, outside surveillance, we assumed a serotype distribution similar to that found among herds under surveillance. Therefore, the herds were divided into a number of herds infected at the same level as in Level 2 and 3 herds and a number of herds with low prevalence or no infection as for herds in Level 1. Accordingly, the proportion of herds infected similar to herds in Levels 2 or 3 were 7 %, or a total of 123 (90% CI 105, 140) herds. It was assumed that the proportion of *S.* Typhimurium among the herds infected similar to level 2 or 3 as well as the proportion of MRDT104 among the *S.* Typhimurium herds was the same as among herds in Level 2 and 3. This gave a total of 2 (90% CI 1, 2) herds infected with MRDT104 (Table 2.1.b).

The rest of the herds were estimated to be infected, similar to the herds in Level 1 (1,060 herds, 90% CI 1,021, 1,098). The proportion of these infected with *S.* Typhimurium was estimated using the serotype distribution from swab sampling of carcasses (Table 2.1.a). This gave an estimated total of 6 (90% CI 0, 25) herds infected with MRDT104. Altogether, we estimated that 8 (90% CI 2,

27) of the herds not included in the surveillance were infected with MRDT104. In comparison, 1 herd not under surveillance were found due to the mandatory MRDT104 trace-back procedure.

In total, we estimated that 47 (90% CI 27, 162) herds producing pigs for slaughter were infected with MRDT104.

2.4 Herds with production of grower pigs

The number of truly MRDT104 infected sow herds includes those detected through follow-up from known positive slaughter pig herds, i.e. herds in Levels 2 or 3, those undetected among herds supplying Level 2 and 3 herds, as well as those also undetected among sow herds supplying growers to Level 1 herds and to herds outside the general *Salmonella* surveillance programme. A scenario tree illustrating the construction of the model is shown in Figure 2.2.

2.4.1 Sow herds supplying Level 2 or 3 herds

During the 1-year period, 476 sow herds were examined through routine follow-up from slaughter pig herds placed in Levels 2 or 3. *Salmonella* positive pen-faecal samples were found in 233 of them. Among these, 164 were found infected with *S.* Typhimurium, four of them with MRDT104. Not all sow herds supplying weaners or growers to herds in Level 2 or 3 are considered infected with *Salmonella*. Assuming that the sensitivity of bacteriological investigation is the same among slaughter pig herds as among sow herds, the bacteriological sensitivity from slaughter pig herds, 65%, was used to estimate the number of salmonella positive sow herds, resulting in a most likely value of 360 (90% CI 338, 385). The proportion of bacteriologically positive herds infected with *S.* Typhimurium was 70%. However, as for slaughter pig herds this proportion is likely to be under-estimated among the bacteriologically negative herds. These herds could be infected up to 100% with *S.* Typhimurium or as little as among the bacteriologically positive herds. This was modelled using a uniform distribution. The proportion of *S.* Typhimurium infected herds with MRDT104 was

Table 2.2.a Estimating the actual number of sow herds infected with MRDT104

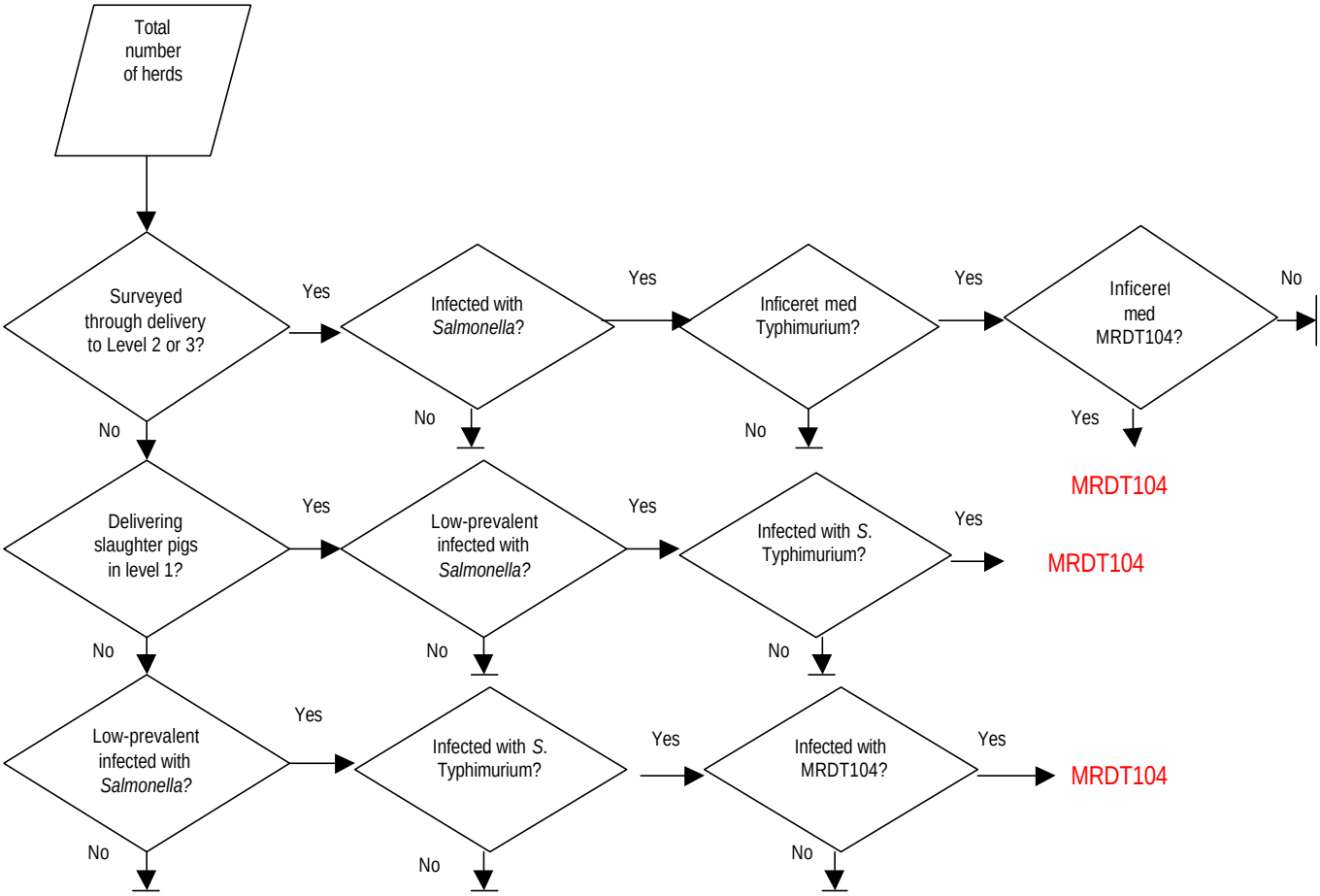
Sow herds supplying weaners or growers to herds in level 2 or 3	**Formula**	**Label**	**Number**	**5th perc.**	**50th perc.**	**95th perc.**
Number of sow herds examined through follow-up		a1	476			
Number of sow herds examined through follow-up found Salmonella positive		b1	233			
Number of sow herds examined through follow-up *S* . Typhimurium positive		c1	164			
Number of sow herds examined through follow-up positive for MRDT104		d1	4			
Proportion of BU positive among tested slaughter pig herds	Fig. 2.1	e1	65%			
Estimated number of Salmonella infected herds*	b1+negbin(b1,e1)	f1		338	360	38
Proportion of Salmonella positive herds infected with *S* . Typhimurium	c1/b1	g1	70%			
Maximum number of possible *S* . Typhimurium infected*	a1-(b1-c1)	h1	407			
Estimated maximum proportion of *S* . Typhimurium in level 2 or 3 herds	beta(h1+1,(a1-h1)+1)	i1		83%	85%	88%
Estimated proportion of *S*. Typhimurium in BU negative Herds	if(i1<=g1;i1; uniform(i1,g1))	j1		71%	78%	85%
Estimated number of BU negative herds with *S* . Typhimurium*	(a1-b1)*j1	k1		173	189	20
Proportion of *S* . Typhimurium positive herds with MRDT104	d1/c1	l1	3%			
Estimated number of undetected MRDT104 herds among BU negative herds*	binomial(k1,l1)	m1		2	6	1
Estimated total number MRDT104 herd in level 2 or 3*	**d1+m1**	**n1**		**7**	**11**	**1**
Sow herds delivering slaughter pigs in level 1						
Number of herds in level 1 only		a2	2698			
Number of herds with low grade Salmonella infection, 1 salm. value >=5+1 salm. Value >1		b2	2020			
Number of Salmonella positive samples found in swab samples among level 1 herds		c2	120			
Number of *S* . Typhimurium positive samples found in swab samples among level 1 herds		d2	36			
Number of MRDT104 positive samples found in swab samples among level 1 herds		e2	0			
Number of MRDT104 positive herds found due to trace-back		f2	2			
Estimated proportion of Salmonella positive herds with *S* . Typhimurium	beta(d2+1,(c2-d2)+1)	g2		24%	30%	37%
Estimated number of *S* . Typhimurium positive herds*	g2*b2	h2		479	610	75
Estimated proportion of *S*. Typhimurium positive herds with MRDT104	beta(e2+1,(d2-e2)+1)	i2		0.1%	1.9%	7.8%
Estimated number of undetected MRDT104 herds in level 1*	if((h2*i2)-f2<0;0; (h2-i2)-f2)	j2		0	9	4
Estimated total number of MRDT104 herds in level 1*	**f2+j2**	**k2**		**2**	**11**	**4**
Sow herds supplying weaners or growers to herds in level 1 or to herds outside surveillance						
Number of sow herds not examined		a3	2723			
Estimated number of herds with *S* . Typhimurium not examined*	Fig. 2.2.b	b3		212	391	63
Proportion of S. Typhimurium positive herds with MRDT104	beta(d1+1,(c1-d1)+1)	c3		1.2%	2.8%	5.5%
Estimated number of undetected MRDT104 herds*	**c3*b3**	**d3**		**5**	**10**	**1**
*Total number of sow herds infected with MRDT104**	*n1+k2+d3*			*22*	*35*	*7*

* Rounded estimated number of herds.

Table 2.2.b Estimating the number of herds infected with S. Typhimurium among sow herds supplying weaners or growers to herds in level 1 or outside surveillance

	Formula	Label	Number
Survey by Kranker et al. (2001)			
Number of herds in sample			69
Number of herds delivering to level 1 or outside surveillance		n	52
Number of herds low-prevalent infected with *Salmonella*			41
Number of low-prevalent herds infected with *S*. Typhimurium		x	7
Sow herds supplying weaners or growers to herds in level 1 or outside surveillance			
Number of sow herds not examined		N	2723
Number of sow herds not examined with *S*. Typhimurium		D	7 to 2723
Estimated proportion of sow herds infected with *S*. Typhimurium	hypgeo(x,n,D,M)	p	
Estimated number of sow herds with *S*. Typhimurium	discrete(D,p)		Table 2.2.a.

Figure 2.2 Scenario tree illustrating the risk of detected or undetected herds with multiresistant *Salmonella* DT104 among sow herds delivering weaners or growers.



3%, Table 2.2.a. Under this assumption, the number of undetected MRDT104 infected sow herds selling weaners or growers to Level 2 or 3 herds was estimated to be 6 (90% CI 2, 10), and the total number of herds 11 (90% CI 7, 15).

2.4.2 Sow herds with slaughter pigs in Level 1

In total, 2,698 farrow-to-finish herds were surveilled by the serology based *Salmonella* surveillance program and placed in level 1. Of these, 2.020 herds were estimated to be infected at a low level by the criteria as described for slaughter pig herds. The proportion of these infected with *S.* Typhimurium and MRDT104 was modelled using data from the surveillance of *Salmonella* in pork in slaughterhouses in the same way as for the slaughter pig herds in level 1. This gave an estimate of 11 (90%CI 2, 48) farrow-to-finish herds in level 1 infected with MRDT104 (Table 2.2.a). In contrast, in this group 2 MRDT104 positive herds were found due to trace-back.

2.4.3 Sow herds supplying Level 1 herds or herds not under surveillance

In total, 1,489 herds of the 1,640 herds with weaner or grower pig production only were not under *Salmonella* surveillance, because they only supplied piglets to slaughter pig herds in Level 1 or to herds not included in the surveillance program. Furthermore, 1,234 farrow-to-finish herds, with a slaughter pig production less than 200 per year as well as less than 7 pen places for slaughter pigs per sow were included in this group, giving a total of 2,723 sow herds not under surveillance. In a survey of 69 Danish sow herds (Kranker et al., 2001), 52 sow herds were supplying Level 1 herds or herds not under surveillance. Forty-one of these herds had 1 serological reaction higher than or equal to 5 as well as one serological reaction higher than 1. We have assumed that a herd with this level of sero-reaction is truly infected with *Salmonella*, although at a low level. In total, 7 of the 41 sow herds from the survey (Kranker et al., 2001) were infected with *S.* Typhimurium. The number of low-prevalent herds infected with *S.* Typhimurium was estimated from these figures using a hypergeometric distribution (Table 2.2.b). On this basis, 267 (90% CI 145, 432) of this group of sow herds were infected with *S.* Typhimurium (Table 2.2.a.).

The probability that a low-prevalent *S,* Typhimurium positive herd was infected with MRDT104 was assumed to be the same as among the herds delivering to level 2 or 3, resulting in an estimate that 10 (90% CI 5, 16) sow herds were infected with MRDT104.

In total, we estimated that 35 (90% CI 22, 71) herds with sows or grower pigs were infected with MRDT104.

2.5 Breeder and multiplier herds

A scenario tree illustrating the construction of the model for breeder and multiplier herds is shown in figure 2.3. We assumed that all breeder and multiplier herds that are required to collect pen faecal samples due to the surveillance program were infected with *Salmonella*, even though the bacteriological examination did not find positive samples. Some breeder and multiplier herds have a production of slaughter pigs and may be surveyed by the general program, so these herds may be included in both models. The breeder and multiplier herds are surveyed in a more restrictive manner than the slaughter pig herds. Ten blood samples are taken from each herd each month from animals 4 to 7 months old, and serology results are reported as S*almonella* values. An index is calculated from the average S*almonella* values from the three preceding months weighted 10:30:60, and the herds are assigned to take pen faecal samples if this index is higher than 5. Consequently, breeder and multiplier herds have a higher chance of being bacteriologically examined. In total, 42 herds had taken pen faecal samples, 29 were *Salmonella* positive, 22 herds were *S*. Typhimurium positive and 3 herds were found infected with MRDT104 during the observation period. Of these, 2 of the herds had the same owner but separate CHR numbers.

The probability that a herd assigned to take pen faecal samples was infected with *S*. Typhimurium was estimated in the same way as for slaughter pig and sow herds, assuming that the proportion of *S*. Typhimurium was higher among the bacteriologically negative (but seropositive) than among the bacteriologically positive herds. In total, 11 (90% CI 11, 12) herds among the bacteriologically negative herds were estimated infected with *S*. Typhimurium using this approach. The probability that a herd found infected with *S.* Typhimurium from pen-faecal samples was positive for MRDT104 was 14% (Table 2.3). This gave a total of 1 (90% CI 0, 4) undetected herd with MRDT104 among the herds assigned to take pen faecal samples.

Ninety-six herds with at least one salmonella value higher than or equal to 5 as well as at least one salmonella value higher than 1 in blood samples were defined as herds with low prevalence of infection. The proportion of these herds infected with *S*. Typhimurium was estimated from the swab

samples of fresh meat at the slaughterhouse excluding Level 2 and 3 herds as described for slaughter pig herds in level 1 resulting in a total of 29 (90% CI 23, 36) herds with *S*. Typhimurium. Using the results from the swab samples we estimate that 1 (90% CI 0, 2) low-prevalent infected herds was infected with MRDT104.

In total, it was estimated that 5 (90% CI 3, 8) breeder and multiplier herds were infected with MRDT104.

Figure 2.3 Scenario tree illustrating the risk of detected or undetected herds with multiresistant *Salmonella* DT104 among breeder and multiplier herds.

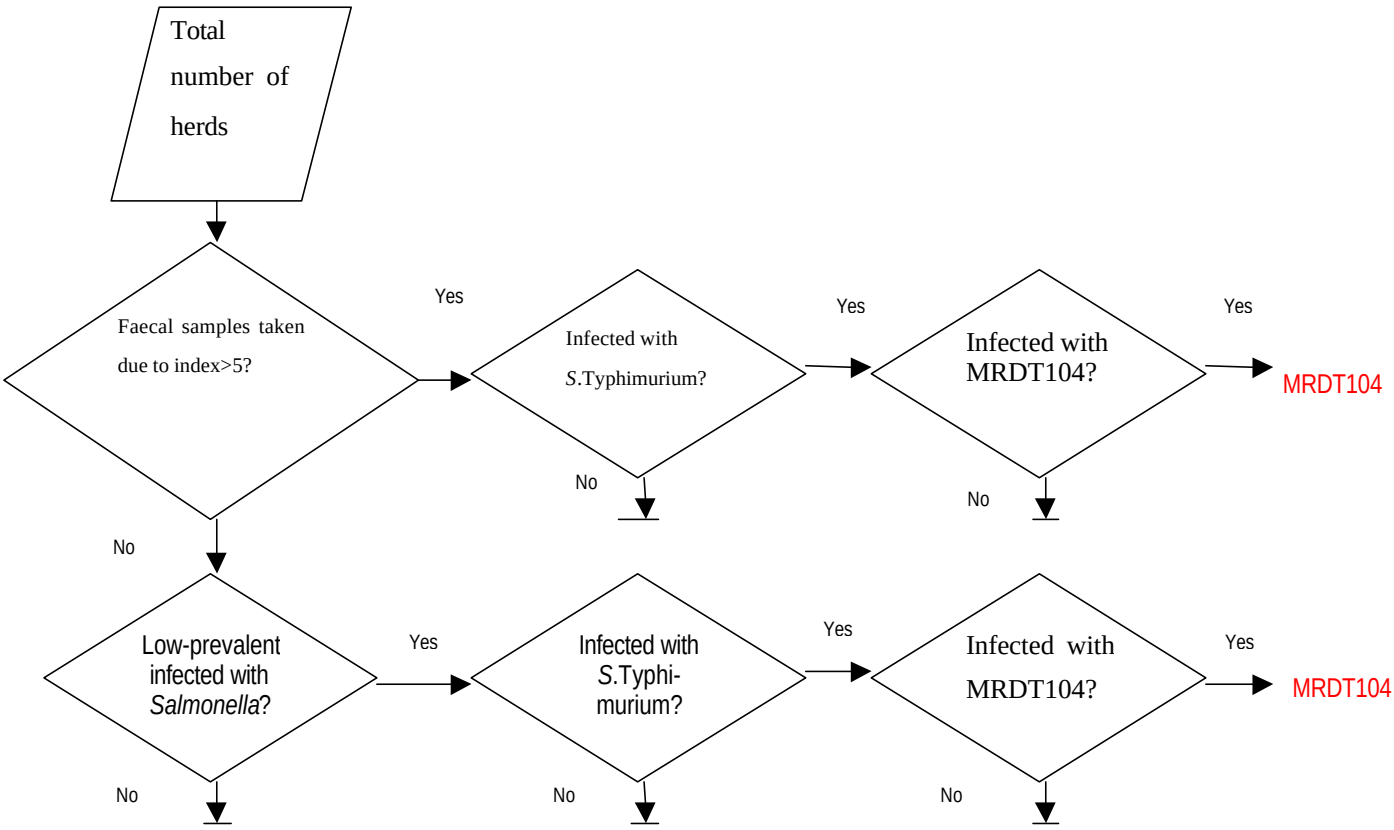


Table 2.3 Estimating the actual number of breeder and multiplier herds infected with MRDT104.

Herds taking pen faecal samples	**Formula**	**Label**	**Number**	**5th perc.**	**50th perc.**	**95th perc.**
Number of breeder and multiplier herds		a1	285			
Number of herds taking pen faecal samples		b1	43			
Number of herds with *Salmonella* positive samples		c1	29			
Number of herds with *S*. Typhimurium		d1	22			
Number of herds with MRDT104		e1	3			
Proportion of *S*. Typhimurium in BU positive herds	d1/c1	f1	76%			
Maximum number of possible *S*. Typhimurium infected	b1-(c1-d1)	g1	36			
Maximum proportion of *S*. Typhimurium in herds taking pen faecal samples	g1/b1	h1	84%			
Estimated proportion of *S*. Typhimurium in BU negative herds	if(h1<=f1;f1; uniform(f1,h1))	i1		76%	80%	83%
Estimated number of BU negative herds with *S*. Typhimurium*	(b1-c1)*i1	j1		11	11	12
Proportion of *S*. Typhimurium also infected with MRDT104	e1/d1	k1	14%			
Estimated number of undetected MRDT104 herds*	binomial(j1,k1)	l1		0	1	4
Number of herds obliged to take pen faecal samples with MRDT104*	**e1+l1**	**m1**		**3**	**4**	**7**
Low prevalent infected herds						
Herds not taking pen faecal samples		a2	242			
Number of herds low prevalent infected (1 *Salm.* value>1+1 *Salm.* value>=5)		b2	96			
Number of *Salmonella* positive samples found in swab samples among level 1 herds		c2	120			
Number of *S*. Typhimurium positive samples found in swab samples among level 1 herds		d2	36			
Number of MRDT104 positive samples found in swab samples among level 1 herds		e2	0			
Estimated proportion of *Salmonella* positive herds with *S*. Typhimurium in swab samples	beta(d2+1,(c2-d2)+1)	f2		24%	30%	37%
Estimated number of low prevalent infected with *S*. Typhimurium*	f2*b2	g2		23	29	36
Estimated proportion of MRDT104 among *S*. Typhimurium level 1 herds	beta(e2+1,(d2-e2)+1)	h2		0.1%	1.9%	7.8%
Estimated number of low prevalent herds with MRDT104*	**g2*h2**	**i2**		**0**	**1**	**2**
*Total estimated number of herds with MRDT104**				*3*	*5*	*8*

*Rounded estimated number of herds.

2.6 Conclusion

Table 2.4 summarises the results of the modelling. In total, we estimate that 97 pig herds were truly infected with MRDT104 during the year between August 1st 2001 and July 31st, 2002 (83, if based on addition of individual model outputs after rounding). However, the true number could be as low as 62 herds or as high as 212 (90 % confidence interval). In comparison, 33 (34 %) were actually detected by the general *Salmonella* surveillance programme and the trace-back procedures applying in cases of MRDT104 infection. The estimate, however, is likely to be underestimated due to a number of herds not under surveillance not included in this model.

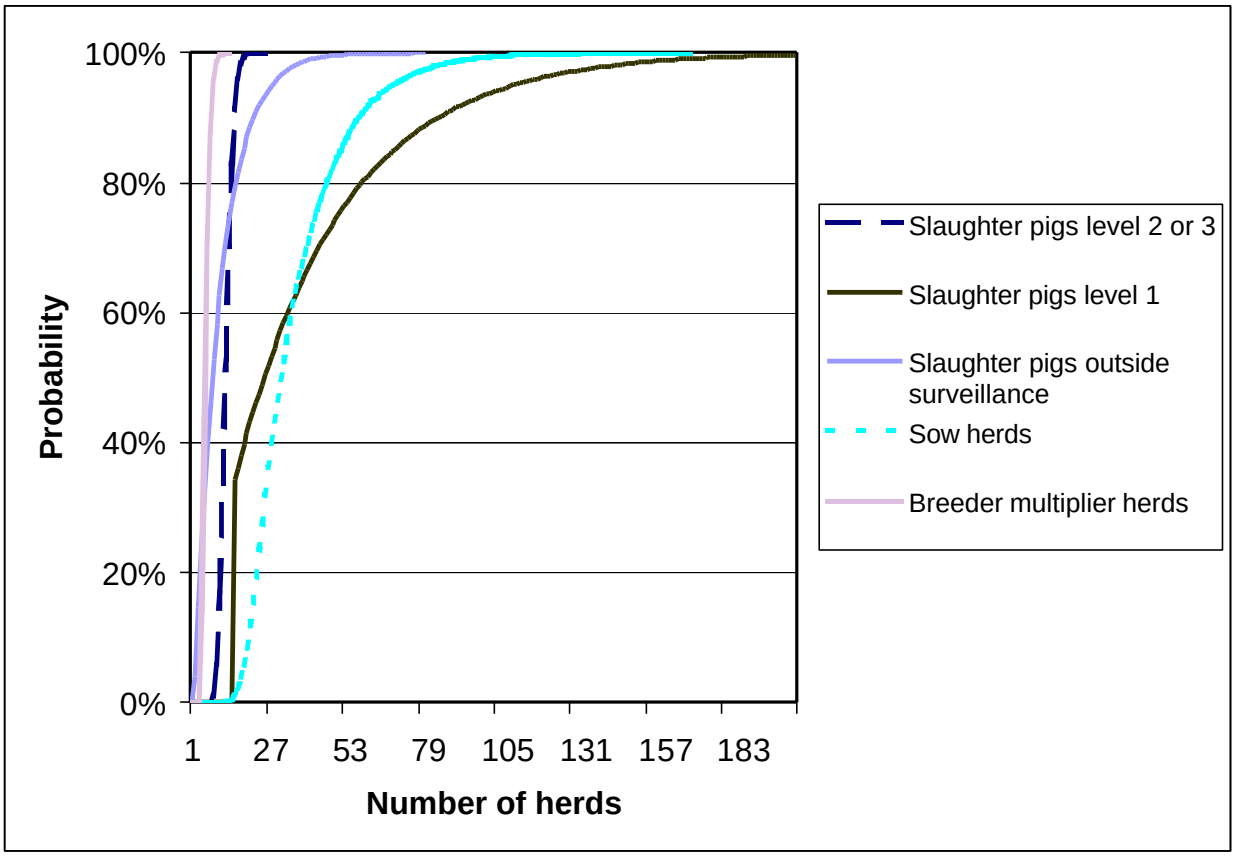
Table 2.4 The estimated number of pig herds infected with multi-resistant *Salmonella* Typhimurium DT104 between August 1st 2001 and July 31st, 2002.

Herd category	Number of herds	Pig herds positive for MRDT104				
		Actually detected	5th percentile	50th percentile	95th percentile	% detected of 50th percentile
Herds producing slaughter pigs						
Level 2 and 3 herds	926	7	8	11	14	63
Level 1 herds	6,888	15	15	29	121	52
Herds outside surveillance	1,881	1	2	8	27	13
Sow herds producing weaners or growers						
Herds supplying Level 2 or 3 herds	476	5	7	11	15	45
Herds delivering slaughter pigs in level 1	2,698	2	2	11	48	18
Herds supplying Level 1 herds or herds outside surveillance	2,723	0	5	10	16	0
Breeder and multiplier herds						
Herds taking pen faecal samples	43	3	3	4	7	75
Low prevalent infected herds	242	0	0	1	2	0
All herd categories	15,877	33	62*	97*	212*	34

* Figures resulting from running all steps of the model together.

The results show that among the slaughter pig herds in Level 2 and 3, the sow herds supplying Level 2 or 3 and the breeder and multiplier herds taking pen faecal samples a rather large proportion of the truly infected MRDT104 herds are actually detected. As all herds in these groups are bacteriologically tested, the herds not detected could mainly be assigned to the poor sensitivity of the bacteriological test. Among the slaughter pig herds in Level 1 the proportion of truly MRDT104 infected herds not found could mainly be assigned to the cut-off level of the Salmonella surveillance program that results in low-prevalent infected herds assigned to Level 1 going undetected. Some slaughter pig herds in Level 1, however, has a chance of being found due to trace-back from the sow herds. Sow herds delivering slaughter pigs in Level 1, however, will not by weaners or growers from other herds and consequently a large proportion of these herds will go undetected. Finally, among the herds outside surveillance a very little proportion of the truly infected herds will be detected, because the herds are not tested. In table 2.4 is shown the cumulated probability curves for the number of herds truly infected with MRDT104 among slaughter pigs in Level 2 or 3, Level1, outside surveillance, sow herds and breeder and multiplier herds.

Figure 2.4 The cumulated probability curves for the number of herds truly infected with MRDT104 in five categories



2.7 References

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MODELLING THE SPREAD OF MRDT104 IN DANISH PIG HERDS THROUGH TRADE CONTACTS – EFFECT OF PROPOSED CHANGES IN THE PRIMARY PIG PRODUCTION

3.1 Introduction

Danske Slagterier (DS) has presented a set of recommendations for the future handling of MRDT104 infected pig herds (see section *ii*). DS suggest focusing the MRDT104 management effort in the slaughter pig producing herds, where fewer restrictions for handling in the primary production should be compensated by extending the number of herds send to hot water decontamination after slaughter.

The objective of this part of the assessment was to develop a risk model describing the transmission of MRDT104 through trade contacts between pig herds, and to estimate the effect of the proposed changes in restrictions on MRDT104 infected herds.

3.2 Estimation method used

3.2.1 General description of the model

The model was programmed in S-PLUS (Version 6.1, Insightful Corp.). We used Monte Carlo simulation to estimate the number of pig herds infected with MRDT104 expected per year if current practices of handling the infected herds are maintained and if the proposed changes are implemented. We also estimate the approximate number of years it will take before the full effect of the proposed changes has been reached.

We have modelled how the infection is transmitted from breeder and multiplier herds via sow herds to the slaughter pig herds (Figure 3.1). For each breeder or multiplier herd (B/M) and sow herds in contact with it, a number of trade contacts is assigned, and the number of MRDT104 infections is simulated. Two versions of the model were run. The *restricted trade model* simulating the current situation and the *continued trade model* simulating the changes proposed by DS. Each simulation runs for 60 months (500 iterations). The output is distributions describing the expected number of

B/M herds, sow herds, Levels 1, 2, 3 herds as well as number of herds not under surveillance infected with MRDT104 after each 12 months period.

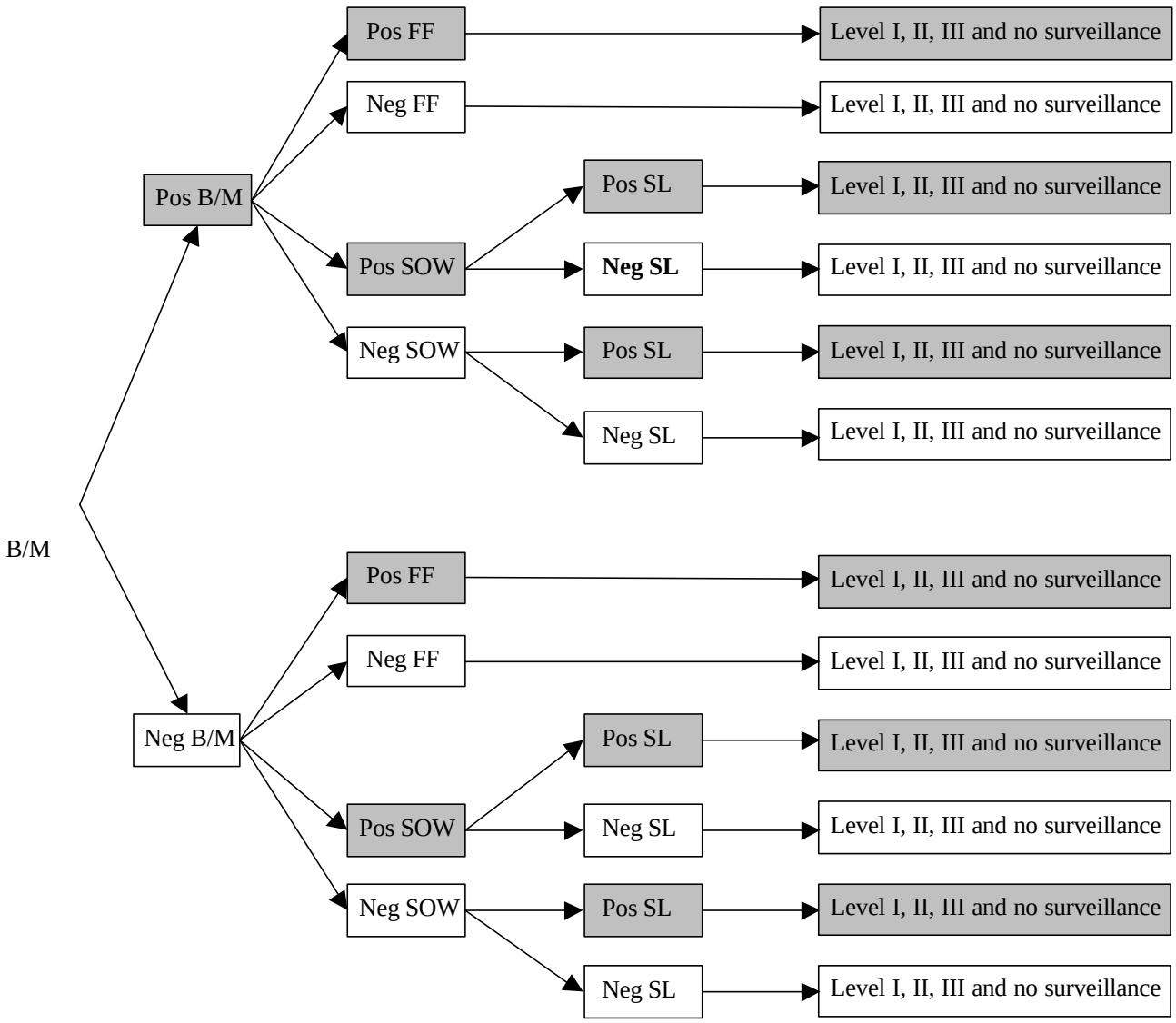


Figure 3.1 Risk assessment model for MRDT104 infections in the primary pig production. Abbreviations: B/M - Breeder and multiplier herds; SOW - Sow herds; FF - Farrow-to-finisher herds; SL- Slaughter pigs only.

Under the current plan, trade is restricted if B/M or sow herds are found infected. This is modelled by allowing contacts in the month where an infection is assigned, and by assigning a number of infected contacts from the previous two months. During the following months, where the herd is

assumed infected, no further contacts will be assigned. DS suggest that trade should continue as normal in case of infection with MRDT104 – this is modelled by allowing contacts during the full period while the infection is assumed to be present (approximately 7 months).

Effects of general changes in the pig industry, for example the expected reduction in numbers of pig herds and their increase in size have not been included in the model. This means that we assume that the distributions describing the number of contacts and the probability of infection per contact remains unchanged during the period of the simulation. However, we recognise that the future buyers of known MRDT104 piglets could be herds that were better protected against a Salmonella infection, i.e. a larger fraction of herds using home-mixed feeds. The 60 month simulation period is not to be interpreted as a 5 year prediction, only as an indicator of the number of year it will take until the level predicted by the *continued trade model* has been reached.

The models are run in two scenarios concerning the relationship between detected and undetected MRDT104 herds.

In scenario I, we assume that the spread of MRDT104 between undetected herds is unknown. But we assume that the proportion of undetected MRDT104 herds, as estimated in section 2 are constant for all years in the simulations, and that the additional number of MRDT104 herds arising from the free trade have the same proportional number of undetected herds as all other detected herds. This is modelled by multiplying the number of detected MRDT104 herds per year by a correction factor describing the proportion of detected herds, in order to attain the estimated ratio between detected and undetected MRDT104 herds.

In scenario II we assume that the rate at which the infection spreads between undetected herd is equal to the rate among detected herds, but that the risk of an undetected MRDT104 breeder and multiplier herds is relatively lower, as estimated in section 2. We also assume that the additional number of MRDT104 herds arising from the free trade leads to no more undetected herds. As the undetected herds have no trade restrictions, the number of undetected herds under the current strategy can be simulated using the *free trade model.* Thus in this scenario the undetected herds will be simulated separately from the detected herds, and independent of the trade restrictions. As the number of MRDT104 herds infected per year represent a small fraction of the total number of

herds, we assume the distributions describing the number of detected herds and the number of undetected herds are additive, even though the distributions are modelled separately.

3.2.2 Available data

The number of breeder or multiplier herds (223 B/M herds, registered under 285 CHR numbers), sow herds (5,897 herds selling weaner and grower pigs), farrow-to-finisher herds (2,319 herds with sows producing slaughter pigs) and the number of slaughter pig herds (7,376 herds with slaughter pigs only), were found in section 2 (table 3.3).

SPF Selskabet provided data on all registered sales of premature gilts, gilts, boars and sows from breeder and multiplier herds (B/M). In total, 221 B/M herds had registered sales in the database in 2001. A distribution of the total number of herds each B/M had contact with during this period is presented in Table 3.1. In the models, B/M herds will for each 12-month period have assigned a number of contact-herds corresponding to this distribution. A proportion (25%) of these contacts is reassigned after each 12-month period. We also assumed that sow herds and farrow-to-finisher herds on average have the same number of contacts to the B/M, therefore the number of contacts was assigned proportionately to the number of herds. So for each B/M herd, 75% of the contacts were assigned to sow herds selling weaners or growers and 25% to farrow-to-finisher herds.

Table 3.1 Probability and distribution of number of trade contacts for breeder and multiplier herds. The width of the ranges has been determined by grouping according to probabilities. Calculations based on data from SPF Selskabet 2001.

Contact-herds per year (range)	B/M herds included	Probability (%)
1 – 12	74	33.5
13 – 16	48	21.7
17 – 24	47	21.3
25 – 39	28	12.7
40 – 90	16	7.2
91- 180	7	3.2
181 – 290	0	0
291 – 309	1	0.5
Total	221	100%

Proportion of contacts changed per year, pert(20,25,30) = 25% (90% CI 22%, 28%)

Table 3.2 Probability and distribution of number contacts per breeder and multiplier herd during 90 days (a) (Calculations based on data from SPF Selskabet 2001), and number of contacts per sow herd during a month (b) (Calculations based on data from Anette Boklund, Danske Slagterier).

a) B/M herds during 90 days

• ***Number of contacts***	Probability
0 contacts	55%
1 contacts	26%
2 contacts	12%
3 to 15 contacts	7%

b) SOW herds per month

Number of contacts	Probability
0 contacts	27%
1 contacts	30%
2 contacts	15%
3 contacts	12%
4 contacts	6%
5 contacts	5%
6 contacts	3%
7 to 12 contacts	0.3%

We assume that infection occurred three months prior to detection, therefore a distribution describing the number of SOW contacts per B/M during 90 days was generated form the SPF data-set (Table 3.2a). Data from a Ph.D. project on the spread of swine fewer (Anette Boklund, Danske Slagterier), were used to generate a distribution describing the number of slaughter pig herds buying from a sow herd (Table 3.2b). As the number of contacts in this study is counted during a 14-day period, the number of contacts during a month was approximately doubled. Accordingly herds, with one contact during 14 days were assumed to have 1-2 contacts during a month, herds with two contacts contact during 14 days were assumed to have 3-4 contacts during a month, etc. The number of herds in each stratum was weighted so that two-thirds of the contacts was assigned to the lower range, one-third to the upper range. We assume that all slaughter pig herds buy weaners or growers every month from their assigned sow herds.

Danske Slagterier provided us with datasheets on the MRDT104 herds and their contacts. The probabilities of MRDT104 infection are based on data from all 85 herds found infected during the period with reduction strategy (August 1st 2000 until July 31st 2002) including 7 herds, which were re-infected after stamping out.

Tables 3.3a-e. Probabilities of MRDT104 infection in pig herds. Data from Danske Slagterier. Outcome of distributions is the 50th percentile. In total 15.815 pig herds.

a) Breeder and multiplier herds and their positive and negative contacts

Breeder and multiplier herds (B/M) (a)	223	Include herds from the DS and PIC.
B/M infected with MRDT104 (b)	2	During 24 month.
Number of contacts (c)	16	During the 3 months from infection to detection.
Number of positive contacts (d)	3	
P_{Bmpos}: beta(b+1, a*24-b+1)	0.05%	Probability of MRDT104 infected B/M per month.
P_{BMi}: beta(d+1,c-d+1)	20%	Probability of MRDT104 infected SOW or FF per quarter when B/M is positive.
% SOW contacts	74%	
% SL contacts	26%	

b) Infected sow herds and their positive and negative contacts, with negative B/M

Sow herds	1,528	Herds with sows only.
Integrated sow herds	4,257	Herds with sows and slaughter pigs, but selling weaners.
7-30 kg producers	112	Herds feeding up weaners, selling them as growers .
Total sow herds (SOW) (e)	5,897	
Infected herds with sows only	6	During 24 month.
Infected integrated sow herds	16	-
Infected 7-30 kg producers	1	-
Total infected SOW (f)	23	-
Number of SL contacts (g)	36	During 24 month.
Number of positive SL contacts (h)	30	-
P_{SOWpos}: beta(f+1, e*24-f+1)	0.05%	Probability of MRDT104 infected SOW per month when BM negative.
P_{SOWi}: beta(h+1,g-h+1)	82%	Probability of infected SL when SOW is positive, during the whole SOW infection period.

c) Infected farrow-to-finish herds, with negative B/M

Farrow-to-finish herds (FF) (i)	1,707	Herds with sows and slaughter pigs.
FF not under surveillance	612	
Total FF	2.319	
Infected FF (j)	3	During 24 month, 1 with positive SOW contacts.
P_{Ffpos}: beta(j+1, i*24-j+1)	0,01%	Probability of MRDT104 infected INT per month when B/M negative.

d) Infected slaughter pig herds, with negative SOW

Slaughter pig herds (SL) (k)	6,107	Herds with slaughter pigs only.
SL not under surveillance	1,269	
Total SL	7,376	
Infected SL (SOW negative) (l)	16	During 24 month.
P_{SLpos}: beta(l+1, k*24-l+1)	0.01%	Probability of MRDT104 infected SL per month when SOW negative.

These data enabled us to estimate the probability of infection when buying animals from an infected vs. non-infected B/M or sow herd (Table 3.3). In the DS data set, the definitions of sow herds and farrow-to-finisher herds were not identical to the definitions used in section 2, so all MRDT104 herds were re-grouped according to the criteria used for the total population in this report. The 84 MRDT104 herds were thus classified as: 2 B/M herds, 49 SL herds, 7 FF, 19 SOW herds also

producing slaughter pigs, 6 SOW herds only producing weaners or growers, and 1 “grower pig producer”.

Information on the frequency and the number of animals purchased at each contact was not available, so the probability of MRDT104 infection during a given time period was assumed to be constant. Herds in contact with an infected B/M during the three-month period prior to detection have a 20% risk of infection (Table 3.3a). The risk is assumed the same for the following 3-months periods, when continued trade is allowed. If the remaining period is less than three months a proportional risk is assigned. In the DS data set, 30 of the 36 slaughter pig herds, which had bought grower and weaner pigs from infected sow herds, were infected (Table 3.3b). We interpret this, as 18% of the slaughter pig herds are able to resist infection. This is modelled by assigning a proportion of the contacts as “resisters”. These slaughter pig herds will remain uninfected throughout the period where their associated sow herds are infectious. All other slaughter pig herds in contact with the infected sow herds will be assumed infected.

The time a herd was assumed infected is based on the average number of days under Zoonosis restriction order. At present, a four month period with a salmonella-index below 20.0 is required for slaughter pig herds before a Zoonosis restriction order can be lifted (ZT-time). Herds with sows test free after two negative bacteriological examinations. Farrow-to-finisher herds also require a one-month period with an index below 20.0. DS suggest that the hot-water decontamination (VVS) should be cancelled when the salmonella-index has been 20.0 or below for a two months period, but if the index during the following six months increase to more than 20.0, the finishers will be VVS slaughtered again. By September 15th 2002, 25 of the 85 herds were still under the Zoonosis restriction order. Some of these herds had ceased to produce pigs, and a cancelling date was entered, but for 17 herds a minimum ZT-time was assigned. The mean number of days in ZT was 216 days, with a standard deviation of 12 days. ZT- time for all herds were drawn from the same normal distribution (LnNormal (5.38, 2.40) / 30 days).

Due to the fact that several herds stopped their production and the application of a minimum ZT-time, the average ZT-time is to some extent underestimated. We assume that the suggested changes for “free-testing” will not change the average number of months a herd is considered infected. Due to the modelling procedure, we indirectly assume that undetected MRDT104 herds also clear themselves of the infection within the same time range as the detected MRDT104 herds, even

though no specific interventions against *Salmonella* have been applied. This is probably not true in all cases. Most of the undetected herds are low-grade infected slaughter pig herds or sow herds selling weaner or grower pigs to Level-1 herds, and it is possible that these low-grade infections persist within the herds.

Danske Slagterier provided us with a data set containing results from the general *Salmonella* surveillance and data on the number of pigs slaughtered per month for all herds registered in the DS slaughterhouse database during the period August 1st 2001 to July 31st 2002. The original herd identification numbers (CHR) were re-coded for confidentiality reasons, but herds found infected with MRDT104 during this period were marked. On the basis of these data we calculated probability distributions for herds with slaughter pigs in Levels 1, 2 or 3 and for herds not under surveillance (Table 3.4). All herds supplying pigs for slaughter were included in the distributions for herds not infected with MRDT104. For each month, the number of herds in each category was counted, and summed for all 12 months. Herds without an assigned level were counted as outside surveillance that month, even though the number of pigs slaughtered during the 12-month period could exceed 200 animals.

The MRDT104 herds were on average seven months under Zoonosis restriction order. Therefore, the probability distributions for the MRDT104 herds are based on surveillance data for the two-month prior to detection of the infection and the following seven months. The MRDT104 herds were categorised according to whether the herds had bought animals from an infected herd (positive supplier) or not (negative supplier). Slaughter pigs from infected farrow-to-finisher herds and integrated sow herds were all categorised as having a positive supplier, as we assumed that the sows were also infected.

The infected herds with negative suppliers are found only if they become Level 2 or 3 herds, whereas infected herds with positive suppliers include cases found via normal surveillance as well as herds found via trace back. In six of the registered MRDT104 herds, no samples for serological testing had been taken during the 12 months, even though Level 1 in some cases was assigned indicating that surveillance had occurred. Of these six herds, three were active slaughter pig herds, and these three herds were assumed to be outside surveillance in the probability distributions for the MRDT104 herds.

Table 3.4 Probabilities of pig herds being in *Salmonella* Levels 1, 2 or 3 or not under surveillance. Calculations based on information in the industry's slaughterhouse database and the Danish Zoonosis Register. Data provided by Danske Slagterier.

3.4a Herds not infected with MRDT104*

	Total herd-months	Probability
Level 1	152161	86.6%
Level 2	3088	1.8%
Level 3	1175	0.7%
No surveillance	19268	11.0%
Total	175692	100.0%

3.4b MRDT104 herds with contact to an infected sow herd or farrow-to-finish herd **

	Total herd-months	Probability
Level 1	125	76%
Level 2	18	11%
Level 3	2	1%
No surveillance***	19	12%
Total	164	100%

3.4c MRDT104 herds with no contact to infected herds **

	Total herd-months	Probability
Level 1	25	40%
Level 2	25	40%
Level 3	13	21%
No surveillance***	0	0%
Total	63	100%

* All herds supplying pigs for slaughter were included in the distributions for herds not infected with MRDT104. For each month, the number of herds in each category was counted, and summed for all 12 months. Herds without an assigned level was counted as outside *Salmonella* surveillance that month, even though the number of pigs slaughtered during the 12-month period could exceed 200.

** The MRDT104 herds were on average 7 months under the Zoonosis restriction order (216 days). Therefore the probability distributions for the MRDT104 herds included the month prior to detection of the infection and the next seven months.

***Six MRDT104 herds had not been tested during the 12 month period, including one slaughter pig herd and two farrow-to-finisher herds. These three herds were assumed to be outside surveillance in the probability distributions for the MRDT104 herds, even though a Level 1 had been assigned.

The model in section 2 estimated that the S*almonella* surveillance program including the trace back procedures detect 34% of the true number of MRDT104 pig herds (Table 2.4).

In scenario I, the distribution estimating the total number of MRDT104 herds is generated by randomly sampling (25.000 times) in the distributions describing the expected number of detected MRDT104 herds per year, and multiplying these numbers with a correction factor randomly sampled from the appropriate distributions describing the proportion of undetected infected herds.

The distribution of the correction factors are generated by dividing the simulation output data from the model estimating the true number of MRDT104 herds (section 2), with the number of herds actually detected during the 12-month period.

In scenario II, the distribution estimating the total number of MRDT104 herds is generated by randomly sampling (10.000 times) in the distributions describing the expected number of detected MRDT104 herds per year, and adding these numbers to the expected number of undetected MRDT104 herds randomly sampled from the distributions generated by a corrected version of the *continued trade model*.

3.3 Input data for model estimating the effect of continued trade from MRDT104 herds

During the two-year period, two breeder and multiplier (B/M) herds were found infected (one herd had two CHR-numbers), giving 0.05% (90%CI: 0.02%, 0.12%) probability of infection per month for detected B/M herds. Twenty percent (20%, 90%CI: 8%, 38%) of the herds, which had bought animals from the two infected B/M herds were also found MRDT104 positive (Table 3.3a). The model estimating the true number of MRDT104 herds found that that three of the estimated five infected B/M herds (CHR numbers) were detected (50^{th} percentile, Table 2.4), indicating that 40% of the infected B/M herds are undetected. Thus we assumed that during the 24 months period there were 1.3 undetected MRDT104 B/M herds, resulting in a 0.038% (90%CI: 0.01%, 0.10%) probability of an undetected MRDT104 infection per B/M per month.

Twenty-two sow herds and one “grower-pig producer” were found infected during the two-year period, giving a general 0.02% (90% CI: 0.01%, 0.023%) probability of infection per month (Table 3.3b). If the sow herd had bought breeding animals from a MRDT104 infected B/M herd, the risk of infection increased to 20% (90% CI: 8%, 38%) (Table 3.3a).

Three farrow-to-finisher herds (i.e. FF herds are assumed not to sell weaners or growers to other herds) were found infected, during the two-year period. As for the sow herds, there was a 20% (90% CI: 8%, 38%) probability of infection if the FF herd had bought breeding animals from an infected B/M herd (Table 3.3a). FF herds buying from negative B/M herds had a 0.007% (90% CI: 0.002%, 0.014%) probability of infection per month (Table 3.3c). During the two-year period,

sixteen slaughter pig herds (SL) were found infected. SL herds buying growers from MRDT104 infected sow herds had an 82% (90% CI: 70%, 91%) probability of infection, whereas SL herds buying from other herds had a 0.008% (90% CI: 0.005%, 0.011%) probability of infection per month (Table 3.3b).

Figure 3.2 Distributions of the correction factors for the undetected MRDT104 herds (Section 2). In scenario I, the estimated numbers of detected MRDT104 herds are to be multiplied by the correction factor in order to estimate the true number of MRDT104 herds.

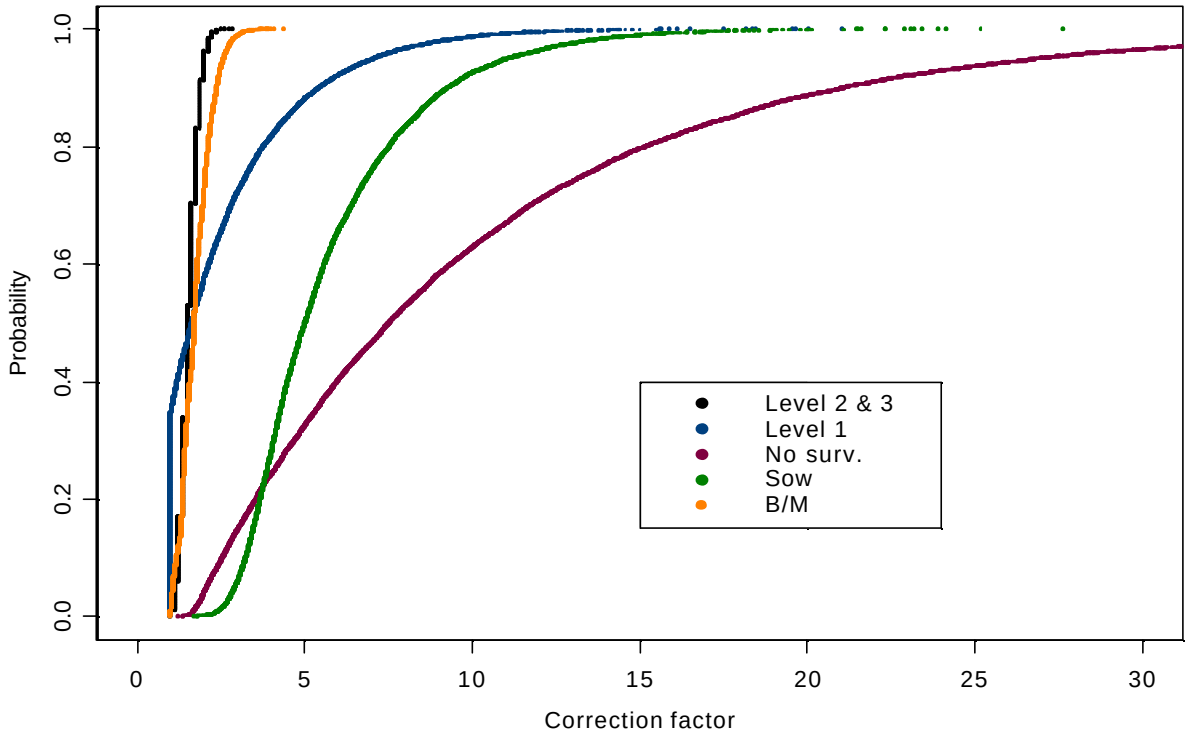


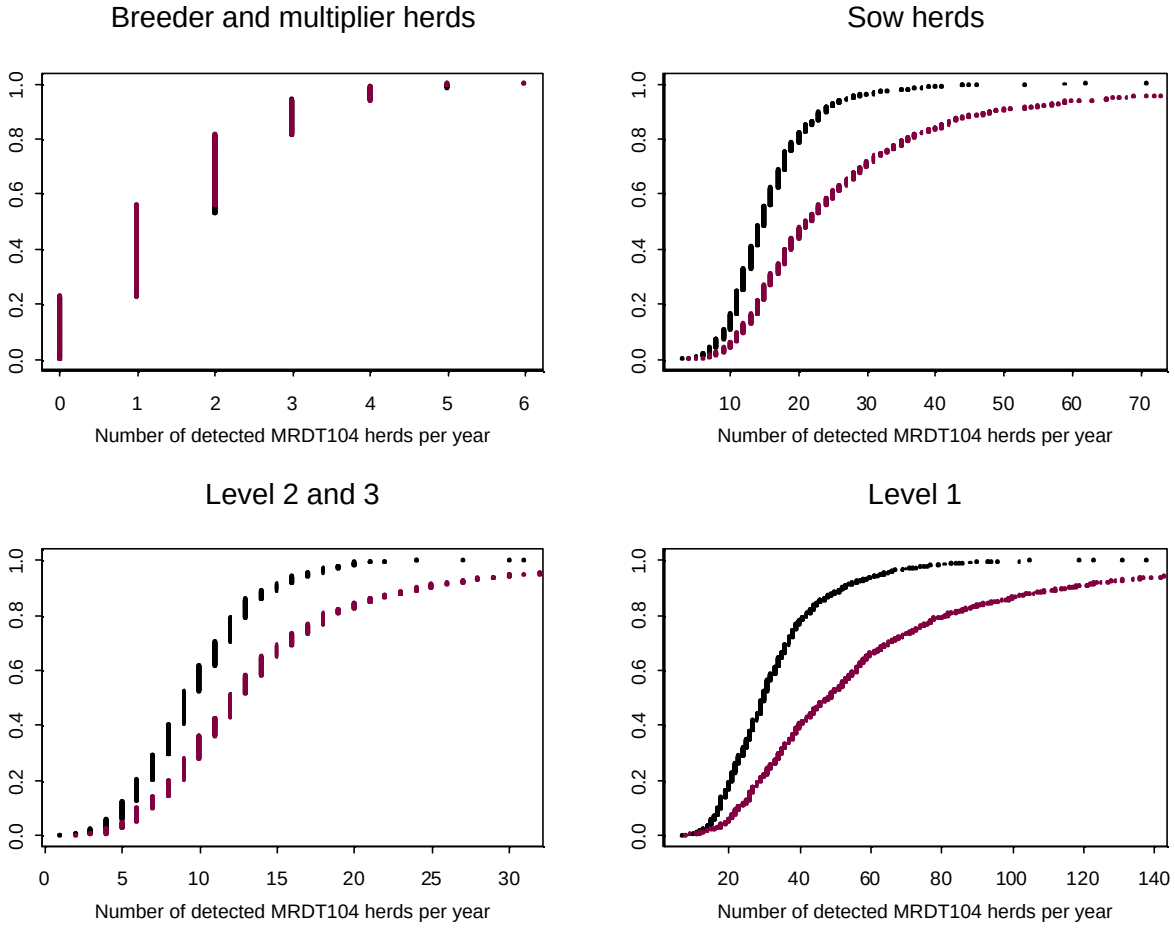
Figure 3.2 show a cumulative plot of the correction factors for B/M herds, sow herds and slaughter pig producing herds. For B/M herds and slaughter pig producing herds under surveillance, the proportion of detected MRDT104 herds is relatively high, and approximately half of the herds are detected. Including 95% of the simulations, the true number of B/M herds is less than 2.6 times the number of detected herds, and the true number of Level 2 and 3 herds are less than 2.0 times higher. If 95% of the simulations are included for the Level 1 slaughter pig producing herds, the true number is less than 7.1 times the number of detected herds. For sow herds and slaughter pig herds not under surveillance, the 50% correction factors are relatively higher (5.0 and 7.5 respectively). Due to lack of surveillance data there is considerable variability and uncertainty surrounding these correction factors, which should be considered when evaluating the total number of MRDT104 herds simulated in scenario I (*This section was corrected 5 Feb. 2003).*

3.4 Output of model estimating effect of continued trade from MRDT104 herds

3.4.1 Number of detected MRDT104 herds

The primary output from the models is the expected number of new MRDT104 herds to be detected during a year (figure 3.3). We simulated five successive years, but the number of detected herds was almost similar for all five years. Thus, the models predict that the increase in detected MRDT104 herds due to continued trade occurs during the first year, where after a steady level is reached. This of course, is significantly affected by the validity of the assumptions that infection is gone, when the Zoonosis restriction order is suspended.

Figure 3.3 Cumulative distributions showing the estimated number of new MRDT104 herds detected per year for the continued trade model (orange) and the restricted trade model (black). Results from the last four years of simulation. Undetected herds not include.



The change in trade pattern had no influence on number of detected MRDT104 breeder and multiplier herds. This was expected, as the probability of infection of B/M herds, is independent of both trade patterns as well as of the total number of infected herds. In 50% of the simulations less than two B/M herds were found infected per year, whereas when including 95% of the simulations the range was between 0 and 4 new infections per year (Table 3.6).

In general continued trade does result in more infected sow herds and slaughter pig producing herds (Figure 3.3). The lower 50 percent of the distributions are relatively unaffected by the change in trade pattern, where the Level 1 slaughter pig producing herds show the most pronounced effect. For the upper part of the distribution a more pronounced effect of continued trade is observed, as the possibility of having years with a very large number of MRDT104 infected herds increase. This is primarily due to a higher frequency of simulation resulting in relatively high numbers.

In the models, there are two ways pig herds can become infected, either the infection is transmitted via trade contacts, or the infections can come from an unknown source, i.e. from the environment or from other contacts than trade. Table 3.5 show the proportion of sow herds and slaughter pig herds infected from trade contact using the two models. When trade is restricted, it is a fairly rare event that a breeder and multiplier herd infect a sow herd. In the *restricted trade model*, 70% of the simulation generated no sow herds infected via trade contacts. Only 35% of the simulations using the *continued trade model* resulted in no trade related infections and in 50% of the simulations, more than 50% of the sow herds were infected via trade contacts.

Table 3.5 Percent of detected MRDT104 herds infected via trade relations.

		Restricted trade			Continued trade	
% MRDT104 herds		Sow herds	Slaughter pig herds		Sow herds	Slaughter pig herds
13-60 months	5th pct	0%	0%	5th pct	0%	0%
	25th pct	0%	0%	25th pct	0%	8%
	50th pct	0%	67%	50th pct	50%	83%
	75th pct	18%	100%	75th pct	84%	100%
	95th pct	100%	100%	95th pct	100%	100%

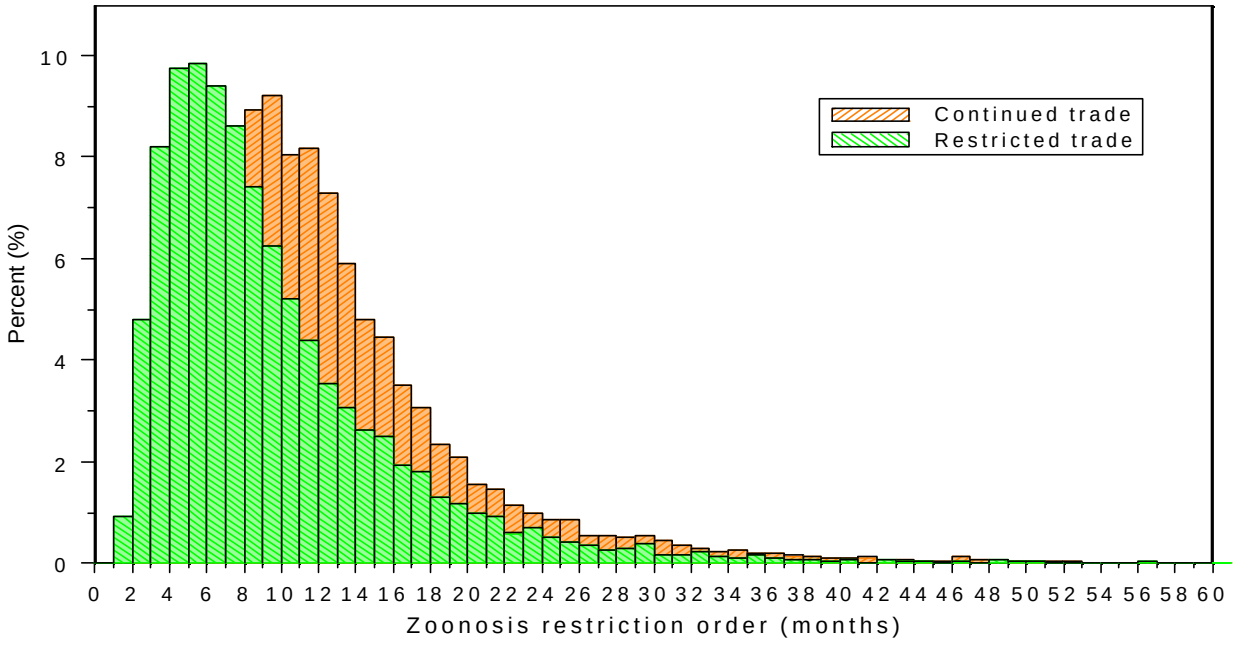
The proportions of slaughter pig herds infected via trade contacts are relatively higher for both models. When trade is restricted, absence of trade related infections were found in only 30% of the simulations, and in 50% of the simulations more than 67% of the slaughter pig herds were infected

by their weaner or grower pig supplier. If continued trade was allowed, the percentages were 20% with no trade related infections and in 50% of the simulations more than 83% of the infections occurred as a result of trade contacts.

At the current strategy most of the sow herds are infected via other sources than contacts to breeder and multiplier herds, but the models predict that continued trade will increase the proportion of trade related infections to approximately 50%. Approximately 70% of the slaughter pig herds are currently infected via trade contacts, and if continued trade is allowed, this proportion increases to about 80%.

The time a herd was assumed infected is based on the average number of days under Zoonosis restriction order. The 84 herds were on average 216 days, or 7.2 months under Zoonosis restriction order. Figure 3.4 is a histogram showing the number of months herds are assumed infected and under Zoonosis Restriction order, for restricted and continued trade. When continued trade was allowed the most likely number of months a herd was assumed infected, increased by four months. The difference is due to the fact that under continued trade the slaughter pig herds is assigned a Zoonosis restriction order at the last month of the "Sow Zoonosis restriction order".

Figure 3.4 Histogram showing the number of months the herds are assumed infected, Zoonosis Restriction order for restricted and continued trade.



3.4.2 Total number of MRDT104 herds

Two different methods have been applied in order to include the undetected MRDT104 herds. In scenario I, we assume that the spread of MRDT104 between undetected herds is unknown, but the proportion of undetected MRDT104 herds is constant. This is modelled by multiplying the number of detected MRDT104 herds per year by the correction factor.

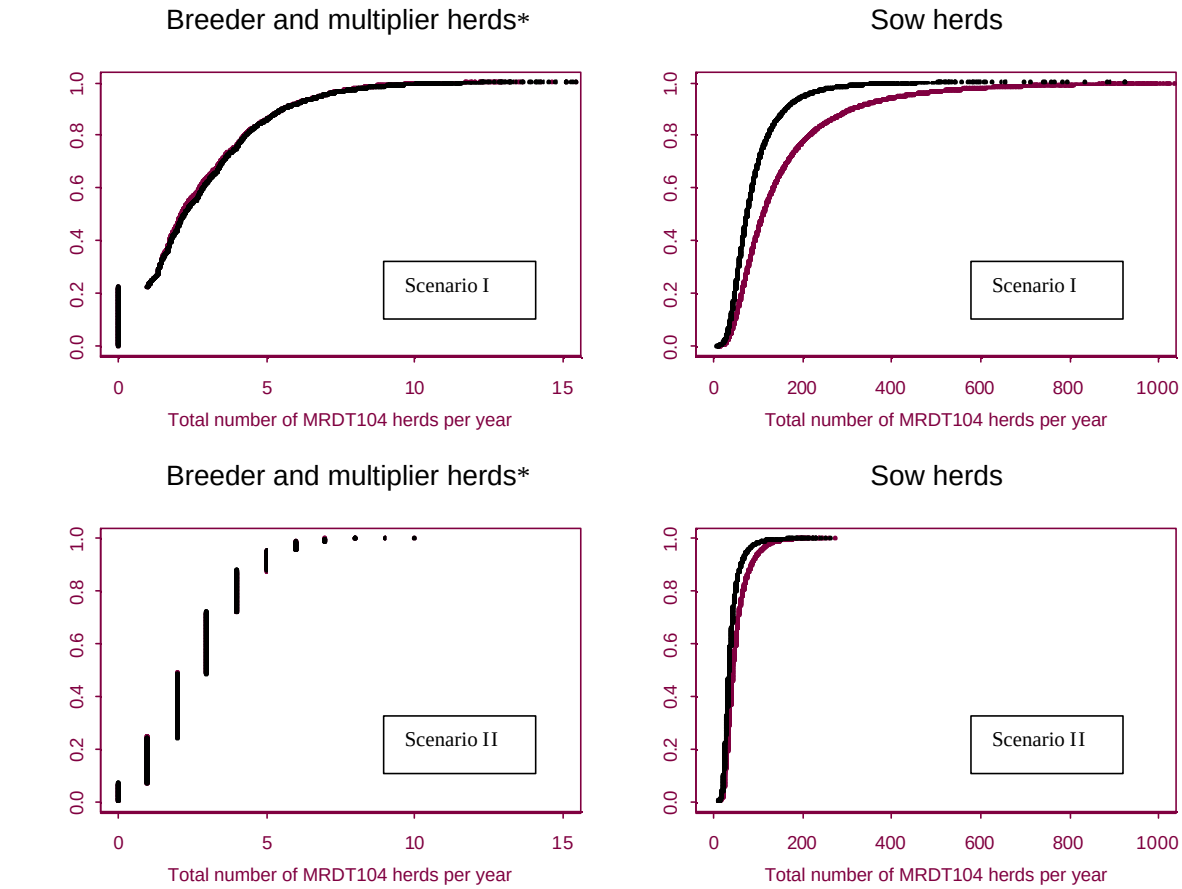
In scenario II, we assume that the rate at which the infection spreads between undetected herds is equal to the rate among detected herds, that the risk of undetected MRDT104 breeder and multiplier herds is relatively lower, and that the additional number of MRDT104 herds arising from the free trade leads to no more undetected herds. Thus, the numbers of undetected herds are the same for both trade models. The number of detected and undetected MRDT104 herds is then added, in order to attain the total number of infected herds.

Scenario I probably overestimates the number of undetected herds, as herds buying animals from known MRDT104 herds, will be able to take extra precautions in order to minimise the horizontal spread of MRDT104, compared to herds not aware that the animals can be infected with MRDT104. In contrast, scenario II probably underestimates the number of undetected herds. This scenario does not take into account that the probability of detecting MRDT104 in sow herds and small slaughter pig herds not under surveillance is relatively smaller than detecting MRDT104 in slaughter pig herds under surveillance.

The total number of MRDT104 breeder and multiplier herds is not influenced by the continued trade or the two different ways to estimate the number of undetected MRDT104 herds (Figure 3.5). This is expected due to the model-input data, and as estimated in section 2 (Table 2.4) the 50^{th} percentile indicates 5 new MRDT104 breeder and multiplier herds per year.

In scenario I, the increased number of MRDT104 sow herds due to continued trade was relatively small for the lower 50% of the distribution (Figure 3.5). But the effect of continued trade on the number of detected MRDT104 herds, was increased when including the undetected herds in the distribution. In 50% of the simulations in scenario I, the *restricted trade model* predicted a total of more than 76 MRDT104 sow herds, whereas the *continued trade model* predicted that more than 111 infected sow herds (Table 3.6).

Figure 3.5 Cumulative distributions showing the estimated total number of new MRDT104 herds per year for the continued trade model (orange) and the restricted trade model (black). Results from the last four years of simulation. In scenario I, the undetected herds are included as an assumed proportion of the number of detected MRDT104 herds. In scenario II, the estimated numbers of undetected herds are added to the number of detected MRDT104 herds.



* The cumulative distribution of infected B/M herds are discontinuous because in some cases the factor correcting for undetected MRDT104 herds were zero in scenario I, and the outcome in scenario II is discrete numbers.

The increase in the total number of MRDT104 sow herds is only marginal in scenario II, as the only difference between the two distributions is the trade related increased number of detected MRDT104 herds. This relatively small difference also reflects that less than 50% of the sow herds are infected via trade relations even if continued trade is allowed (Table 3.5). In 50% of the simulations in scenario II, the *restricted trade model* predicted a total of more than 36 MRDT104 sow herds, whereas the *continued trade model* predicted more than 45 infected sow herds (Table 3.6).

Table 3.6 Estimated number of detected MRDT104 breeder and multiplier herds (B/M) and sow herds per year from the restricted trade model and the continued trade model. Number of undetected herds according to scenario II and I. Results from the last four years of simulation. From each distribution are listed: 5th percentile, 50th percentile, 95th percentile. The estimated numbers include both detected and undetected MRDT104 herds.

a) Detected MRDT104 herds

		Restricted trade			Continued trade	
Primary model		**B/M herds**	**Sow herds***		**B/M herds**	**Sow herds**
13-60 months	5th pct	0	8	5th pct	0	10
	50th pct	1	15	50th pct	1	22
	95th pct	4	28	95th pct	4	68
Actually detected during 24 months		*2*	*26*			

* Sow herds producing slaughter pigs are also included

b) Total MRDT104 herds

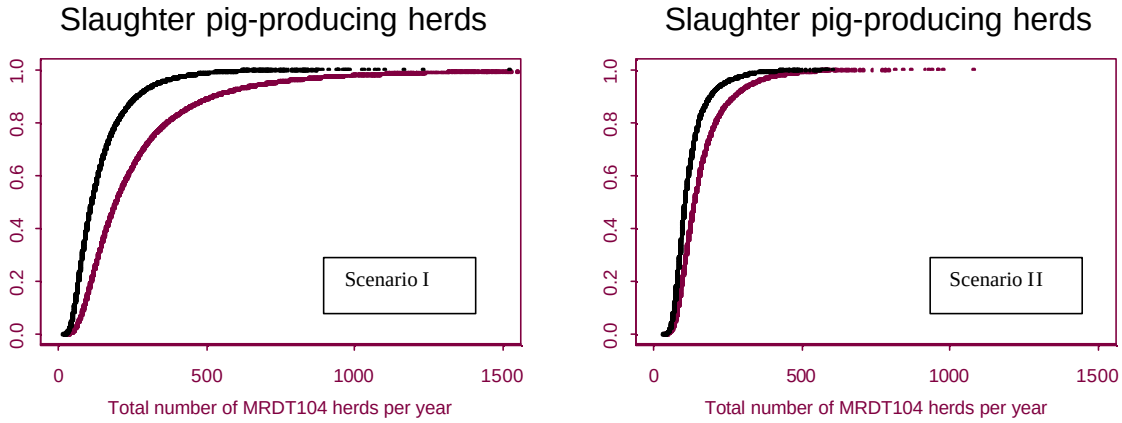
		Restricted trade			Continued trade	
Scenario I		**B/M herds**	**Sow herds**		**B/M herds**	**Sow herds**
13-60 months	5th pct	0	32	5th pct	0	41
	50th pct	3	76	50th pct	2	111
	95th pct	7	210	95th pct	7	437

c) Total MRDT104 herds

		Restricted trade			Continued trade	
Scenario II		**B/M herds**	**Sow herds**		**B/M herds**	**Sow herds**
13-60 months	5th pct	0	21	5th pct	0	24
	50th pct	3	36	50th pct	3	45
	95th pct	5	77	95th pct	5	107

MRDT104 infected slaughter pig producing herds constitute between 50 and 75 percent of the total number of infected pig herds. In scenario I the effects of continued trade is more pronounced than for the other herd types (Figure 3.6). In 50% of the simulations in scenario I, the *restricted trade model* predicted a total of more than 114 MRDT104 slaughter pig producing herds, whereas the *continued trade model* predicted more than 196 truly infected herds (Table 3.7).

Figure 3.5 Cumulative distributions showing the estimated total number of MRDT104 herds per month in scenario II for the continued trade model (orange) and the restricted trade model (black). Results for the last four years of simulation. Undetected herds simulated separately using the continued trade model, assuming that 60% of the MRDT104 B/M herds are detected.



This relatively large effect is primarily because this grouping includes slaughter pig producing herds in Level 1 and herds not under surveillance - where the proportion of undetected MRDT104 herds is relatively high (Figure 3.2). Scenario I predicts that when trade is restricted 70% (75 of 114 herds, Table 3.7) of the new MRDT104 infection among slaughter pig producing herds, will occur among Level 1 herds or in herds not under surveillance – where detection can occur by trace back from an infected sow herd. If continued trade is allowed, the proportion is the same, but the number of infected Level 1 herds and herds outside the surveillance increase to approximately 138 MRDT104 herds per year. Even though a small proportion of these will be recognised due to trade contact to known MRDT104 sow herds, detection of most of these herds will rely on the trace-back procedures.

Table 3.7 Estimated number of detected MRDT104 herds producing slaughter pigs, divided into salmonella Level 1, Level 2, Level 3 and herds not under surveillance per year from the restricted trade model and the continued trade model. Number of undetected herds according to scenario II and I. Results for the last four years of simulation. From each distribution are listed: 5th percentile, 50th percentile and 95th percentile. The estimated numbers include both detected and undetected MRDT104 herds.

a) Detected MRDT104 herds*

		Restricted trade **					Continued trade			
Primary model		**Level 1**	**Level 2**	**Level 3**	**No surveillance**		**Level 1**	**Level 2**	**Level 3**	**No surveillance**
13-60 months	5th pct	15	3	0	1	5th pct	20	4	0	2
	50th pct	30	7	2	4	50th pct	49	10	2	7
	95th pct	64	15	5	10	95th pct	157	28	6	26

* Sow herds producing slaughter pigs are also included.
** During 24 months, 75 slaughter pig producing herds were found infected.

b) Total MRDT104 herds

		Restricted trade					Continued trade			
Scenario I		**Level 1**	**Level 2**	**Level 3**	**No surveillance**		**Level 1**	**Level 2**	**Level 3**	**No surveillance**
13-60 months	5th pct	19	5	0	2	5th pct	25	6	0	7
	50th pct	52	11	3	27	50th pct	89	15	4	49
	95th pct	252	23	8	147	95th pct	527	44	10	319

c) Total slaughter pig producing MRDT104 herds

		Restricted trade			Continued trade	
		Scenario I	**Scenario II**		**Scenario I**	**Scenario II**
13-60 months	5th pct	47	64	5th pct	72	75
	50th pct	114	107	50th pct	196	139
	95th pct	344	224	95th pct	731	343

3.5 Assumptions

Models as these are based on a large number of assumptions, and the estimated numbers of MRDT104 herds particularly depend on the assumptions about:

- The number of months a herd can transmit the infection compared to the time under Zoonosis restriction order.
- The dependency between the horizontal infection rate and the actual number of infected herds: In the present model the infection rate is independent of the number of herds infected.
- The assigned number of trade contacts for sow and farrow to finisher herds.
- The correction for undetected MRDT104 herds.

A list of the assumptions are presented in the appendix (section 3.7).

An evaluation of the assumption about trade relations and transmission rates can be done by comparing the number of detected herds simulated by the *restricted trade model* with the actual number of detected herds during the last two years. Assuming that the 50th percentile is representative for the number of detected herds, the model predicts 15 infected sow herds per year (Table 3.6), compared to an average of 13 infected herds detected per year (26 MRDT104 herds during 24 months). The model predicts approximately 43 infected slaughter pig producing herds, including sow herds producing slaughter pigs (Table 3.7), whereas only 37-38 herds were actually detected per year (75 MRDT104 herds during 24 months). So the models probably overestimates the number of detected sow herds and slaughter pig-producing herds by approximately 14%.

3.6 Conclusion

The change in trade pattern and method for estimating undetected MRDT104 herds had no influence on number of MRDT104 breeder and multiplier herds, and in 50% of the simulations less than two new B/M herds were predicted to be found infected per year.

At the current strategy most of the sow herds are infected via other sources than contacts to breeder and multiplier herds, but the models predict that continued trade will increase the proportion of trade related infections to approximately 50%. Approximately 70% of the slaughter pig herds are currently infected via trade contacts, and if continued trade is allowed, this proportion increases to about 80%.

The lower 50 percent of the distributions are relatively unaffected by the change in trade pattern, but for the upper part of the distributions an important effect of continued trade is observed, as the possibility of having years with a very large number of MRDT104 infected sow herds increase. The effects are most pronounced in scenario I, where the herds infected via their trade contacts during the period of continued trading, are assumed to result in more undetected herds.

The models probably overestimates the number of detected sow herds and slaughter pig-producing herds by approximately 14%.

3.7 Appendix

1. In scenario I, we assume that the spread between undetected MRDT104 herds is unknown and that the additional number of MRDT104 herds arising from the free trade have the same proportional number of undetected herds all other detected herds. The proportion of MRDT104 herds that are undetected, as estimated in section 2 is assumed to be constant for all years in the simulations.

2. In scenario II, we assume that the rate at which the infection is spread between MRDT104 undetected herds is equal to the rate among detected herds and that the additional number of MRDT104 herds arising from the free trade leads to no more undetected herds. The risk of undetected infection among breeder and multiplier herds is constant for all years in the simulation and as estimated as in chapter two. The 50% correction factor is used to estimate a probability of infection for undetected B/M herds.

3. As the number of MRDT104 herds infected per year represents a small fraction of the total number of herds, we assume the distributions describing the number of detected herds and the number of undetected herds are additive, even though the distributions are modelled separately.

4. Effects of general structural changes in the pig industry, for example the expected reduction in numbers of pig herds and their increase in size have not been included in the model. The distributions describing the number of contacts and the probability of infection per contact remains unchanged during the period of the simulation.

5. Sow herds are defined as herds with sows registered in the CHR-register, but where the ratio between pen places for sows vs. slaughter pigs is 1:7 or more. Herds with a sow:slaughter-pig ratio smaller than 1:7 is defined as a farrow-to-finisher herd. Herds with no registered sow are defined as slaughter pig herds.

6. The herds are assumed to be infectious during the month of detection and two months before.

7. The distribution of contact-herds to each breeder and multiplier herds registered in SPF database for 2001, is representative of all B/M herds and their contacts.

8. A distribution describing the number of SOW-contacts per B/M during 90 days generated from the SPF data-set is representative of all B/M herds and their contacts.

9. A proportion (25%) of a B/M contact-herds is changed each year.

10. Sow herds and farrow-to-finisher herds are assumed to have the same number of contacts to B/M herds.

11. Data on the number of slaughter pig herds buying from a sow herd during 14 days, is approximately half the number of contact-herds a sow herd would have during a month.

12. We assume that all slaughter pig herds buy weaners or growers every month from their assigned sow herds.

13. The probabilities of MRDT104 infection based on data from herds found infected during the period August 1st 2000 until July 31st 2002 is representative of future infections also during continued trade.

14. When trade is allowed, the risk of infection is assumed to be the same as during the period when the infection wasn't recognised.

15. A proportion of the slaughter pig herds are able to resist the infection throughout the period when their associated sow herds are infectious. All other slaughter pig herds in contact with the infected sow herds will be assumed infected.

16. The time a herd was assumed infected is the same as the average number of days under Zoonosis restriction order. ZT- times for all herds were drawn from the same normal distribution.

17. The suggested changes for “free-testing” will not change the average number of months a herd is considered infected.

18. Probability distributions for slaughter pig producing herds in Levels 1, 2 or 3 and for herds not under surveillance based on monthly salmonella surveillance data for all herds registered in the DS slaughterhouse database during the period August 1st 2001 to July 31st 2002, were assumed representative of all slaughter pig producing herds.

19. All herds supplying pigs for slaughter were included in the distributions for herds not infected with MRDT104. Probability distributions for the MRDT104 herds are based on surveillance data for the month prior to detection of the infection and the following seven months.

4. SURVIVAL OF MRDT104 IN THE ENVIRONMENT AND RISK OF HORIZONTAL TRANSMISSION

Farm animal waste is widely recognised as an important vehicle for the transmission of *Salmonella*. Deposition of solid manure (a mixture of feces and straw as opposed to slurry which is a mixture of urine and feces with minimal straw) seems to constitute less of a risk of infection with, for example *Salmonella*, as it undergoes a composting process with significant reduction of the pathogen load. *Salmonella* in animal waste has been the subject of numerous studies, published in the international scientific literature. In Denmark, at major study was commissioned by the Danish Veterinary Service in the late 1970s and carried out by the Royal Veterinary and Agricultural University in Copenhagen (H. Errebo Larsen og B. Munch: Sygdoms- og miljømæssige problemer i forbindelse med behandling og spredning af flydende husdyrgødning (gylle). Rapport, 1981). More recently, a number of projects carried out by the DVI in collaboration with external partners have studied some of the aspects involved.

Regulations concerning deposition of animal wastes

Knowledge generated by these projects form the basis for the present requirement that herds under zoonosis restriction order due to infection with MRDT104 must plough slurry into the soil immediately after deposition on the field. The legal basis for this is contained in Bek. nr. 435 om ændring af bek. om salmonella i kvæg og svin, mm. (2001). The objective of the requirement is to remove infective material from the surface of the field and thus remove the risk of accidental transmission, directly or indirectly, to farm or companion animals or to humans. The basis is a general statement in the above regulation that the zoonosis restriction order may include ‘specific precautions to reduce disease transmission during handling of slurry and use of farm implements shared between farms. There appear to be no special requirements for minimum distances to for example neighbouring farms or to fields with crops, or other such restrictions.

The requirement to plough in slurry is not absolute, as exemptions can be granted if the concentration of MRDT104 bacteria is below 1 CFU per gram. If there is more than one slurry tank on the farm, multiple exemptions can be issued. In total, 30 exemptions were given during 2001, to a total of 26 herds. Until November 2002, 48 exemptions were given to 31 herds (Table 4.1). Among all herds under Zoonosis restriction order, between 0% and 50% of the MRDT104 pig herds

were given exemption each month. In 2001 approximately 40% of the MRDT104 herds were granted exemptions, compared to 60% in 2002 (until 1. November).

Table 4.1 Exemptions from ploughing in MRDT104 infected pig slurry in 2001 and 2002. The columns show the number of exemptions given, the number of herds under Zoonosis restriction order in the current month, and the number and percentage of herds given exemptions the current month. Some herds have been granted more than one exemption.

Year	Month	Medium DT104 0.1-1 CFUs /g	Low MRDT104 < 0.1 CFUs/g	Herds under ZT	Herds per month	
2001	March	4	5	32	9	(28%)
2001	April	9	10	37	18	(49%)
2001	September	0	2	26	2	(8%)
2002	January	1	5	32	4	(13%)
2002	February	6	5	28	8	(29%)
2002	March	2	16	26	13	(50%)
2002	April	0	6	28	4	(14%)
2002	May	4	0	27	4	(15%)
2002	August	0	2	24	2	(8%)
2002	October	1	0	22	1	(5%)
Total 2001				64	26	(41%)
Total 2002, until 1. November				51	31	(61%)

The risk of *Salmonella* transmission presented by animal waste is influenced by the survival of *Salmonella* following deposition of manure or slurry in the fields and by the probability of contact between infective slurry or soil and farm animals, either directly or via vectors, such as rodents, flies or pet animals.

This section reviews some of the results available in the literature on survival of *Salmonella* in and on soil. The review also includes a very recent and not yet published study of the fate of MRDT104 following deposition on agricultural land. The issue of transmission to farm animals is addressed, although mainly indirectly, in the form of a tempero-spatial analysis of the occurrence of farms infected by specific DNA subtypes of MRDT104 in Denmark.

4.1 Survival of Salmonella following deposition on farmland

The reduction time for *Salmonella* is described by T_{90}, the time taken for reduction of bacteria by 90% or 1 $\log_{10}$ unit. Accordingly the time taken for the land to become un-infective depends on the number of *Salmonella* bacteria deposited. The rate of reduction is influenced by a number of factors, for example whether the slurry is deposited on grass or on bare soil, and also on the

composition of the soil, in addition to meteorological parameters, such as humidity and amount of ultra-violet light.

A study by Temple et al. (1980) showed that fecal samples inoculated with 10^5 *Salmonella* Typhimurium per gram and buried in soil declined to around 10^3-10^4 per gram feces in 8 weeks.

Platz (1980) found that salmonellae covered by soil had about twice as long survival time as bacteria remaining on the surface (45 days – range 7-120 days – compared to 26 days – range 3-69 days). The study also showed that heavier soil types gave a statistically significant better survival of *Salmonella* than did sandy types.

Schlundt (1982) found that for *Salmonella* Typhimurium deposited on grass during the period from August to November, T_{90} ranged between 24 and 18 days, depending on whether the *Salmonella* bacteria were located close to the ground or near the top of the grass, respectively.

Morse and his colleagues (1982) found that *Salmonella* Typhimurium could be recovered for more than 450 days in fine loamy soil under field conditions in the American Mid-west following application of a 24-hour broth culture.

Baloda et al. (2001) studied persistence of *Salmonella* Typhimurium DT12 following deposition of naturally contaminated (level of contamination not given) pig slurry on agricultural soil. They were able to recover *Salmonella* bacteria for 2 weeks after deposition and cautions that the apparent absence of *Salmonella* at later sampling occasions may to some extent reflect a lack of sensitivity of the method used for recovery. They also quote unpublished results as showing that under controlled conditions they have demonstrated that DT104 and DT12 can survive up to 299 days in soil.

Natvig et al. (2002) found that following incorporation of bovine manure spiked with *Salmonella* Typhimurium into soil to a level of 4-5 log CFU/g soil, *Salmonella* could be recovered for 2-3 months in 15-25 g samples of soil after storage under simulated conditions similar to the weather in the American Mid-West. Their results indicated that the rate of decline of *Salmonella* might be more rapid in sandy soils than in silty clay loam.

A further, as yet unpublished study has been carried out in collaboration between the DVI, DS and RVAU to determine the effects of various soil treatments on the recovery of *Salmonella* (Boes et al., under preparation). During April 2002, a field study testing the recovery of *Salmonella* from land plots where slurry from a MRDT104 herd was deposited at a level of 40 tons per hectare using four different methods:

1. Deposited by hose-applicator, followed by ploughing and harrowing
2. Deposited by hose-applicator on ploughed land, followed by harrowing
3. Deposited by hose-applicator on field with winter wheat seedlings
4. Injected into the soil in a field with winter wheat seedlings.

The concentration of *Salmonella* in the slurry was 0.1-1 CFUs /g, making the farm eligible for exemption for the requirement to plough in the slurry. No salmonellae were found in samples of soil and plants from the plots prior to deposition of slurry. Within the test plot, 8 samples (10 x 15cm, including approximately 1 cm topsoil) were collected immediately after deposition, as well as after one, two, three and four weeks. *Salmonella* from a 5g sample was isolated using a semi-quantitative method including an enrichment step. Assuming a concentration of max 1 CFU per g slurry, approximately 4000 CFU's were deposited per m2, resulting in max 60 CFU's per 10x15cm sample, or 0.2 CFU per gram soil (assuming a soil mass density of 1.8 g /cm3), or 1 CFU per sample analysed.

Table 4.2 Recovery of *Salmonella* in agricultural soil from deposited slurry

Method	Day 0 Positive/total	week 1 Positive/total	week 2 Positive/total	Week 3 Positive/total	Week 4 Positive/total
1. Ploughing + harrowing	0/8	0/8	0/8	0/8	0/8
2. Harrowing alone	3/8 (38%)	0/8	0/8	0/8	0/8
3.1 Hose-applicator (soil)	4/8 (50%)	0/8	0/8	0/8	0/8
3.2 Hose-applicator (plants)	1/8 (13%)	0/8	0/8	0/8	0/8
4. Injection	1/8 (13%)	1/8 (13%)	0/8	0/8	0/8

The results are shown in Table 4.2. *Salmonella* was not recovered in the soil where deposition was followed by ploughing. This was to be expected as the ploughing removes the slurry from the surface where the samples were taken. For the other treatments, where the slurry was included in the samples with soil, *Salmonella* was recovered in 13-50% of the samples at the sampling right after deposition. The relatively low recovery rate (13-50%) possibly reflects the sensitivity of the

sampling and isolation methods used, as it appears unlikely that the bacteria would decimate to a significant extent during the two-hour period between deposition and sample collection. During the subsequent four weekly samplings, *Salmonella* was isolated only from one sample on week 2 from the injection plot.

While the absence of *Salmonella* positive samples from week 3 onwards should not be confused with the absence of *Salmonella* in the soil it nevertheless is a strong indication that the level of *Salmonella* was low, as would be expected from the number of CFU's in the slurry. The study, however, provides little useful information about the effects of the soil treatments used.

Thermo tolerant *E. coli* behaves similarly to *S.* Typhimurium when deposited on agricultural land. The same study as described above modelled the T_{90} for *E. coli* to be used as a surrogate for *Salmonella* T_{90} (Boes and Alban, unpublished). They found a rapid decrease in the number of CFU's of thermo tolerant E. coli between first sampling immediately after deposition of slurry and the sampling on day 7 (T90=2.8 to 6.4 days, depending on the method of deposition), followed by a much slower rate of decrease between day 7 and day 28 (T90=15.5 to 61 days). One possible explanation for this difference may be that an initial, rapid die-off of bacteria exposed to ultraviolet light and drying-out is followed by a much slower rate of decay of bacteria protected by particles of straw or manure.

4.1.1 Salmonella in non-food animals

A project still in progress has attempted to evaluate the role of non-food animals in the maintenance and introduction of *Salmonella* in food animal herds. Preliminary results of the study (Baggesen et al., 2002) indicate that wild birds, rodents and flies captured in or near farms where *Salmonella* is not present are negative. In contrast, flies, mice and wild birds were shown to be infected at a low prevalence on farms with *Salmonella*. In these instances, identical types of *Salmonellae* were usually found in food and non-food animals.

Table 4.3 shows results of Salmonella surveillance in animals belonging to the wild fauna (Dietz and Andersen, 1999). The results seem to suggest that mammals or birds living or foraging in the vicinity of humans or domestic animals may relatively often carry *Salmonella*, while animals that do not, such as hares or deer tend to be *Salmonella* negative.

Table 4.3 Recovery of *Salmonella* from animals in the wild fauna in Denmark, 1995-1998. Data: Dietz and Andersen, 1999. *Hedgehogs originated from nursing homes with unknown interspecies contacts.

Species	No. examined	Percent positive
Water fowl, gulls	166	2.4
Other species of birds	112	0
Birds of prey	44	6.8
Small birds (Passerines)	19	15.8
Other mammals (eg. brown hare, roe deer)	261	0
Foxes	250	2.0
Badgers	169	0
Hedgehogs*	152	23.6
Total	1173	3.7

While these investigations do not provide information about the direction of transmission of *Salmonella*, the absence of *Salmonella* in non-food animals from farms where *Salmonella* had not been detected in production animals, and the results of Dietz and Andersen (1999) seem to indicate that non-food animals mainly become infected through contact with food animals. Therefore, it appears likely that those same vectors may become contaminated or infected by contacting *Salmonella* contaminated slurry deposited in the field and may transmit the infection between adjacent farms. However, on the basis of the available data it is not possible to differentiate between transmission by non-domestic animals acquiring the infection on a farm, or from deposited slurry.

4.2 Spatio-temporal analysis of the occurrence of herds infected with MR DT104

For approximately half of the primary case herds infected with MRDT104, trace-back sampling did not demonstrate trade contacts as likely sources of infection. While this may in part reflect the sensitivity of the testing procedure, it may also indicate that a relatively high proportion of the known positive farms are infected horizontally, for example through the wild fauna or rodents or the sharing of farm implements, even though such transmission has not been identified in very many cases. In Section 1.3.1 we have briefly described how a study found communal use of farm implements or machinery to be the most likely means of transmission of MRDT104 between farms in one of the known clusters.

MRDT104 is highly clonal and in general DNA based typing methods have insufficient discriminatory power. An as yet uncompleted study at the DVI has attempted to combine results of DNA with information about geographical location and time of detection for infected herds, as well as data

from epidemiological trace-backs in an analysis of the DT104 epidemic (Skov et al., under preparation).

The study involved 166 MRDT104 isolates, each representing 1 herd, detected between July 1996 and July 2002. The herds included primary cases as well as those detected by compulsory trace-back of trade contacts. The distance in straight lines between all farms was calculated on the basis of their latitude and longitude. The herds were categorized into two groups: those belonging to clusters and those where trace back had not revealed a source of infection. For each group, the distance between herds with the same DNA-type was calculated and the cumulative distribution plotted with the distance between herds constituting the X-axis.

The authors tested the hypothesis that the herds were located randomly in time and space, meaning that the distribution of distances from herds not belonging to a known cluster to herds infected with the same DNA type within a time window of 180 days would not differ from the distribution of distances between herds in clusters.

The hypothesis was rejected ($p<0.001$). In other words, herds sharing the same DNA type of MRDT104 tended to be closer to each other in time and geographical location than did herds where the DNA types differed. Among herds with identical DNA types, 50% were located within a distance of 20 km from herds infected with the same DNA type and 25% within a distance of about 7 km, compared to 90 and 60 km, respectively, for all MRDT104 herds. This is an indication that some farms where compulsory trace-back revealed no likely source of infection may have become infected through horizontal transmission of MRDT104, although it cannot be ruled out that some of them may have become infected through trade contacts not detected.

We note that as mentioned in Section 1 of this report, MRDT104 has been demonstrated in two broiler flocks where vertical transmission can almost certainly be ruled out. Furthermore, *Salmonella* Typhimurium DT12, which used to be found exclusively in pigs, now occasionally can be isolated from broiler flocks at ante mortem examination, most likely results from horizontal transmission.

Further temporal analysis of the clustering revealed that the time lapse between detection of infections is more highly auto-correlated than would be expected to happen by chance. This is

natural, given that a proportion of known positive herds are detected by the trace-back procedures. In contrast, looking only at the index cases there is no evidence of clustering in time. This is an indication that during the 6-year period that MRDT104 has been present in Danish herds, there has been no 'runaway' epidemic spread of the infection. The analysis, however, provides no information about whether this is a result of the specific MRDT104 intervention measures, or a result of the general *Salmonella* control programme.

4.3 Conclusions

Salmonella bacteria can survive for extended periods following deposition on farmland. In a recent study involving pig slurry naturally contaminated with MRDT104 at a low concentration, sampling did not recover DT104 from soil after 1 week, using a method with limited sensitivity. Had the concentrations of *Salmonella* applied been higher, the time to final recovery would have been longer.

Wildlife most often is *Salmonella* free but may become infected through contact with infected farm animals or their waste. It is likely that flies, rodents, birds or other animals can transport MRDT104 from slurry deposited on farmland into the farm environment. However, the risk has not been quantified.

An analysis combining data on the DNA types of MRDT104 from 166 Danish farm with information about their geographical location and the time of detection indicated that horizontal transmission may have played a role in the infection of some farms where epidemiological trace-back did not show trade contact as a likely cause. Among farms most likely infected horizontally are two broiler flocks.

4.4 References

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