Prevalence estimates for waterborne zoonotic pathogens in Sweden: a review of available information

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Content

Introduction

Water is a powerful medium for transport and transmission of infectious agents. Several zoonotic pathogens can infect humans and animals through water consumption or from using water for recreation and irrigation. The pathogens can originate from both domestic and wild animals, and may reach the water either directly or indirectly through run-off from grazing areas, nesting sites or farm land treated with manure-based fertilisers. However, lack of comprehensive information about the occurrence and prevalence of zoonotic pathogens in different Swedish animal species renders risk estimation difficult. This in turn has major implications for the current understanding of the role of water in epidemiology and for assessment of risk minimisation measures to prevent the spread of infection to humans and animals via water.

To achieve a good understanding of current knowledge about the occurrence and prevalence of a number of zoonotic pathogens in different Swedish animal species (cattle, sheep, poultry, pigs, horses and wild animals) a careful inventory of existing published information (scientific or grey literature) was performed. The pathogens were selected based on previous knowledge regarding their capacity to spread disease through water. The inventory covered the following pathogens:

- *Campylobacter*
- *Cryptosporidium*
- *Giardia intestinalis/lamblia*
- Hepatitis E virus (HEV)
- *Salmonella*
- Verotoxin-producing *Escherichia coli* (VTEC)

For each of these pathogens, veterinary epidemiologists at the National Veterinary Institute of Sweden (SVA) performed a thorough review of published studies on occurrence in Sweden over the past 20 years (from 1994 to 2014). The literature review was complemented with information from the following sources: strategic documents published in the past 10 years, reports from the Swedish Board of Agriculture in the past 10 years (from 2004 to 2014), a review of projects in FOKA (SVA's internal system for documentation of research projects), SVA's surveillance reports in the past 10 years (from 2004 to 2014) and contact with in-house experts to check whether any other sources of information were available. Since the inventory was completed in 2014 data published after this is not included in the following report.

All available information was reviewed in order to document epidemiological conclusions regarding the validity of the findings as disease measures in the population, i.e. whether the findings could be used in risk assessments as prevalence estimations. This report presents the results of this evaluation. Studies which provide valuable prevalence estimates are reported. All other studies that contributed information on the occurrence of the named waterborne pathogens in animal populations in Sweden, but which cannot be used as prevalence estimates, are also presented and their shortcomings discussed. The validity of studies for prevalence estimation was based on an epidemiological evaluation of the study design,

considering aspects such as target population and sampling design (number of samples and, most importantly, selection of animals and herds).

The inventory presented below, which deals separately with the individual pathogens, summarises the epidemiological information collected and highlights knowledge gaps, centred on four themes: prevalence in herds, prevalence in animals, zoonotic aspects and age-related patterns.

The results from the inventory clearly shows that the current knowledge about prevalence of zoonotic pathogens in Swedish animals varies between different pathogens and also within pathogen between animal species. Knowledge gaps about prevalence exists for all pathogens in some species. The most solid documentation regarding prevalence in Sweden is found for *Salmonella*. Several surveillance activities regarding *Salmonella* are performed each year in food producing animals in Sweden to ensure a low prevalence. Other pathogens where surveillance activities are performed regularly are *Campylobacter* and VTEC. No surveillance is carried out for the rest of the pathogens covered in this report.

Variations in the host range (i.e. zoonotic potential) between species and subtypes of pathogens included in this report can occur. In many cases included studies do not investigate if the pathogens found are of a zoonotic type. Note that lack of subtyping may limit the possibility to determine the role of zoonotic transmission in epidemiology and to perform risk assessment.

The information gathered and presented in this report can be useful in qualitative and quantitative risk assessments and also as input in modelling the potential spread of the pathogens to and from water. **However, it is important to bear in mind that prevalence estimates vary depending on the sensitivity of the tests used, as well as the surveillance effort and thus it is recommended that any use of the information presented here for such purposes be carried out in cooperation with veterinary epidemiologists with knowledge in this field.**

Campylobacter

No published peer-reviewed prevalence studies for *Campylobacter* in Swedish farm animals were found. Results of surveillance in abattoirs are reported below for poultry and results from reports and unpublished data for cattle. These two reservoirs are perhaps the most important sources of *Campylobacter* when considering the implications for human health. We also report some studies and data for dogs, sheep, pigs, wild birds and other wild animals.

CATTLE

In a study by Waldenström *et al.* (2007) faeces samples from grazing cattle were tested for presence of *Campylobacter*. In the study, only a single herd was tested, on Gotland in 2001, and *C. jejuni* was identified in 9/71 samples (originating from 25 cattle), while one sample tested positive for *C. lari*. Note, however, that the study was not designed to estimate prevalence and that the testing of a single herd provides no epidemiological information about the presence of *Campylobacter* in the cattle population in Sweden.

A report was identified where 426 faeces samples were collected at two abattoirs from cattle originating from 249 herds. The herd-level prevalence is described in Table 1. The results show seasonal variation in the prevalence of *Campylobacter* in beef cattle and higher prevalence in younger animals than in animals closer to slaughter age. In the study, the authors reported that only 53/192 isolates were successfully typed, but of those 48 were *C. jejuni*, four were *C. coli* and one was *C. lari* (Blixt *et al.,* 2001).

Table 1. Herd-level prevalence of *Campylobacter* spp. reported in 249 Swedish beef herds, 1999-2000 (Blixt *et al.,* 2001)

Age category	Prevalence	
	Summer	Winter
Calf rearing	24/30 (80.0%)	20/31 (64.5%)
Young stock	21/45 (46.7%)	10/52 (19.2%)
Finishers	10/48 (20.8%)	13/53 (24.5%)

From October 2011 to October 2012, SVA was responsible for systematic sampling of dogs, pigs, sheep, cattle and poultry in order to compare the types of *Campylobacter* found in those species to those found in humans. This project and the data collected as part of it are referred to as the '2012 *Campylobacter* source attribution project'. In the project, *C. jejuni* was identified in 46/203 (22.7%) cattle faecal swabs at slaughter, 6/203 (2.96%) tested positive for *C. coli* and 2/203 were untypeable. A farm identifier was available for 156 samples representing 136 farms. Of these, 37/136 (27.2%) farms were positive for *C. jejuni*, 4/136 (2.9%) for *C. coli* and 1/136 for an untypeable strain of *Campylobacter* (unpublished results). **The herd-level prevalence of 27.2% for *C. jejuni* should be considered the most reliable estimate for Swedish cattle, but it should be borne in mind that there is evidence of seasonal variation in prevalence.**

PIGS

No published Swedish prevalence investigation of *Campylobacter* at farm level was identified in the recent literature (1994-2014). There was one study that tested pig carcasses at slaughter and identified 1% (six samples; four *C. coli* and two *C. jejuni*) carcass prevalence after swabbing 541 carcasses at 10 slaughter plants in the country in 2007 (Lindblad *et al.*, 2007). However, this is not a good estimate of the prevalence of *Campylobacter* at farm level, since no information is given in the report regarding the herd of origin of the sampled carcasses and the study was primarily designed to estimate contamination at slaughter, and not prevalence.

Unpublished data from the 2012 *Campylobacter* source attribution project, based on slaughter faecal swabs, indicated that most pigs are positive for *Campylobacter* spp. (149/190, 78.4%) in Sweden, as in other regions of the world (Varela, Friendship & Dewey, 2007). Of the 149 positive samples, two were *C. jejuni,* 135 were *C. coli* and 12 were untypeable. The herd of origin was recorded for 176 samples, representing 133 herds. Of these, 106/133 herds were positive for *Campylobacter* spp., 2/133 for *C. jejuni,* 98/133 for *C. coli* and 7/133 for untypeable *Campylobacter*. **A reliable estimate of herd-level prevalence of *C. jejuni* in Swedish pigs is approximately 1.5%.**

SHEEP

In a study (only published as a conference poster) by Engvall *et al.* (1999), sheep were sampled at slaughter and faeces samples were examined for presence of *Campylobacter*. In total, 583 ewes and 404 lambs were sampled at seven different abattoirs in Sweden. The results showed that approximately 10% of the samples contained *Campylobacter* spp., with slightly more in samples from lambs (13%) than from ewes (7%).

As part of the 2012 *Campylobacter* source attribution project, SVA collected faeces samples from 425 sheep (lambs) at slaughter and tested these for presence of *Campylobacter*. In total, 62 samples contained *Campylobacter*, 58 (13.6%) of which were identified as *C. jejuni,* three as *C. coli* and one as *C. lari*. A farm identifier was available for 382 of these samples, which originated from 237 farms, and 47 (19.8%) of these farms were positive for *Campylobacter* spp., while three farms were positive for *C. coli* and 44 for *C. jejuni*. The *C. jejuni* types identified are also commonly found in humans. Only two of the 58 isolates were types that were not also identified in humans during the same period. **A herd-level prevalence in 2012 of 19.8% is a reliable estimate of herd prevalence of *Campylobacter* spp., with 18.6% *C. jejuni.***

POULTRY

Poultry are perhaps the most important reservoir of *Campylobacter* when considering the implications for human health. This is because poultry are commonly colonised with *C. jejuni*, which is the most common cause of human campylobacteriosis. The published literature contains a prevalence estimate based on the Swedish surveillance system results of 2004-2005 (Hansson *et al.*, 2010). The samples were collected at slaughter and are summarised at the slaughter batch level, and represent 37 animal-holding locations. The batch level prevalence was 21.3%, but during the study period 34/37 (91.9%) farms delivered a positive batch. The batch-level prevalence has decreased since 2004. According to data collected in the 2012 *Campylobacter* source attribution project, 205/2377 (8.6 %) slaughter batches tested by coecal

swab were positive for *Campylobacter*. Of the positive batches, most (202/205, 98.5%) were *C. jejuni,* while the remaining three positive samples were *C. coli.*

There is a marked seasonality in *Campylobacter* presence in poultry in Sweden. The Swedish surveillance data from 2001-2005 indicate that late summer and early autumn have higher *Campylobacter* rates than winter and spring (Hanson *et al.*, 2007). In 2014, thermophilic *Campylobacter* spp. were detected in 363 (11.5%) of the 3162 broiler flocks at slaughter in the national campylobacter programme. **The flock-level prevalence estimates from the surveillance programme in broilers are a good measure of the flock-level prevalence in Swedish broilers.**

WILD BIRDS

The occurrence of *Campylobacter* in wild birds in Sweden has been investigated in both the urban shore bird population of Malmö (Broman *et al.*, 2002) and in migratory birds passing over the Baltic Sea at Öland (Broman *et al.*, 2004; Waldenstrom *et al.*, 2005; Waldenstrom *et al.*, 2009) and Gotland (Waldenstrom *et al.*, 2007). In the study that sampled blackheaded gulls in Malmö, 786 faeces samples were collected in 1999 and 2000, of which 235 (29.9%) tested positive for *C. jejuni*. The study found no significant difference in colonisation by age of the bird, but found a seasonal pattern in the prevalence, the highest in autumn and the lowest in winter. The paper does not report relative odds between the seasons (Broman *et al.*, 2002). The study of migratory birds on Gotland was completed in late spring to early summer 2001 and included faeces samples from 247 birds, primarily redshank and barnacle geese (Waldenstrom *et al.*, 2007). Of these, seven (2.8%) were found to be positive for *C. jejuni* and 112 (45.3%) for *C. lari*, which is not responsible for many human infections. The study of migratory birds at Öland was conducted in 2000 and included 1794 individual birds, of which 89 (4.9%) were *C. jejuni*-positive (Waldenstrom *et al.*, 2012). The isolates from the study were further subtyped as part of another publication, where they were compared to *C. jejuni* isolates recovered from humans and were found to be dissimilar from the human strains (Broman *et al.*, 2004).

In a study of antimicrobial susceptibility of *Campylobacter* from Swedish wild birds, a total of 137 isolates of *C. jejuni* were investigated, of which 20 (14.6%) were resistant to metronidazole, five (3.6%) to amoxicillin and one each to ciprofloxacin, nalidixic acid and doxycycline (Waldenstrom *et al.*, 2005). In the study, shore birds had a higher prevalence of resistant isolates than thrushes and raptors.

Within the 2012 *Campylobacter* source attribution project, SVA collected faeces samples from migratory birds from Öland and tested them for *C. jejuni*. A total of 1480 samples were collected, of which 125 (8.4%) were positive. These isolates were typed and compared to human isolates obtained from Swedish human *C. jejuni* cases in the same period; only seven of the 125 (5.6%) isolates were types that were also present in humans.

OTHER WILDLIFE

Wahlström *et al.* (2003), published a study on the occurrence of *Campylobacter* species in hunted wildlife and found that 7/31 pooled samples from 66 wild boar were positive for *Campylobacter* (three *C. coli*, two *C. jejuni*, two other); 1/38 pooled samples from 86 moose was positive (*C.*

jejuni); 7/69 pooled samples from 172 roe deer were positive (five *C. jejuni,* one *C. hyointestinalis,* one other*)*; 1/47 pooled samples from 118 hares was positive (*C. coli)*; and none of 90 red deer tested had *Campylobacter*. Some of the samples were frozen prior to analysis, which may have had an effect on the number of positive samples.

DOGS

A study of 91 healthy dogs older than 5 months in 2001 identified *C. jejuni* in 10 dogs (11.0%) (Engvall *et al.*, 2003). In 2011-2012, SVA collected faeces samples from 180 dogs as part of the *Campylobacter* source attribution project. The samples were tested for *C. jejuni* and seven (3.9%) were found to be positive for *C. jejuni*. The types identified are also commonly found in humans. Fifty-two (28.8%) faeces samples contained *C. upsaliensis*, which rarely causes human illness (Holmberg *et al.*, 2015).

HORSES

No published studies on the occurrence of *Campylobacter* in Swedish horses could be found from the past 20 years.

RODENTS

Rodents may play a role in the maintenance and spread of *C. jejuni* in farm environments. A study of the prevalence of several zoonotic pathogens in rodents found in proximity to poultry and pig farms identified *C. jejuni* in 3/114 rodents caught close to pig farms and 5/58 rodents caught around poultry farms (Backhans *et al.*, 2013). In the study 19/114 and 1/58 rodents close to pig and poultry farms, respectively, tested positive for *C. coli*.

Cryptosporidium

The zoonotic potential varies between different *Cryptosporidium* species and also between subtypes within the species. Prevalence studies of *Cryptosporidium* spp. in Swedish animals were only found for cattle and showed high prevalence of the species *C. bovis*, which is not zoonotic. Strong age-dependent patterns were observed. *Cryptosporidium parvum*, a zoonotic type, is only found in calves up to 2 months, with low prevalence.

CATTLE

Presence of *Cryptosporidium* in the cattle population

A cross-sectional study of cattle **dairy** herds (Silverlås *et al.*, 2009) showed that *Cryptosporidium* spp. oocysts morphologically similar to *C. parvum* were present in up to 90% of herds (microscopic examination does not allow differentiation of *C. parvum* from two other morphologically identical, but genetically different species, *C. bovis* and *C. ryanae,* both of which are not zoonotic). However, the prevalence was highly age-dependent (see "Age-related patterns"). *C. andersoni* oocysts were found in at most 8% of herds (see "Age-related patterns"). Within-herd prevalence cannot be estimated from the study design.

Another study focusing on suckler beef calves detected *Cryptosporidium* spp. in calves under 90 days of age in 29 out of 30 herds (97%) (Bjorkman *et al.*, 2015). It is important to note that there is a strong age-related pattern in the distribution of this pathogen (as detailed in the "Age-related patterns" section), and that the study focused in the most affected age group (calves). It is also important to note that the study used a convenience sample of only 30 herds in two regions of Sweden (Halland and Uppsala-Örebro), and therefore caution is needed when generalising the findings to prevalence estimates in the Swedish beef cattle population.

Species of *Cryptosporidium*

Molecular characterisation of the *C. parvum*-like oocysts found in **dairy** cattle in the study by Silverlås *et al.* (2009) was performed in a subsequent study by those authors (Silverlås *et al.*, 2010b). The results showed that *C. bovis*, which is non-zoonotic, was the dominant species (see "Age-related patterns" for exact numbers).

In a later study (Silverlås *et al.*, 2013), 782 samples from routine diagnostics at SVA, i.e. samples from calves with diarrhoea submitted for diagnosis, were tested for *Cryptosporidium*. Of those samples, 198 were positive for *Cryptosporidium* spp., the species being identified as *C. parvum* in 178 samples, *C. bovis* in six samples and both species in seven samples. *C. parvum*-positive calves were younger than *C. bovis*-positive calves. Besides this age-dependent pattern, it should be noted that this is a sample from animals with diarrhoea and therefore not representative of the healthy cattle population. In particular, *C. parvum* is associated with diarrhoea, while *C. bovis* is not (Silverlås *et al.*, 2010a), and therefore samples submitted for diagnostics can be expected to be biased towards a higher prevalence of *C. parvum*.

In a study which focused on beef cattle (Bjorkman *et al.*, 2015), species was determined in 113 out of 122 positive samples. In total, *C. parvum* was identified in 27 samples, which represented

8.1% of the total 332 tested, and *C. bovis* was identified in 89 samples (26.8% of all samples). Again, note that the focus was on young animals.

The information from these studies (which cannot be used as a prevalence estimate) shows that *C. bovis* is the most common species in Swedish cattle, but *C. parvum* seems to be associated with diarrhoea in very young calves.

Age-related patterns

The prevalence of *Cryptosporidium* spp. is higher in younger animals. Silverlås *et al.* (2009) found *C. parvum*-like oocysts (which, as mentioned before, could be of the species *C. parvum, C. ryanae* or *C. bovis*, the latter two non-zoonotic) in calves (<2 months) in 45 out of 50 dairy herds (90%), in young stock (4-12 months) in 41/50 (82%) herds and in cows (1 week pre-partum to 2 weeks post-partum) in 38/50 (76%) herds. In the same study, *C. andersoni* was found in calves in 1/50 herds (2%) and in young stock in 4/50 herds (8%), and was not found in cows.

In a subsequent study (Silverlås *et al.*, 2010b), 110 positive samples from the previous study were subjected to molecular characterisation (results shown in Table 2). ***C. parvum* was only dominant in very young calves** (less than 14 days). In all other ages, *C. bovis* was the dominant *Cryptosporidium* spp.

Table 2. Age-related *Cryptosporidium* spp. detection in dairy cattle (Silverlås *et al.,* 2010b). Note that percentages refer to the proportion of positive samples identified as each species, (NOT percentage of positive samples)

Age	*C. parvum*	*C. bovis*	*C. ryanae*
<15 days (n=21)	52%	43%	5%
15-21 days (n=10)	20%	70%	10%
22-28 days (n=9)	11%	89%	0%
29-42 days (n=21)	5%	86%	9%
43-59 days (n=12)	0%	100%	0%
4-12 months (n=33) (plus 2 samples with *C. andersoni*)	0%	82%	18%
Cows pre- and post-partum (n=2)	0%	100%	0%

Bjorkman *et al.* (2015) found a lower prevalence of *Cryptosporidium* in beef calves compared with dairy calves. On each farm tested, the number of positive calves was between 6.3 and 75% (median 42.3%). In the study, cattle over 90 days of age were not tested.

Zoonotic link

All *C. parvum* subtypes identified by Silverlås *et al.* (2010b) were zoonotic. However, note that the findings previously presented demonstrate that this species is only prevalent in very young calves, and is associated with diarrhoea.

Within-herd prevalence

None of the studies reviewed provides evidence to estimate ***within-herd prevalence***.

OTHER SPECIES

No prevalence studies were found in other animal species in Sweden.

A study on a farm with human cases (Silverlås *et al.*, 2012) identified four positive chickens from 27 investigated, with the species present identified as *C. meleagridis*. *Cryptosporidium* spp. were not detected in samples from pigs, calves, sheep and goats on the same farm.

Backhans *et al.* (2013) placed rodent traps on 16 pig farms, five chicken farms and seven non-farm locations. Of 207 rodents tested, 11% were positive for *Cryptosporidium spp.* cysts, mainly on pig farms, but a zoonotic link was not found in molecular characterisation.

Between 2009 and 2013, positive *Cryptosporidium* spp. samples have been found at SVA in the following animals (positive/samples tested): Rodents in general (4/101), camels (1/79), sheep (5/35), deer (3/23), alpacas (1/16) and monkeys and apes (1/8).

OTHER STUDIES NOT RELEVANT FOR PREVALENCE ESTIMATION

Björkman *et al.* (2003): This study carried out in 1998-1999 (Skaraborg) and involving 75 cattle farms found that 17 farms (22.7%) were positive for *Cryptosporidium* oocysts (*parvum*-like). Oocysts were found in 16/146 (11%) of calves (up to 90 days) with diarrhoea and 6/124 (4.8%) of calves without diarrhoea. However, this was not a prevalence study. Animals were chosen based on presenting diarrhoea, and healthy animals were matched to those on farms where animals were showing clinical signs.

Torsein *et al.* (2011): This was a case-control study, where 30 cases and 30 controls were selected based on high and low mortality in calves. The study found 32.4% of animals positive for *Cryptosporidium*, but only five animals per herd were tested and the study was NOT designed for animal prevalence.

Giardia

There are no prevalence studies of *Giardia intestinalis*, which is the species of Giardia that is associated with giardiasis in humans, in Swedish animals but it is known to occur. To date *Giardia intestinalis* is divided into 8 genetic groups (assemblages), assemblage A to H. Assemblage A and B are found in humans. Assemblages also contain a number of genetic variants, forming so called sub-assemblages that are more or less common in humans and different animal species.

CATTLE

It is known that *Giardia* is present in cattle in Sweden, but there is no good estimate of the prevalence.

Björkman *et al.* (2003) found *Giardia* on 38 of 75 farms enrolled in a study, but this was not a representative sample intended to study prevalence, but rather a case-control study investigating the causes of diarrhoea. Only calves were tested, and *Giardia* spp. was found in the faeces of 42/146 (28.8%) calves with diarrhoea, while in calves not presenting diarrhoea the number of positives was 29/124 (23.4%). No genotyping was performed in the study.

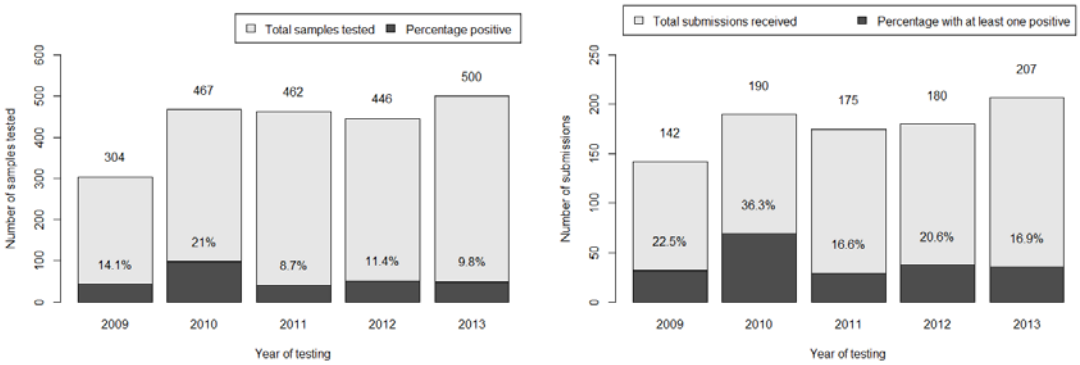

Figure 1. Results of tests performed at SVA on cattle. Percentage of confirmed *Giardia* spp. in different years relative to number of samples tested (left) and number of submissions (right).

SHEEP

It is known that *Giardia* is present in sheep, but there is no good estimate of the prevalence. There is only one study available from 2001 (Ljungström *et al.*, 2001) where lambs older than 7 weeks were tested for presence of *Giardia* cysts in faeces samples. Seventeen farms were chosen based on the presence of diarrhoea, together with 11 control farms, so the **study was not intended to calculate the prevalence** of herds with *Giardia* in Sweden. Twelve of the herds with diarrhoea were found to be positive for *Giardia* (70.6%) and seven of the herds without diarrhoea (63.6%) were also positive. These figures were important in showing that there was no statistical difference in prevalence between the farms with and without diarrhoea, but due to the purposeful selection of herds with diarrhoea (and a non-explained process of selection of herds without diarrhoea) the figures are not useful for prevalence estimation. The *within*-herd prevalence is not reported. It is reported that 16 of 64 (25.0%) lambs with diarrhoea were positive, as well as 10 of 55 lambs without diarrhoea on problem farms (18.2%)

and 10 of 44 lambs on farms without diarrhoea problems (22.7%), but these numbers are not detailed by farm. No genotyping was performed in the study.

DOGS AND CATS

It is known that *Giardia* is present in dogs, but there is no good estimate of the prevalence. It is known that the prevalence is higher in young animals. In two documented studies (Castor and Lindqvist 1990; Florén 2008), ***no zoonotic*** link was found. In these two studies, the prevalence in puppies was found to be 33% and in adult dogs 0-1%, but these studies were based on convenience sampling and small samples, and are therefore not representative of any dog population in Sweden.

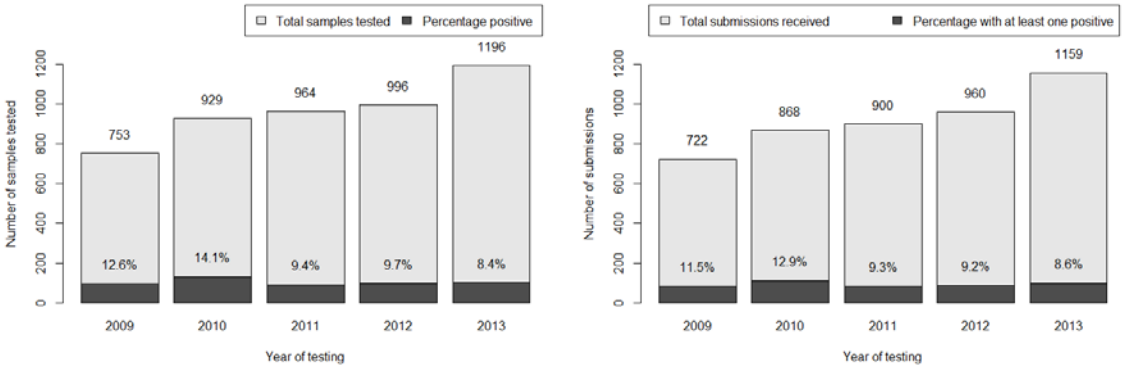

Figure 2. Results of tests performed at SVA on dogs. Percentage of confirmed *Giardia* spp. in different years relative to (left) number of samples tested and (right) number of submissions.

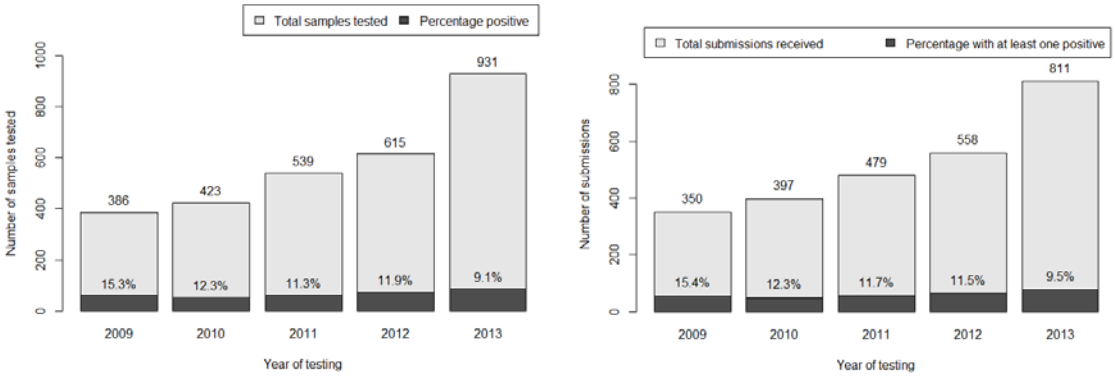

Figure 3. Results of tests performed at SVA on cats. Percentage of confirmed *Giardia* spp. in different years relative to (left) number of samples tested and (right) number of submissions.

OTHER SPECIES

Backhans *et al.* (2013) placed rodent traps on 16 pig farms, five chicken farms and seven non-farm locations. Of 207 rodents tested, 13% were positive for *Giardia* cysts, mainly on pig farms, but a ***zoonotic link was not found*** in molecular characterisation.

Lebbad *et al.* (2010) examined 114 samples sent to diagnosis or from studies in rodents, including the following animal species: dogs, cats, sheep, cattle, moose, yalk, deer, guinea pig, rabbit, rats, mice and non-human primates. No sub-assemblages closely related to A II, the

most common A sub-assemblage in humans, were found. The following assemblages which can also affect humans were found:

- Assemblage A: Found in cat, dog, sheep, fallow deer, moose
- Assemblage B: Found in a rabbit (whose owner had a history of *Giardia* diarrhoea, but was not tested), guinea pig and monkey
- Assemblage E: Found in three sheep (which also had *Giardia* assemblage A). Not zoonotic.

Reviewing all samples tested at SVA from 2009 to 2013, *Giardia* spp. oocysts have been demonstrated in 40/101 rodents tested, 26/79 camel samples, 5/35 sheep, 6/21 cheetah, 8/19 deer, 1/16 alpacas, 1/16 lemur, 1/7 monkeys; 1/4 lizards, 3/4 chinchillas and 2/2 hamsters.

Hepatitis E virus

There has been no prevalence study of hepatitis E virus (HEV) in the animal population in Sweden, but the pathogen has been investigated in a number of studies. Relevant epidemiological findings are summarised below.

PIGS

Piglets

Norder *et al.* (2009) tested 18 piglets found positive for HEV in other studies in Denmark and Sweden (the work did not state how many pigs from each country). The authors stated that "Phylogenetic analyses of the genotype 3 strains showed geographical clades and high similarity between strains from patients and pigs from the same area", concluding that "even though most hepatitis cases in Nordic areas are acquired in Asia, there are autochthonous cases probably due to contact with pigs or foodborne transmission".

Widen *et al.* (2011) tested 240 animals in 22 farms in Sweden and found HEV in 26.9% of animals and 72.7% of farms (all were genotype 3 strains). The number of positives per samples tested, broken down per area, was: 10/10 Dalarna, 15/20 Örebro, 13/50 Västmanland, 26/140 Uppsala and 0/10 Stockholm. However, the process used to select these 22 farms is not explained and therefore these estimates should be used with care. Note also that only young animals were tested (which are expected to have higher circulation of HEV than older animals). The authors concluded that there is "probable endemic circulation of genotype 3 in Sweden".

In summary, the risk of zoonotic transmission cannot be measured based on current studies.

Adults

Banks *et al.* (2004) selected 294 samples from a surveillance programme for Aujeszky's disease (selection process not explained) in Sweden and found 58% SERO-positive for HEV (presence of ANTIBODIES, not of the virus). However, the zoonotic link was not investigated. As the selection process is not explained, these numbers cannot be used as a prevalence estimate.

WILD ANIMALS

Wild boar

Widen *et al.* (2011) tested serum from 159 wild boars (40% piglets and 60% yearlings) sent in by hunters in southern Sweden and found HEV in 14.8% of piglets and 4.8% of yearlings (total of 8.2% positive animals). The number of positives per samples tested, broken down per area, was: 1/2 Värmland, 0/8 Västra Götaland, 0/6 Jönköping, 7/56 Halland, 2/20 Skåne, 1/30 Kronoberg, 2/34 Östergötland, 0/12 Sörmland and 0/1 Uppsala. Prevalence estimates based on samples sent by hunters can be used as approximate estimates of prevalence if it is assumed that the animals hunted are a random sample of the wild boar population and that the infection status of the animals does not affect (positively or negatively) their chance of being caught by a hunter. Under those assumptions, the sample size used in this study would allow 4% precision in the prevalence estimated with 95% confidence, i.e. the 95% confidence

interval (CI) for the true prevalence is 4-12%. Note, however, that this confidence interval refers to the apparent prevalence found and it disregards possible imperfections in the tests used to identify positive animals (false positives or false negatives could exist).

A poster from 2009 (Widen *et al.,* 2009) mentions a possible zoonotic link, suggesting evidence of close phylogeny among cases in humans, wild boar and swine. No more details were not given (poster format).

Moose

Lin *et al.* (2015) tested serum from 231 Swedish moose (51 of which also had available faeces samples) for the presence of HEV RNA and anti-HEV antibodies. All samples were provided by hunters or through their assistance. The moose samples were obtained from seven Swedish counties: Öland, Småland, Västergötland, Södermanland, Västermanland, Värmland and Västerbotten. The number of samples reactive for HEV RNA was 34 (apparent prevalence 14.7%, 95% CI 10.5-20.1%). The apparent seroprevalence (which identifies animals previously exposed to the virus, so it is a cumulative effect of past and ongoing infections and not a representation of the prevalence of currently infected animals) was 18.6% (95% CI 13.9-24.4%). The prevalence of samples considering all HEV markers investigated (antibodies and RNA) was 29% (95% CI 23.3-35.4%).

As mentioned above, prevalence estimates based on samples sent by hunters can be used as approximate estimates of prevalence if it is assumed that the animals hunted are a random sample of the moose population and that the infection status of the animals does not affect (positively or negatively) their chance of being caught by a hunter. An additional concern is that the number of samples is not determined beforehand to estimate prevalence with a desired precision. However, the confidence intervals presented above reflect the uncertainties associated with the prevalence estimated, given the sample size achieved. Again, note that this confidence interval is for the apparent prevalence found and it disregards possible imperfections in the tests used to identify positive animals (false positives or false negatives could exist).

No statistically significant differences were found among age groups or between genders. All samples were collected during autumn, after the mating period.

Infection markers were found in moose from all regions of Sweden apart from the north of the country. However, it is not clear whether this refers to regional differences in the prevalence of HEV, or is a reflection of the different densities of moose across the country. The number of samples sent by hunters from Västerbotten was only eight.

The zoonotic potential of the virus found is not known. Lin *et al.* (2015) concluded that "the phylogenetic relationship demonstrated that the moose HEV belonged to the genotype 1-6 group, which includes strains that also infect humans". Therefore, zoonotic potential cannot be excluded, but was not demonstrated in the study.

Salmonella

Based on available data, it can be concluded that the prevalence of *Salmonella* in the major food-producing animals and also wildlife is very low, and the major food-producing animals can be considered to be virtually free from *Salmonella*. It can also be concluded that neither domestic food-producing animals nor domestic wildlife are an important (direct or indirect) source of *Salmonella* for humans in Sweden.

It should be borne in mind that prevalence estimates will vary depending on the sensitivity of the tests used, as well as the surveillance efforts. This is also the case in this summary. For example, the general surveillance, which is the basis for the official statistics, is the result of combining different surveillance activities which vary over time. Furthermore, different tests give different results. Serological tests reflect past exposure (animals with antibodies to *Salmonella*) and this is not the same as infected and or infectious herds. Bacteriological tests reflect that the bacterial species (infectious agent) is present, while isolation in faeces truly demonstrates infectious animals. However, if lymph nodes are examined, a positive test indicates an infected, but not necessarily infectious, animal. Finally, different *Salmonella* serotypes (there are >2000) have differing epidemiology, and knowledge about this is required to estimate the within-herd prevalence. **If modelling or risk analysis are to be performed based on the figures presented in this report, it is recommended that this is done in cooperation with veterinary epidemiologists with knowledge in this field.**

CATTLE

The incidence of *Salmonella*-infected cattle herds is very low. It has decreased and, since the late 1990s, seems to be on a steady low level. The surveillance at slaughter supports this conclusion. There is no estimate of the number of *Salmonella*-infected individual cattle, but it can be concluded that it is very low and that it is most probably stable. Details and available data are given below.

Herd prevalence

The Swedish salmonella control programme is described at www.sva.se. The number of detected infected herds is given in Figure 4. To account for the decreasing number of herds in Sweden (but not for the increasing average number of cows per herd), the number of reported cases/1000 farms was calculated and is reported in Figure 5.

As the sensitivity of the surveillance is not 100% (it detects only parts of the infected herds), the number of newly detected infected herds can be used as the lower bound of the incidence of *Salmonella* in cattle herds. Furthermore, surveillance varies over time. For example, in 2009 a serological survey of all dairy herds increased the sensitivity of the surveillance system.

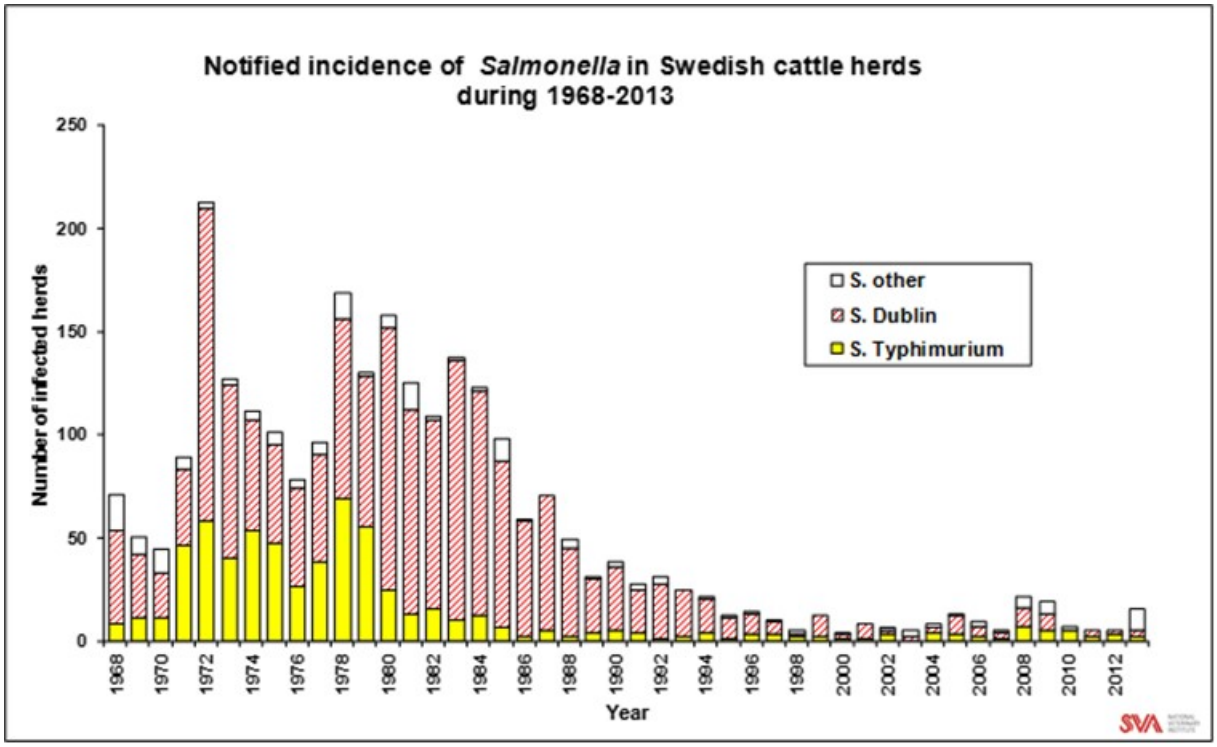

Figure 4. Number of cattle herds infected with *Salmonella* detected in Sweden per year.

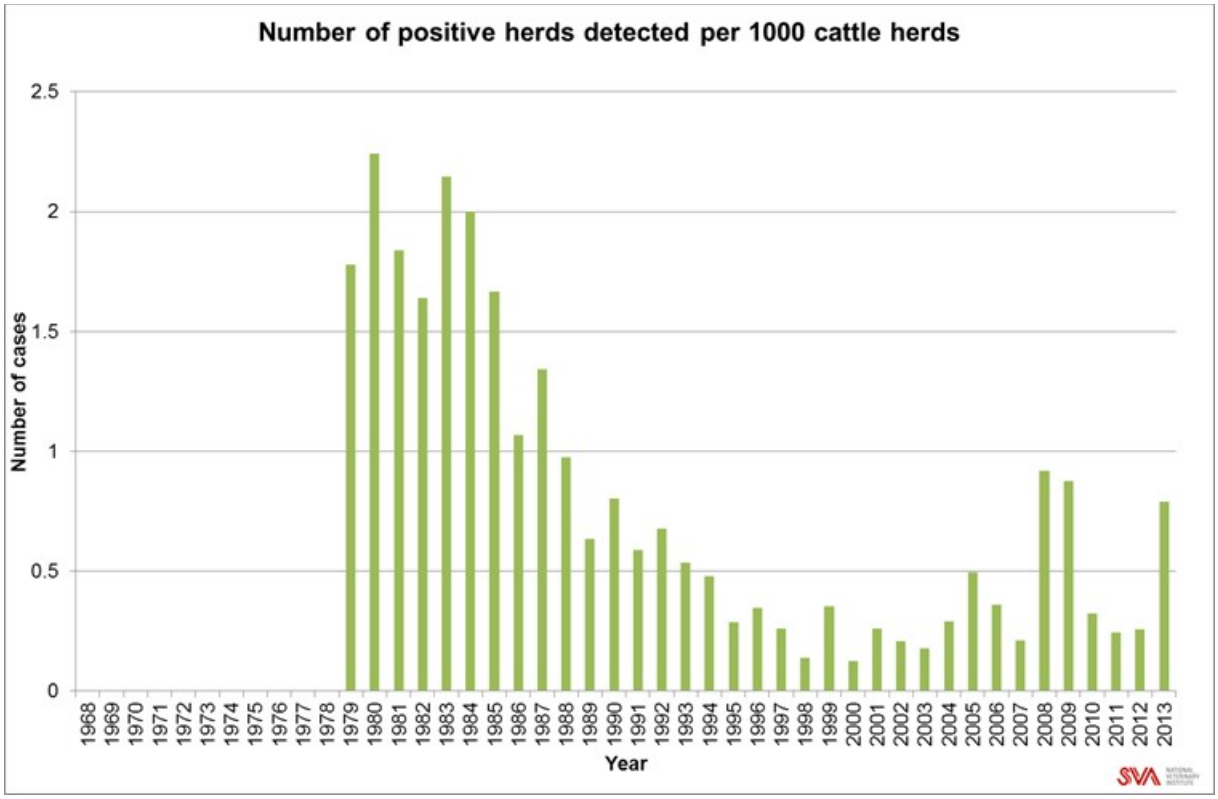

Figure 5. Number of cattle herds infected with *Salmonella* detected per 1000 cattle herds in Sweden per year. Data for the period 1968-1978 are not available.

During 2007, national **serological** monitoring using **bulk-milk testing was performed on dairy herds** (it can detect herds exposed to *Salmonella*, but cannot determine whether animals are truly infected) by randomly including one in every six dairy herds in Sweden (n=1013). Bulk tank milk samples were analysed with two different and indirect ELISA tests, one detecting mainly *S*. Dublin (Dublin ELISA) and a second detecting mainly *S*. Dublin and *S*. Typhimurium (mixed ELISA). Although the manufacturer of the ELISA recommended a cut-off at PP 35, a more conservative cut-off at PP 20 was used (Ågren 2012). If a higher cut-off had been used, the proportion of positive herds would have been lower. This has to be considered when comparing the prevalence estimates reported here with those in other studies.

During 2013, national serological monitoring including all dairy herds (n=4683) was conducted (census) using the same tests as reported in 2007 (Dublin and mixed ELISA and a cut-off at PP20; Ågren, 2012; Ågren *et al.,* 2016).

In 2009, surveillance of all dairy herds on the island of Öland (n=204), a region with a higher prevalence of *Salmonella*, was carried out using Dublin ELISA and a cut-off at PP 35. The results of this study cannot be compared with those of the national serological monitoring, as a different cut-off was used. Further bacteriological investigations of 32 seropositive herds (PP>35) showed that 7/32 (22%) were bacteriologically positive (Ågren, 2010). It should be pointed out that this figure probably varies depending on the serotype to which the herd is positive and that a larger proportion can be expected to be positive if repeated bacteriological sampling is performed.

In the national screening in 2013, 175 herds from Öland were included and 17.7% (0.129-0.444) were positive (15% were positive for *S*. Dublin). It was concluded that the prevalence in this region is still higher than in the rest of the country (Ågren, 2012).

The results of the national bulk milk tests have been compiled at SVA and are shown in Table 3. In the 2007 study, 1.3% of herds were positive in the Dublin ELISA and 4.0% in the mixed ELISA. Corresponding figures for 2013 were 0.9 and 3.0 %. The difference in results between 2007 and 2013 is not significant. The samples in 2007 were taken in the autumn and in 2013 herds were sampled in March and April. It is known, and has been shown e.g. under Danish conditions, that the proportion of bulk milk positive herds is highest in autumn (Nielsen and Dohoo, 2012).

Table 3. Number of surveyed tank milk samples from Swedish dairy companies in 2007 and 2013 and the number and proportion of positive samples in Dublin ELISA and mixed ELISA

Test used and year	Number of samples tested	Number of positive samples	Percentage of positive samples	95% confidence interval
Dublin ELISA, 2007	1013	13	1.3	0.6-2.0
Dublin ELISA, 2013	4683	41	0.9	-- (census)
Mixed ELISA, 2007	935	37	4.0	2.7-5.2
Mixed ELISA, 2013	4683	141	3.0	-- (census)

The Mixed ELISA detected *Salmonella*-positive herds in all counties except Västmanlands County. Using the Dublin ELISA, antibody-positive bulk milk samples were found in nine counties. The highest percentage of positive samples was from Kalmar County (5.8%). The reason for this is the higher prevalence in Öland. The results are in accordance with previous knowledge about salmonella infection in cattle, where infection with *S.* Typhimurium is spread across the country, but at a low level, while infection with *S.* Dublin is primarily concentrated in Kalmar County.

Slaughter animal prevalence

As mentioned above, surveillance varies over time. For example, surveillance at sanitary slaughter, which was an important part of the surveillance, has decreased as sanitary slaughter has virtually ceased since the end of 1990s. In 1995, surveillance at slaughterhouses was initiated as a requirement for Sweden to obtain additional European Union (EU) guarantees. Although designed to estimate the prevalence, surveillance of lymph nodes may also detect infected herds.

Figures 6 and 7 show the results of the systematic surveillance performed at slaughterhouses (required by the EU). Sampling of lymph nodes reflects the number of infected individual cattle, but it does not reflect the number of infectious cattle (i.e. cattle contaminating the environment). Sampling of carcasses by swabs reflects the efficiency of pre-harvest control of *Salmonella*, allowing detection of whether *Salmonella*-excreting cattle are slaughtered and the hygiene at slaughter. It is also a measure of the exposure in humans. It cannot be used as a prevalence estimate of the number of infected individual cattle. It should also be highlighted that the carcass swabbing performed in Sweden uses a large swabbing area and this sampling can therefore be expected to be more sensitive than the swabbing carried out in many other countries.

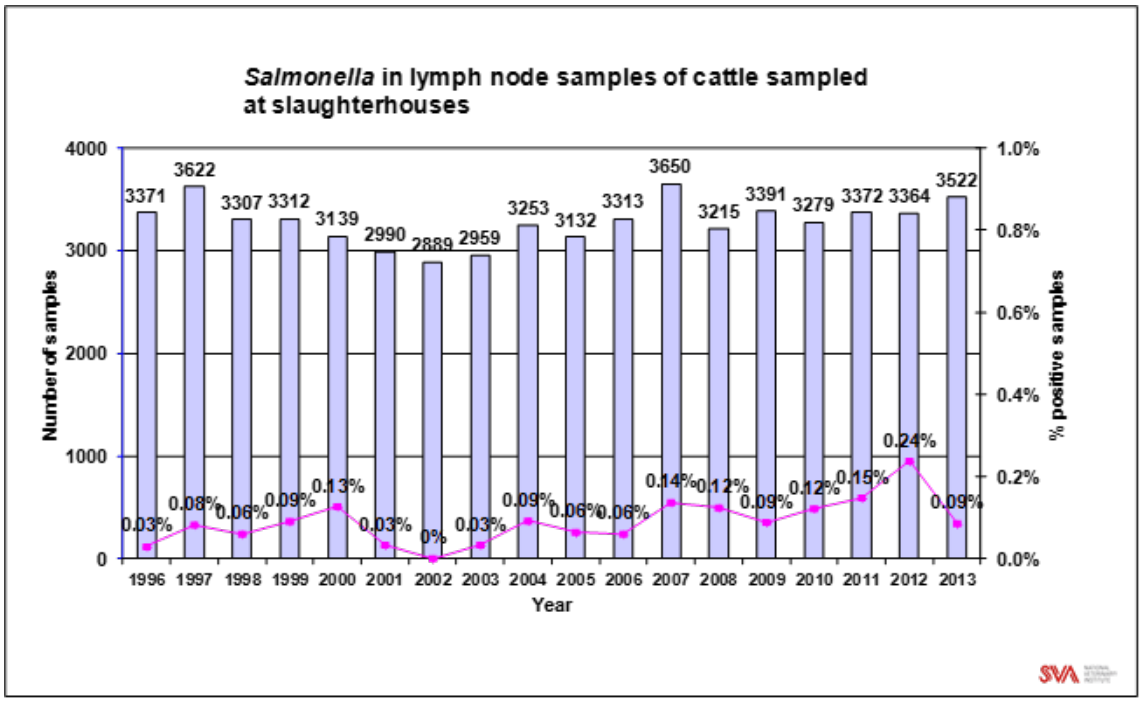

Figure 6. Number of samples of lymph nodes from cattle collected during slaughter and tested for *Salmonella* in Sweden per year, and percentage of positives.

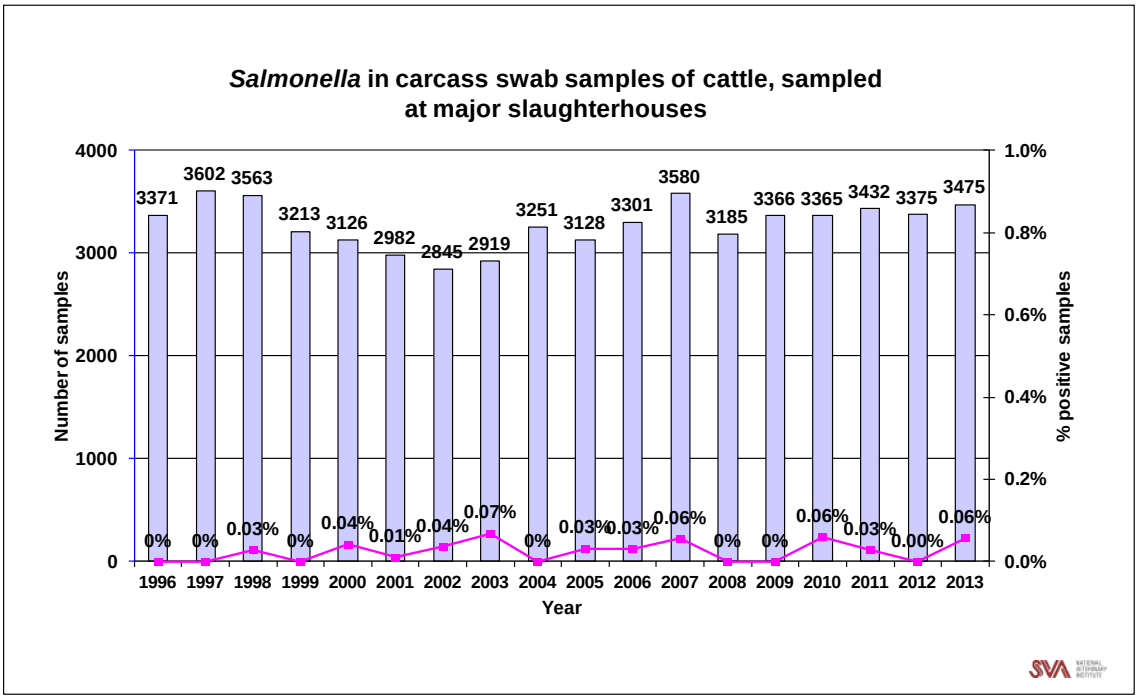

Figure 7. Number of carcass swab samples from cattle collected during slaughter and tested for *Salmonella* in Sweden per year, and percentage of positives.

Within-herd (animal) prevalence

There is no estimate of the number of *Salmonella*-infected individual cattle within herds. As the number of infected herds seems to be stable and the average herd size is increasing, the number of infected individual cattle could be expected to increase (given that within-herd prevalence has not changed over time). However, based on the surveillance at slaughterhouses, there is no indication that this is the case.

PIGS

The incidence of *Salmonella*-infected pig herds in Sweden is extremely low. It has decreased and since the late 1970s seems to be on a steady low level. The surveillance at slaughter supports this conclusion. There is no estimate for the number of *Salmonella*-infected individual pigs in pig herds. However, it can be concluded that it is very low and that it is most probably stable. Detailed numbers and available data are presented below.

Herd prevalence

The Swedish *Salmonella* control is described at www.sva.se. The number of detected infected herds is shown in Figure 8. As the sensitivity of the surveillance is not 100%, the number of newly detected infected herds can be used as the lower bound of the incidence of *Salmonella* in pig herds. Furthermore, surveillance varies over time, resulting in a higher number of detected cases when more surveillance actions are implemented.

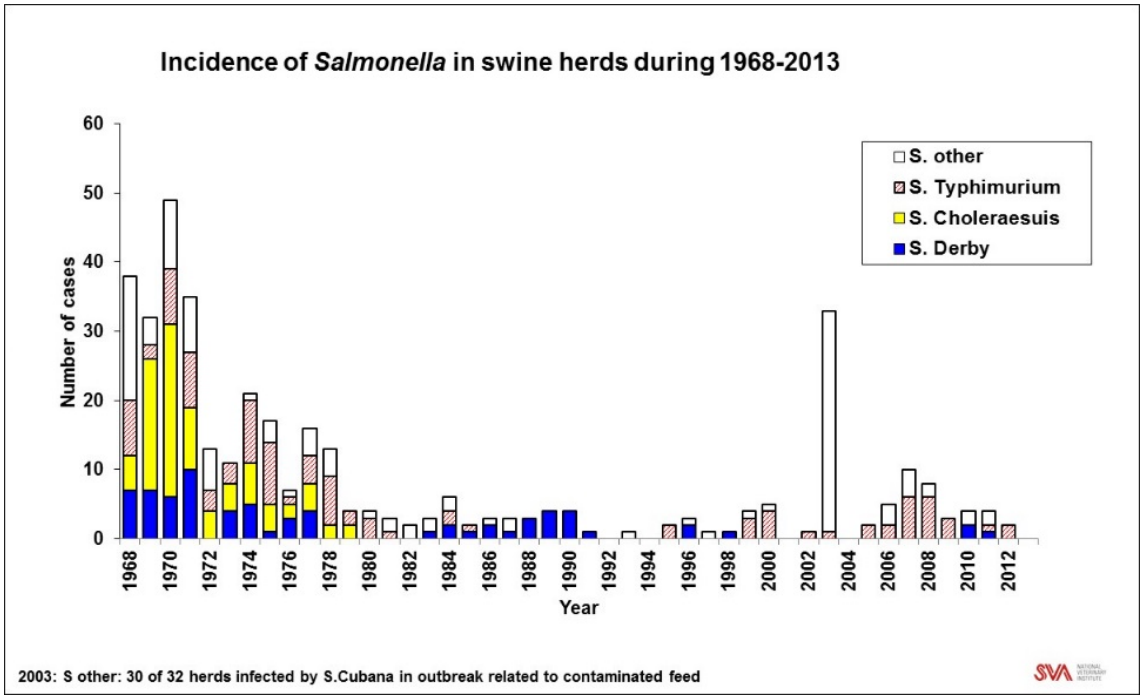

Figure 8. Number of detected cases of *Salmonella* in swine herds in Sweden per year.

Slaughter animal prevalence

Figures 9 to 12 show the results of the systematic surveillance performed at slaughterhouses (required by the EU). Sampling of lymph nodes (Figures 9 and 10) reflects the number of infected individual fattening pigs and adult pigs, respectively, sent to slaughter. It does not reflect the number of infectious pigs (i.e. it does not represent the number of pigs that are effectively contaminating the environment). Sampling of carcasses (Figures 11 and 12) reflects the efficiency of pre-harvest control of *Salmonella*, i.e. it reveals whether *Salmonella*-excreting pigs (fattening pigs and adult pigs, respectively) are slaughtered and also the slaughter hygiene. It is also a measure of the exposure in humans. It cannot be used as a prevalence estimate of the number of infected individual pigs. It should also be highlighted that the carcass swabbing performed in Sweden uses a large swabbing area and this sampling can therefore be expected to be more sensitive than swabbing carried out in other countries.

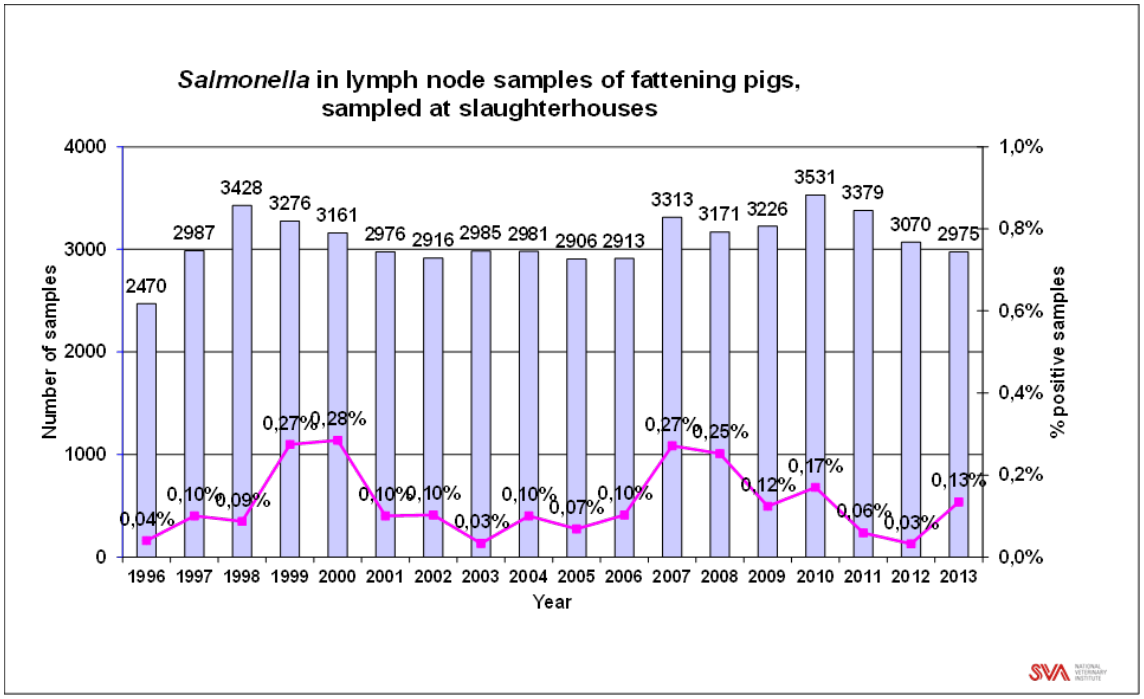

Figure 9. Number of samples from lymph nodes collected from fattening pigs at slaughter in Sweden per year, and percentage of samples positive for *Salmonella.*

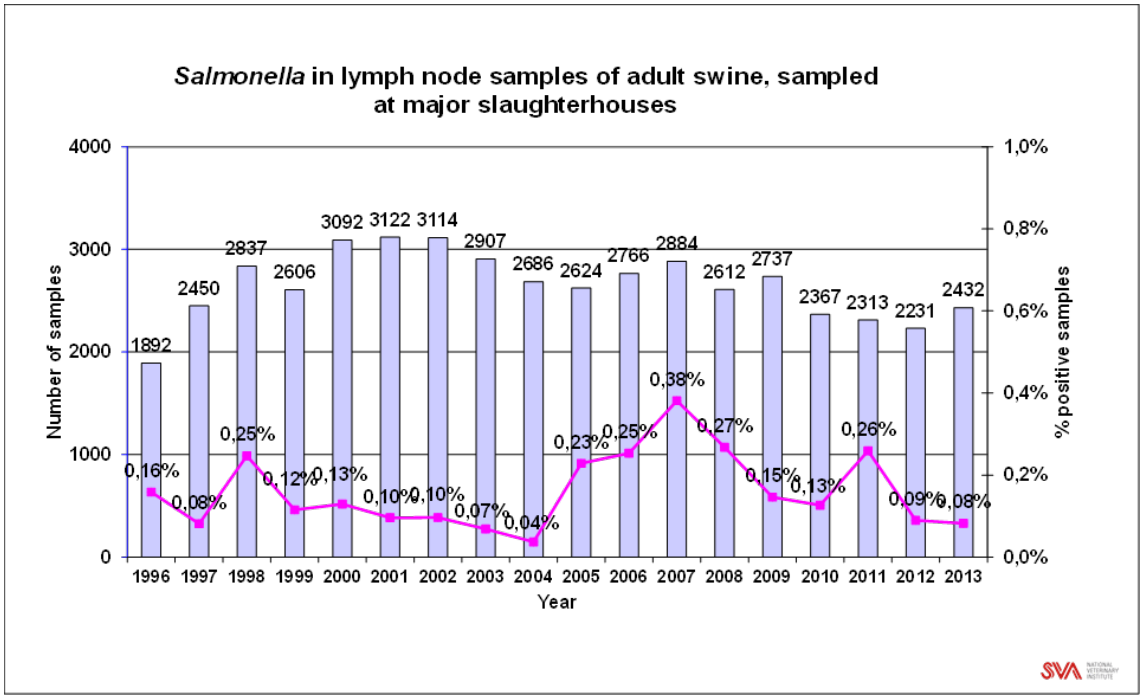

Figure 10. Number of samples from lymph nodes collected from adult pigs at slaughter in Sweden per year, and percentage of samples positive for *Salmonella*.

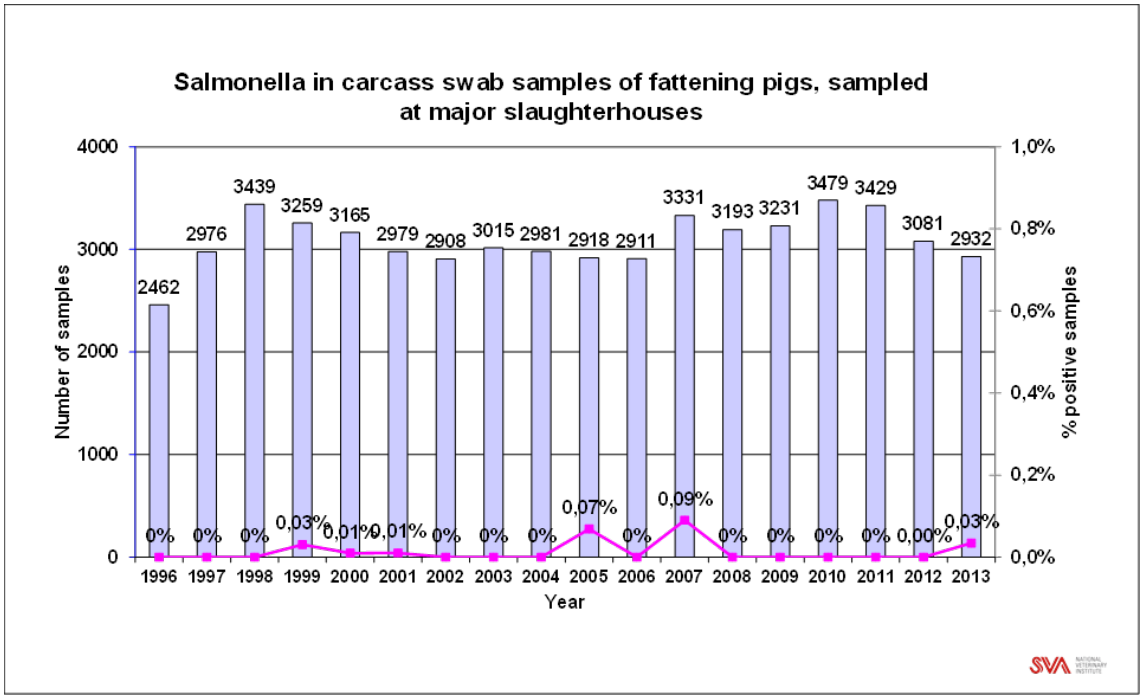

Figure 11. Number of carcass swabs collected from fattening pigs at slaughter in Sweden per year, and percentage of samples positive for *Salmonella*.

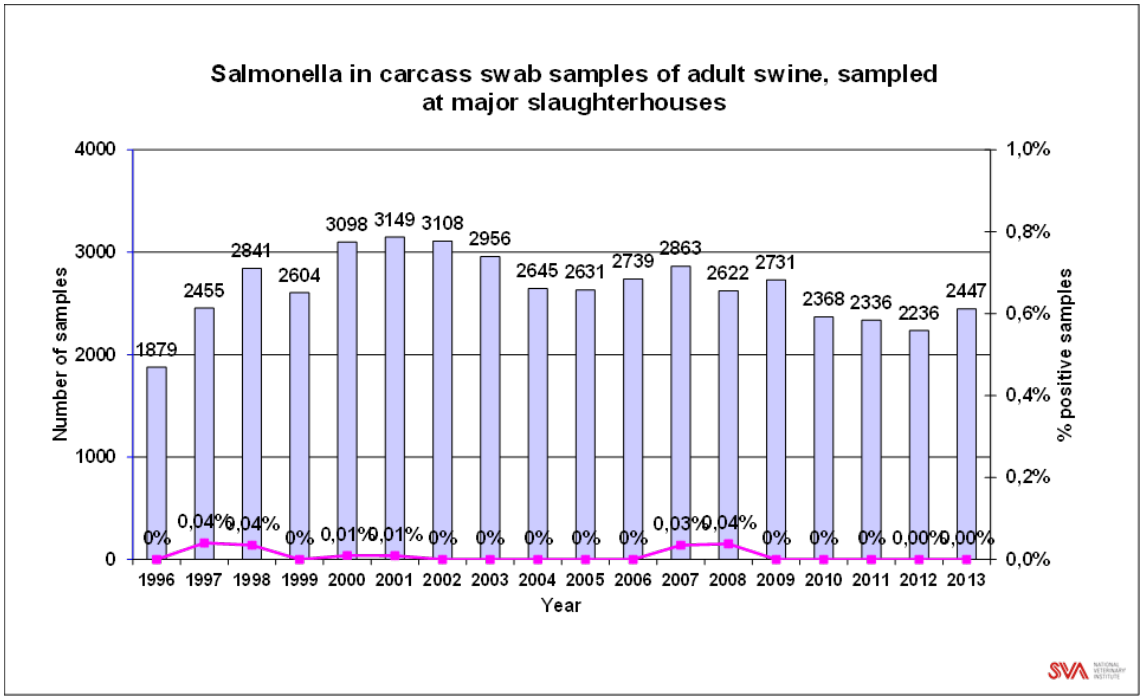

Figure 12. Number of carcass swabs collected from adult pigs at slaughter in Sweden per year, and percentage of samples positive for *Salmonella*.

Within-herd (animal) prevalence

There is no estimate of the number of *Salmonella*-infected individual pigs within herds. As the number of infected herds seems to be stable and the average herd size is increasing, the number of infected individual pigs could be expected to increase (given that within-herd

prevalence has not changed over time). However, based on the surveillance at slaughterhouses there is no indication that this is the case.

POULTRY

The incidence and prevalence of *Salmonella* in commercial poultry is extremely low. In geese and ducks the prevalence seems to be higher, but is still very low in an international perspective. Moreover, these production types represent a very small part of the poultry production in Sweden.

Herd prevalence

The Swedish *Salmonella* control is described at www.sva.se. The numbers of infected holdings detected are given in Figures 13 and 14 for layers and broilers, respectively. For geese, turkeys and ducks, the numbers of notified holdings are given in Table 4.

In contrast to *Salmonella* surveillance in other food-producing animals, surveillance in poultry covers all herds and all flocks of commercial poultry. Therefore, the number of notified infected holdings is considered to be a good estimate of the present incidence of *Salmonella* in poultry production. The frequency of sampling varies among flocks, however. In laying hens, surveillance was intensified in the late 1990s and an increased number of holdings infected with *S.* Livingstone was detected (Figure 13), but since the late 1990s the incidence has been very low and stable. In broilers the incidence decreased during the late 1980s and since the mid-1990s it has been very low (Figure 14).

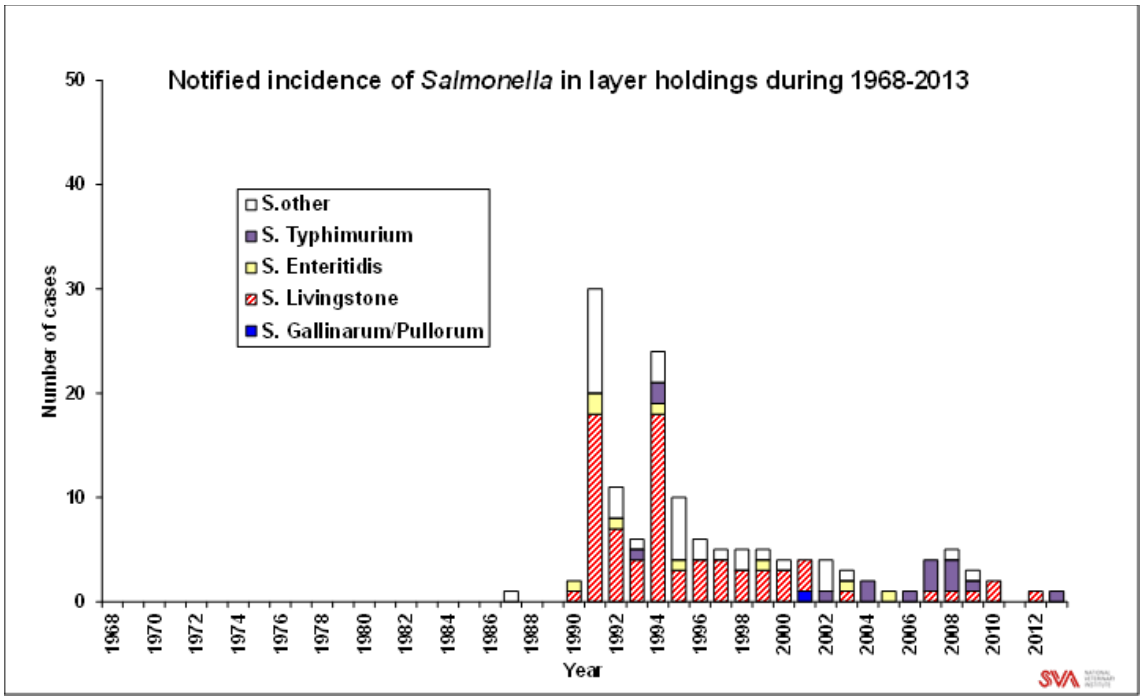

Figure 13. Number of infected laying hen holdings detected positive for *Salmonella* in Sweden per year. Data for years before 1990 are not available or scarce.

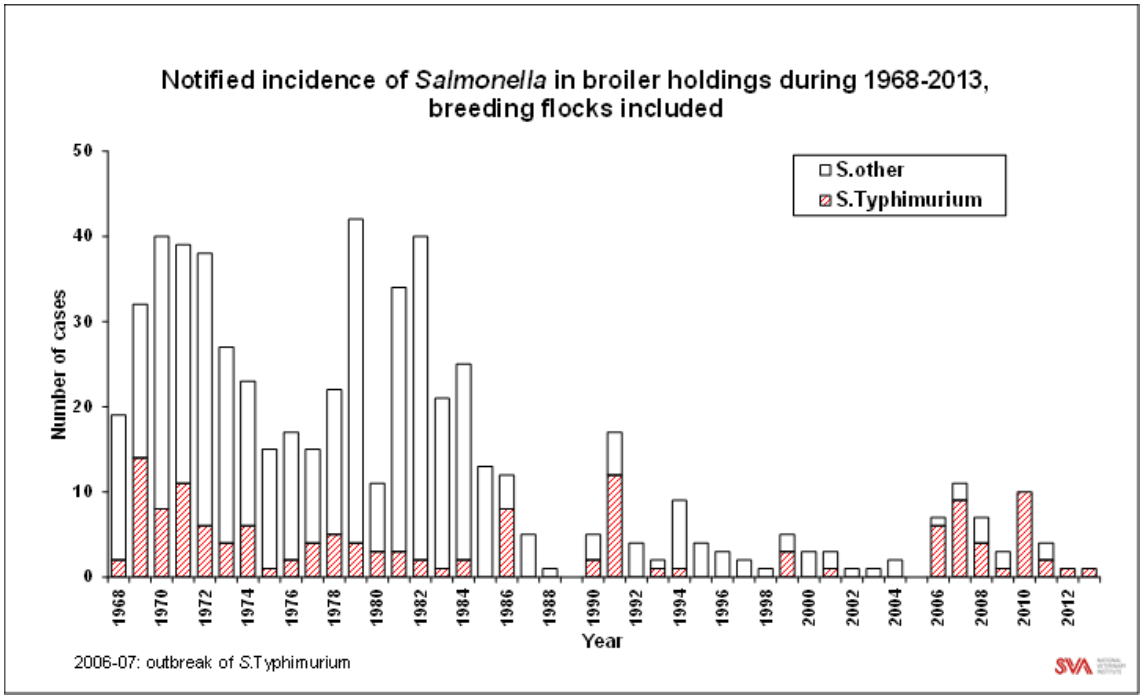

Figure 24. Number of infected broiler holdings detected positive for *Salmonella* in Sweden per year.

Table 4. Number of notified cases of *Salmonella*-infected holdings of turkeys, geese and ducks in Sweden per year

Animal Species	Isolated	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Turkeys	*S.* Typhimurium	0	0	0	0	1	1	0	0	0	0
	Others	0	0	0	1	1	1	0	0	0	0
	Total	0	0	0	1	2	2	0	0	0	0
Geese	*S.* Typhimurium	2	0	0	2	0	5	1	0	1	0
	Others	0	0	0	0	0	0	0	1	0	0
	Total	2	0	0	2	0	5	1	1	1	0
Ducks	*S.* Typhimurium	0	0	2	0	0	1	0	0	1	0
	Others	1	0	0	3	0	0	0	0	1	0
	Total	1	0	2	3	0	1	0	0	2	0

The numbers of notified holdings of geese, turkeys and ducks are also low. However, this population is very small. For example, in 2013 there were 126 holdings with turkeys, compared with more than 4000 holdings with laying hens older than 20 weeks.

Slaughter animal prevalence

Figure 15 shows the results of the systematic surveillance performed at slaughterhouses (required by the EU). Sampling of neck skin reflects the efficiency of pre-harvest control of *Salmonella*, demonstrating whether *Salmonella*-excreting poultry are slaughtered and hygiene at slaughter. It is also a measure of the exposure in humans. It cannot be used as a prevalence estimate of the number of infected individual birds in the holdings. The figure includes all poultry slaughtered at the major slaughterhouses and this is dominated by broilers. As seen in Figure 15, the prevalence is extremely low, reflecting that pre-harvest control is efficient.

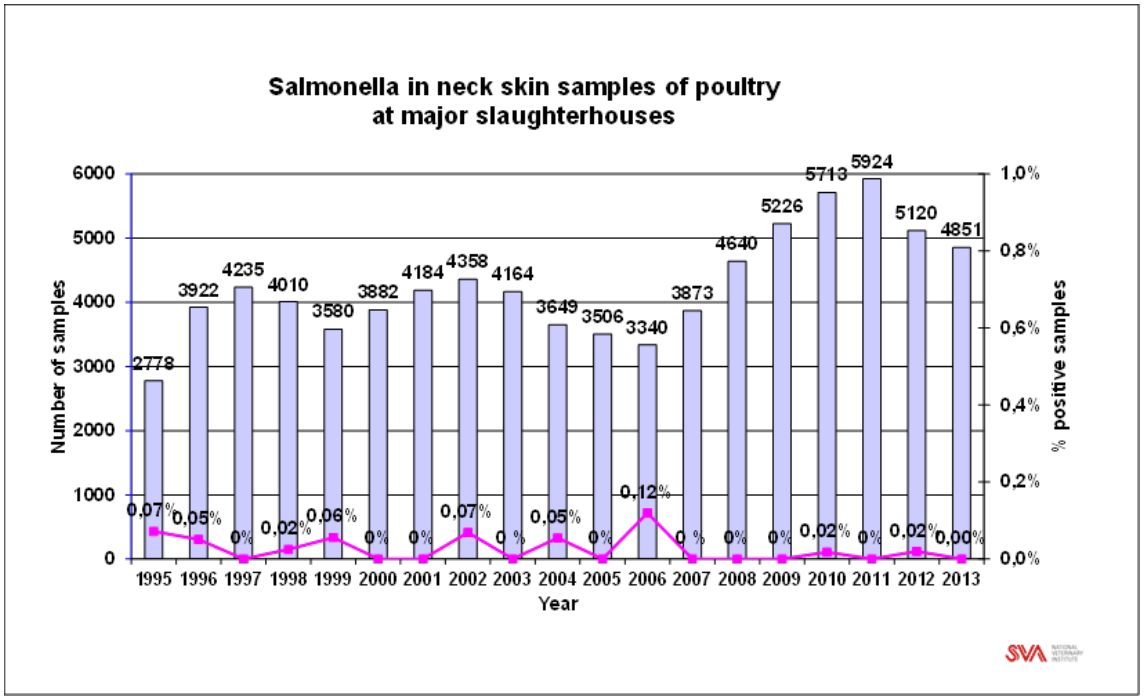

Figure 35. Number of neck skin samples collected from poultry at slaughter in Sweden per year, and percentage positive for *Salmonella*.

Within-herd (animal) prevalence

There are no estimates of the number of *Salmonella*-infected individual poultry within flocks.

SHEEP

Herd prevalence

A study by Sörén *et al.* (2015) aimed to detect herd prevalence of at least 1% with 95% confidence. The herd size distribution was skewed, as 79.9% of the herds were considered small, with 30 sheep or less, and 20.1% had between 31 and 1425 sheep. To avoid having most testing done on small herds, sampling was stratified by herd size in two groups; small herds with ≤30 sheep and large herds with >30 sheep. In each stratum, 237 herds were selected at random. A total of 40 out of 100 (40%) large herds and 17 of 144 (12%) small herds were positive. The overall adjusted prevalence was 17.6% (95% CI 12.9-22.2). Sheep-associated *S. diarizonae* was detected in all Swedish counties (n=21). No difference in geographical distribution could be observed. No other *Salmonella* type was found.

In contrast to cattle, swine and poultry, there is no active surveillance for *Salmonella* in sheep. Instead, the surveillance relies on passive surveillance, including post mortem examinations.

OTHER STUDIES NOT RELEVANT FOR PREVALENCE ESTIMATION

Studies targeting different livestock species

Boqvist *et al.* (2003) present figures for *Salmonella* isolated from samples collected between 1993 and 1997. The study includes all primary isolates from infected herds/flocks and also positive findings from other surveillance activities (autopsies, sanitary slaughter and surveillance at slaughterhouses), even if *Salmonella* could not be re-isolated at follow-up tests

in the herd of origin. A total of 555 isolates were recorded from animals; 115 from cattle (nine different serotypes), 18 from swine (eight serotypes), 21 from broilers and 56 from layers. In conclusion, the study describes a number of notified cases of *Salmonella*, including serotypes, in different animal species, ***but the prevalence is not estimated***.

Lewerin *et al.* (2011) reported data on all pig, cattle and sheep herds found to be infected with *Salmonella* during 1993 to 2010, based on information obtained from the Swedish Board of Agriculture (SJV). A herd was considered a case if *Salmonella* was isolated from at least one faeces sample in the herd. Each holding was only included once, even if investigations revealed that several animal species were infected. The case species was set to the animal species from which *Salmonella* was first identified. In the study period there were a total of 267 holdings where *Salmonella* was isolated from the animals. One large foodborne outbreak involving 31 infected pig herds occurred during the study period and only the first case of this outbreak was kept in the dataset (i.e. 30 of these herds were excluded). The final dataset included 13 sheep flocks, 200 cattle herds and 54 pig herds. Analysis of the data was performed for all *Salmonella* spp., as well as separately for the most common serotypes. Furthermore, serotypes were grouped into cattle-, pig- and sheep-adapted types (*S.* Dublin, *S.* Derby and *S. diarizonae*) and "other", representing mainly feed-associated serotypes, and summarised by animal species. Finally, analyses were also carried out for serotypes associated with small passerine birds (*S.* Typhimurium DT U277 and DT40). The most common serotype in cattle was *S.* Dublin (n=124) followed by *S.* Typhimurium (n=45); in pigs it was *S.* Typhimurium (n=31) and in sheep *S. diarizonae* (n=10).

The *Salmonella* cases in pigs seemed to be geographically associated with the density of pigs. The cases of *Salmonella* Dublin in cattle were located mainly in the south-east of Sweden, while the majority of the *Salmonella* Typhimurium cases were in the very south and the other serotypes were more evenly distributed. The overall clustering matched the cattle density. The number of infected sheep herds was too few to observe any geographical clustering.

Some seasonal variation was seen in cattle, as shown in Figure 16, but available data were not sufficient for further analyses.

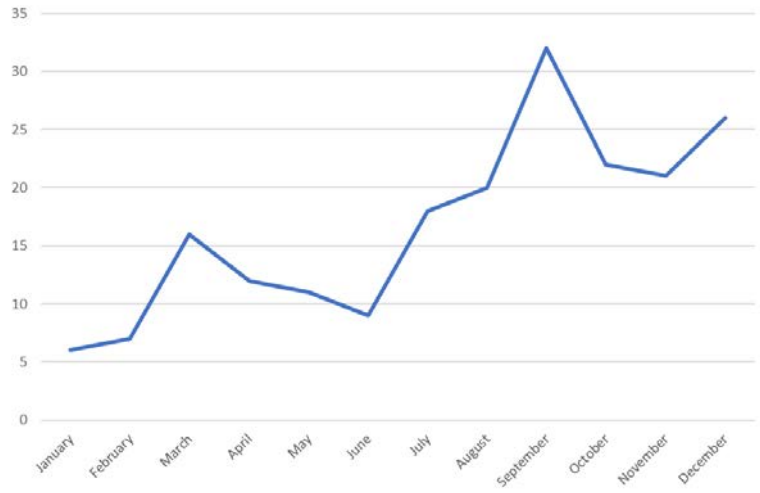

Figure 16. Number of *Salmonella*-infected Swedish cattle herds detected per calendar month in the study by Lewerin *et al.* (2011).

The study by Lewerin *et al.* (2011) was based on herds found to be infected in any part of the *Salmonella* control, i.e. a combination of passive and active surveillance. Apart from *S.* Dublin in cattle, which is more common in south-east Sweden, the prevalence of *Salmonella* in cattle and pig herds is related to the cattle and pig population density. It should be noted that the number of infected pig herds is very low and their contribution to *Salmonella* contamination of the environment can be expected to be very small. The number of infected sheep herds was too low to permit analysis.

Comparison of different production types of herds in cattle

Wahlström *et al.* (2008) reported a study aimed at clarifying whether the risk of being reported as a *Salmonella* case differed between different production systems. The population at risk was Swedish cattle herds during 1993-2004. This population was divided into five groups: 1) specialist beef herds (SBH) buying >150 calves from >5 herds; 2) SBH buying >150 calves from <6 herds; 3) SBH buying <150 calves annually; 4) dairy herds; and 5) beef cow herds. The number of *Salmonella*-infected herds per 1000 cattle-herd-years varied between 0 and 7.8. The risk of cattle herds in group 1 of becoming infected was 14-305 times higher than that for herds belonging to groups 3-5. The most common serotype in groups 1, 3 and 4 was *Salmonella* Dublin, causing 67% (8), 100% (8) and 71% (75) of all infections, respectively. However, in group 5 none of the herds was infected with *S.* Dublin.

These results reflect the importance of live animal movement as a source of *Salmonella* infection. This was also supported by the fact that the cattle-adapted serotype *S.* Dublin was the most common serotype in groups 1, 3 and 4. The difference in serotype distribution might also reflect a difference in epidemiology in less (group 5) compared with more (groups 1-4) intensively managed herds.

ZOONOTIC LINK

Wahlström *et al.* (2011) performed a source attribution that allocated sporadic domestic human cases of *Salmonella* in Sweden between 1 July 2004 and 31 June 2006 to the expected sources of *Salmonella*. Cases allocated e.g. to cattle included cases infected directly from cattle, from food originating from cattle (beef/milk) and from indirect infection, for example by environmental contamination by cattle in turn contaminating produce.

The study showed that food-producing animals in Sweden are not an important source of *Salmonella* in humans. Only 0.5% (95% CI 0.3-0.8%) of the human cases could be attributed to Swedish food-producing animals. A similar proportion (0.6%; 95% CI 0.-0.9%) was attributable to wildlife (small passerine birds, seagulls and hedgehogs). Imported food was an important source of *Salmonella,* responsible for 6.4% (95% CI 5.8-7.1%) of cases. No source could be allocated for 7.7% (95% CI 7.1-8.3%) of the human cases. Table 5 is reproduced from the paper by Wahlström *et al.* (2011).

Cases associated with outbreaks totalled 2.9% of all cases, and most of these were most probably caused by imported food, as the majority (90%) of *Salmonella* outbreaks attributed to defined food commodities between 1992 and 2009 (n=40) were considered to be due to imported food (SLV, unpublished results). Finally, 82% of all cases were acquired abroad.
In conclusion, neither domestic food-producing animals nor domestic wildlife are an important source of *Salmonella* for humans in Sweden.

Table 5. Human domestic sporadic *Salmonella* cases reported between 1 July 2004 and 31 June 2006 (mean percentage and 95% credibility interval) attributed to nine different sources and an unknown source. Cases attributed to groups of courses (food-producing animals and wildlife) are also detailed. Percentage of travel-related cases and of cases due to domestic outbreaks are also given. Reproduced from Wahlström *et al.* (2011)

	Attributed human cases (mean and 95% credibility interval)		
Source	Mean (%)	2·5%	97·5%
Imported food	6·4	5·8%	7·1%
Food-producing animals			
Pigs	0·08	0·002%	0·28%
Cattle	0·10	0·003%	0·30%
Layers	0·16	0·08%	0·27%
Broilers	0·09	0·01%	0·19%
Geese	0·04	0·01%	0·10%
Wild life			
Small passerine birds	0·22	0·05%	0·37%
Seagulls	0·09	0·02%	0·17%
Hedgehogs	0·30	0·04%	0·49%
Unknown source	7·7	7·1%	8·3%
Outbreaks	2·9	—	—
Travel-related cases	82	—	—
Total	100		
All food-producing animals	0·5	0·3%	0·8%
All wildlife	0·6	0·3%	0·9%

OTHER SPECIES – NOT LIVESTOCK

Horses

Salmonella is rarely detected in Swedish horses, but sporadic cases occur every year. There is no active surveillance for *Salmonella* in horses. Lewerin (2010) sampled 79 horses submitted for necropsy to SVA and the Swedish University of Agricultural Sciences (SLU). Samples from the intestinal wall, intestinal content and intestinal lymph nodes were cultured for *Salmonella*. *Salmonella* could not be isolated from any of the horses. These results indicate that *Salmonella* is not a common part of the intestinal flora of Swedish horses and that *Salmonella* carriers may be rare. There is no prevalence estimate available for horses in Sweden.

Cats

Tauni and Osterlund (2000) described an outbreak of *Salmonella* Typhimurium infection associated with wild birds in cats and humans in the Swedish county of Värmland in 1999. The results cannot be used as an estimate of prevalence, considering that this was an outbreak investigation. It can only be concluded that besides being present in small passerine birds, this serotype can also be found in cats. However, cats are not considered to be a reservoir.

Wild birds

Hernandez *et al.* (2003) reported a study focusing on the migratory bird fauna of the North Western Palearctic. Apparent healthy birds on active migration were trapped at the Ottenby Bird Observatory during the migration periods July-November 2001, March-May 2002 and July-December 2002. In total, 2377 samples from 110 species of migratory bird were sampled.

Only one of the isolates, obtained from a mistle thrush (*Turdus viscivorus*) in 2002, carried *Salmonella*. The serotype was *S*. Schleissheim, a rare *Salmonella* serotype. The failure to find *Salmonella* was probably not caused by technical problems. The sampling methods used, with faeces samples from fresh droppings or cloacal swabs, are established techniques for studying *Salmonella* prevalence in birds. These results suggest that the natural occurrence of *Salmonella* in healthy birds during migration in Sweden may be low.

In an earlier study, Palmgren *et al.* (1997) tested stool samples from 151 wild birds (50 gulls and 101 passerines) just entering Sweden from their winter grounds. Two isolates of *Salmonella* Typhimurium, with multiple antibiotic resistance, were found in gulls.

Data from all tests performed at SVA between 2007 and 2013 showed a total of 12 gulls (in Swedish *måsfåglar*) that were positive for *Salmonella*, 11 *S*. Typhimurium and one that could not be serotyped. Based on phage typing of *S*. Typhimurium, the most common serotype was DT41, which is in agreement with data from earlier years (Expert opinion, H. Wahlström). Since 2012, phage typing has been replaced by MLVA typing. At present, there are insufficient data to permit conclusions to be drawn on the MLVA types that can be expected to be found in seagulls. Apart from DT41, gulls can also reflect *Salmonella* serotypes that might occur in the environment, for example at refuse dumps. Wahlström *et al.* (2003) found a low prevalence of salmonella in gulls, with an estimated 4% of 111 gulls infected with *Salmonella* from one of four serotypes (*S*. Typhimurium*, S*. Oranienburg*, S*. Livingstone *and S*. Agona). Three of the four samples were from gulls shot near refuse dumps, reflecting that this was probably the source of infection (Wahlström *et al.*, 2003).

In dead/sick small passerine birds found at feeding tables during winter, infection with *S*. Typhimurium DT40 and the closely related DT NST(U277) are commonly reported. Small passerine birds can also be considered to be a reservoir for this subtype of *S*. Typhimurium. They are considered to be a source of infection for cats eating such birds and humans can also become infected when handling contaminated bird feeding places. The number of dead/sick birds submitted to SVA varies between years, indicating that prevalence varies between years.

The most common MLVA types among small passerine birds and also cats in Sweden during 2010-2014 have been 2-13-3-NA-212 and 2-12-3-NA-212, while several similar profiles also occur. Together, these comprise most of the isolates from this group (Robert Söderlund, SVA, personal communication).

Hedgehogs

Data from tests performed at SVA show that *Salmonella* can be found in hedgehogs. From 2008 until 2014, approximately 45 positive animals were detected (*S*. Typhimurium and *S*. Enteritidis). Most of the positive animals found in 2013 and 2014 originated from Gotland, where *Salmonella* in hedgehogs has been identified in a hedgehog rescue centre. Hedgehogs seem to be a domestic reservoir for certain types of *Salmonella*. The overall distribution in the country is not known and there is no prevalence estimate.

Wild boar

In an evaluation study of capture traps (Sanno *et al.*, 2014), 80 wild boar were captured in traps placed close to existing artificial feeding areas for free-living wild boar in two different hunting counties in central Sweden. Three animals were shot in close proximity to the traps in the evaluation study. In addition, a gilt submitted to the Norwegian Veterinary Institute for necropsy and four animals shot close to a farm with free-range domestic pigs infected with *S.* Derby were included. For the first 36 individuals, both tonsils (with one exception) were removed and stored separately and 5-20 g faeces were collected. For the remaining 44 individuals and for the eight animals not caught in traps, the sampling also included the ileocaecal lymph nodes. A total of 11 of 88 (10%) were PCR-positive, nine samples from tonsils and none from faeces. Of the PCR-positive samples, seven were bacteriologically positive (six tonsils, one ileocaecal lymph node). This estimative is NOT representative of the risk to humans or environmental contamination, as none of the animals was proven to be infectious (no faeces samples were positive). Moreover, as these pigs are artificially fed, they are not considered representative of the wild boar population.

In a study during 2003 (Wahlström *et al.*, 2003) that tested wild boar shot all over Sweden, faeces samples were analysed for *Salmonella*. Samples from a total of 68 wild boar were obtained, and all were negative for *Salmonella*.

VTEC

Based on available data, it can be concluded that the prevalence of VTEC O157 is about 3-3.5% in cattle and that there are regional differences, with the majority of infected cattle found in southern Sweden. It can also be concluded that a specific strain of VTEC O157 called clade 8 is present in cattle. Human cases of EHEC with the complication Haemolytic Uraemic Syndrome (HUS) are often caused by clade 8, with MLVA profiles consistent with that in Swedish cattle.

National prevalence studies for VTEC O157 in cattle faeces have been conducted at the major slaughterhouses since 1996. About 3-3.5% of Swedish cattle shed VTEC O157 in faeces. Most infected animals are found in southern areas of the country. In the latest survey, the positive samples were further characterised and it was found that 25% of infected cattle carried the clade 8 strain. There are regional differences in VTEC O157 at herd level and Halland is considered a high-prevalence area (Eriksson *et al.,* 2005). However, in recent sampling on Öland, a high proportion of cattle herds were found to be positive, which indicates a regional trend in VTEC O157 presence. In addition, many of the positive samples were of the clade 8 strain. The high presence of clade 8 in this area is a public health concern. It has been shown that cases that progress to HUS are mostly caused by clade 8, with MLVA profiles consistent with samples from Swedish cattle (Söderlund *et al.,* 2014).

Animals can be infected with VTEC, but show no clinical symptoms. Cattle are considered to be a main reservoir of the bacteria. Most studies performed to investigate the prevalence in Sweden have focused on O157 (Albihn *et al.,* 2003; Eriksson *et al.,* 2005; Boqvist *et al.,* 2009; Widgren *et al.,* 2013). However, there are some studies targeting O26, O103, O145 and O121. Results and epidemiological considerations are presented below.

CATTLE

Slaughter animal prevalence of VTEC O157:H7 in cattle

Nationwide surveys of VTEC O157 in cattle faeces samples collected at slaughter have repeatedly been conducted to monitor the prevalence. The study design is reported to detect prevalence in the population of at least 0.1% with 90% confidence (Albihn *et al.,* 2003; Boqvist *et al.,* 2009). In each survey, 2000-3000 faeces samples were collected from about 15-16 slaughterhouses covering 90% of slaughtered cattle in Sweden (Albihn *et al.,* 2003; Boqvist *et al.,* 2009).

Prevalence studies for VTEC O157 in cattle faeces samples were performed annually at the major slaughterhouses between 1996 and 2002 *(*Anonymous, 1997, 1998, 1999, 2000, 2001, 2002*;* Albihn *et al.,* 2003; Anonymous, 2003) and the results showed that the prevalence was around 1% (Table 6). As very small changes in the prevalence were observed during these years, it was decided to conduct such studies every third year. The following prevalence study (Anonymous, 2006, 2007; Boqvist *et al.,* 2009) was conducted during the period 2005-2006 and the prevalence was 3.4% (Table 6). This figure cannot be compared with the previous values, as the laboratory methodology had been slightly modified (Boqvist *et al.,* 2009). In addition, ear samples were included to evaluate whether they could be used to assess VTEC O157:H7 contamination at slaughter. Fifty-four (12%) of 446 ear samples tested positive for

VTEC O157:H7. In the prevalence study conducted in 2008-2009 (Anonymous, 2010), the prevalence was 3.3% (Table 6) and 41 (8.2%) of 500 ear samples tested positive for VTEC O157:H7. The latest survey (2011-2012; (Anonymous, 2012) showed a similar prevalence of 3.1% (Table 6). Moreover, approximately 25% of the positive samples in the 2011-2012 survey were identified as the hypervirulent strain clade 8. Figure 17 shows the geographical distribution.

In these studies it was established that the bacterium is isolated from cattle in the south of Sweden, but very rarely in the northern two-thirds of the country. In the 2008/09 survey, one ear sample from Luleå, in northern Sweden, was positive. This was the most northerly positive sample in the slaughterhouse surveys. It was also shown that the prevalence was higher in younger animals compared with adult cattle (Boqvist *et al.,* 2009).

Table 6 Results of nationwide surveys of VTEC O157 in cattle faeces samples collected at slaughter during 1996–2012

Year	Faeces samples	Positive	Ear samples	Positive
1996–1997	3 071	37 (1.2%)	-	-
1997–1998	2 308	7 (0.3%)	-	-
1999	2 057	14 (0.7%)	-	-
2000	2 001	34 (1.7%)	-	-
2001	1 998	36 (1.3%)	-	-
2002	2 032	29 (1.4%)	-	-
2005–2006	1 758	60 (3.4%)	446	54 (12.1%)
2008–2009	1 993	65 (3.3%)	500	41 (8.2%)
2011–2012	2 376	73 (3.1%)	-	-

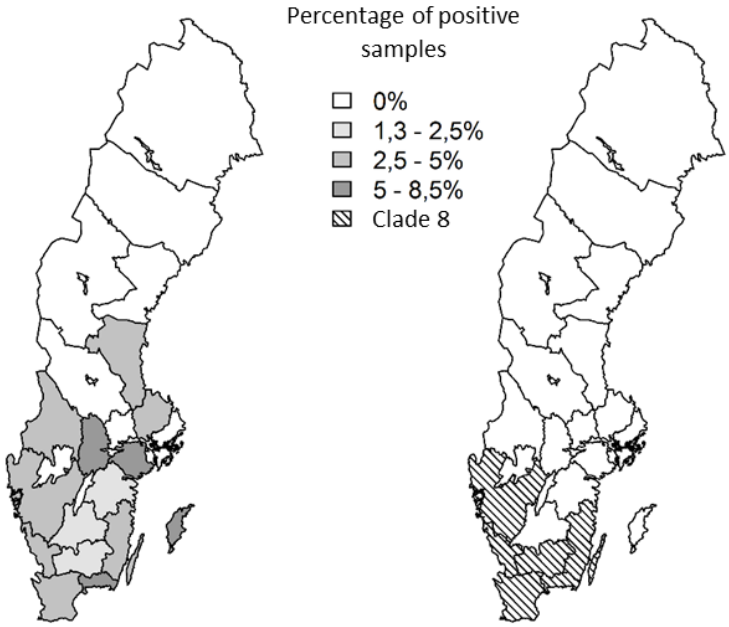

Figure 17. Geographical distribution of positive samples from the nationwide survey 2011-2012 of VTEC O157 in cattle faeces samples collected at slaughter. In total, 2376 samples were analysed. Prevalence of VTEC O157 by county (left) and presence of clade 8 by county (right).

Herd prevalence of VTEC O157:H7 in cattle

Faeces samples were collected from 371 randomly selected dairy herds to estimate the prevalence of VTEC O157:H7 and to ascertain whether there are differences in herd prevalence between different regions (Eriksson *et al.,* 2005). In total, 7397 individual faeces samples were collected and VTEC O157:H7 was isolated from 33 (8.9%) of the 371 herds investigated. The prevalence was higher (23.3%) in Halland County, which is situated in south-west Sweden, than in the rest of Sweden. No farms from the north of Sweden were positive.

Slaughter animal prevalence of VTEC O103 and VTEC O26 in cattle

Samples collected in the nationwide survey 2011-2012 were also analysed for VTEC O26 and VTEC O103. VTEC O26 was detected in 8 (0.6%) of 1308 faeces samples and in 15 (4.5%) of 336 ear samples. VTEC O103 was detected in three (0.3%) of 1000 faeces samples and three (0.6%) of 500 ear samples.

Other studies that give information on the presence of VTEC in cattle farm environments

These studies are not designed for prevalence estimations.

In a study to evaluate environmental sampling to detect VTEC O157 in 31 dairy cattle herds, samples were collected from individual animals (Widgren *et al.,* 2013). The individual samples were pooled at the laboratory (pool size = 3) within the age categories calves, young stock and adults, before being analysed. The within-herd pool prevalence ranged from 0 to 57%. Pools from calves and young stock were significantly more likely to be positive. The individual prevalence was not estimated from the pool prevalence.

In a longitudinal observational study including 126 cattle farms in four regions in southern Sweden (Halland, Västra Götaland, Gotland and Kronoberg county) conducted in 2009-2013, the farm environment was repeatedly sampled (Widgren *et al.,* 2015). The herds represented a convenience sample selected by the regional livestock association. Risk factors for detecting VTEC O157:H7 in the herd environment were: a preceding positive sample, herd size, infected neighbouring farms, recent introduction of animals and autumn season. A subset of these herds (n=115) was also analysed for VTEC O26, O103 and O121 (unpublished data). VTEC O26 was detected in five herds, VTEC O103 in seven and VTEC O121 in 14 herds. During 2012, samples (n>400) were collected from proximal water sources, such as streams and ditches, of positive herds (Szántó, 2012). VTEC O157:H7 was not detected in any sample (Szántó, 2012).

SHEEP

Slaughter animal prevalence of VTEC O157:H7

In a nationwide survey, faeces and ear samples were collected at nine slaughterhouses during 2007-2008 (Söderlund *et al.,* 2012). VTEC O157 was detected in nine (1.8%) of 492 faeces samples and two (1.9%) of 105 ear samples. It was only detected in animals younger than 6 months (85% of the samples) from southern Sweden.

PIGS

Slaughter animal prevalence of VTEC O157:H7

The prevalence of VTEC 0157:H7 in slaughtered fattening pigs was investigated in a study in 1998-1999 (Eriksson *et al.,* 2003). Faeces samples were collected at five slaughterhouses. VTEC O157:H7 was detected in two (0.08%) of 2446 samples. The results of the study show that fattening pigs are a reservoir for VTEC O157. However, the prevalence of VTEC O157:H7 is low.

HORSES

There are no studies in Sweden concerning horses and presence of VTEC. Horses are very rarely tested for VTEC and no positive samples were found at SVA during the period 2008-2014. Horses are not considered a reservoir for VTEC.

WILD ANIMALS

In a study in 2003 (Wahlström *et al.,* 2003), samples collected from 778 wild animals (Canada geese, roe deer, hares, moose, wild boar and gulls) shot during hunting were examined for VTEC 0157. With the exception of one positive isolate from a wild boar, VTEC 0157 was not isolated from any of the animals. The results of the study suggest that the wild animal species investigated are not reservoirs for VTEC 0157.

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