

ORIGINAL ARTICLE

Transmission of *Salmonella* between wildlife and meat-production animals in DenmarkM.N. Skov¹, J.J. Madsen², C. Rahbek^{2,3}, J. Lodal⁴, J.B. Jespersen⁴, J.C. Jørgensen⁵, H.H. Dietz⁵, M. Chriél⁶ and D.L. Baggesen¹

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Introduction

Many investigations have shown that contaminated food of animal origin is the main source of human infections (Bezanson *et al.* 1983; Holmberg *et al.* 1984; Tacket *et al.* 1985; Humphrey 2000). *Salmonella* is one of the most prevalent causes of human gastroenteritis in industrialized countries. Since the presence of *Salmonella* in animal food products is associated with the occurrence of *Salmonella* in primary animal production, strategies to control the introduction and spread of infection within herds are considered important (Wegener *et al.* 2003).

Epidemiological investigations have indicated that both trade of subclinically infected animals and the use of con-

Abstract

Aims: To investigate the transmission of *Salmonella* spp. between production animals (pigs and cattle) and wildlife on production animal farms in Denmark.

Methods and Results: In the winter and summer of 2001 and 2002, 3622 samples were collected from *Salmonella*-infected and noninfected herds of pigs and cattle and surrounding wildlife. *Salmonella* was detected in wildlife on farms carrying *Salmonella*-positive production animals and *only* during the periods when *Salmonella* was detected in the production animals. The presence of *Salmonella* Typhimurium in wild birds significantly correlated to their migration pattern and food preference.

Conclusions: *Salmonella* was transmitted from infected herds of production animals (cattle and pigs) to wildlife that lived amongst or in close proximity to them.

Significance and Impact of the Study: *Salmonella* in animal food products is associated with the occurrence of *Salmonella* in primary animal production. Strategies to control the introduction and spread of infection should include wildlife management, as the nearby wildlife may act as reservoirs for *Salmonella* spp. and/or may be passive carriers of the bacteria.

taminated feed are the main risk factors for introducing *Salmonella* infections to herds (Steger *et al.* 1997; Stärk *et al.* 2002). However, *Salmonella* has a broad range of animal hosts and has been isolated from several wildlife species such as birds (Refsum *et al.* 2002a, 2002b), rodents (Healing 1991), hedgehogs (Nauerby *et al.* 2000; Handeland *et al.* 2002), and insects (Olsen and Hammack 2000; Mian *et al.* 2002). Transmission of *Salmonella* from infected wild birds to the environment, as well as to other animal species, has been reported (Kapperud *et al.* 1998). In some countries a relatively high prevalence of *Salmonella* in wildlife has been reported (Henzler and Opitz 1992; Refsum *et al.* 2002a) and it has been documented that wildlife may contribute to the horizontal

transmission of *Salmonella* (Tauni and Österlund 2000; Warnick *et al.* 2001; Liebana *et al.* 2003).

In Denmark, however, routine wildlife disease surveillance has revealed a very low prevalence of *Salmonella* in wildlife (Dietz *et al.* 1998) relative to levels reported from other countries (Healing 1991; Hubalek *et al.* 1995; Hudson *et al.* 2000; Bailey *et al.* 2001; Millan *et al.* 2004). This suggests that there are fewer sources of *Salmonella* transmission in Denmark. Thus, evaluating the transmission of *Salmonella* between wildlife and production animals in Denmark is relatively more straightforward because there are fewer routes of transmission to consider.

Other investigations have described the presence of *Salmonella* in wild birds, although most of these were screening studies (Kapperud and Rosef 1983; Handeland *et al.* 2002; Refsum *et al.* 2002a,b) that did not include additional data, i.e., contact of the birds with farm animals and their *Salmonella* status. Although some investigators have studied the presence of *Salmonella* in wild birds living near contaminated locations, such as refuse dumps (Cizek *et al.* 1994) or at *Salmonella*-infected animal production farms (Cizek *et al.* 1994; Kirk *et al.* 2002), they did not include longitudinal sampling. Thus, factors such as season, direction of transmission, and incidence of clinical disease among production animals have not been thoroughly evaluated.

The aim of the present study was to describe and analyse the transmission between production animals and wildlife living at or near *Salmonella*-infected production animal herds.

Materials and methods

We studied the incidence of *Salmonella* in wildlife living at or near *Salmonella*-infected, as well as noninfected, cattle and pig farms in Denmark. A 2-year longitudinal study was conducted during the winter and summer months of 2001 and 2002. Samples were obtained from both cattle herds with clinical outbreaks and pig herds with persistent infections. In addition, samples were obtained from wildlife at locations geographically distant from production animals, such as urban areas, grain-producing farms, and from areas with a large concentration of migratory songbirds. The isolates from different animal species and sampling sites were characterized and compared using both serotyping and phage-typing techniques, as well as pulsed-field gel electrophoresis (PFGE).

Study design and selection of farms

A total of 13 production herds, five pig and eight cattle, were included in the study. The herds were geographically located within a radius of approximately 90 km in the

central part of Denmark (west Zealand 2, Fun 1, south-east Jutland 3, and east Jutland 7). Each of the 13 herds was visited either once (two of the herds), twice (six herds), three times (two herds), or four times (three herds) (Table 1). The visitation frequency for a particular herd was determined by the influence of intervention measures against infection in the cattle herds, as well as by limitations in access to some herds due to a concurrent outbreak of Newcastle disease (Anon 2003). Eight controls were included: four farms without animal production, two houses in village areas, and two locations with a high concentration of migratory songbirds.

The selection of pig herds was based on data from the Danish *Salmonella* Surveillance Program, which encompasses serosurveillance of slaughter pigs (Nielsen *et al.* 2001; Wegener *et al.* 2003). The infected herds had been afflicted with a subclinical *Salmonella* infection for at least one year prior to the study. On the other hand, the non-infected herds had not been diagnosed seropositive for *Salmonella* for at least 2 years prior to the study. Selection of cattle herds was based on the diagnosis of acute clinical salmonellosis, while the selection of noninfected herds was based on information from local veterinarians. Prior to inclusion in the study, the *Salmonella* status of each herd was verified by bacteriological examination of 20 pooled, fecal samples. Each pool consisted of five replicate samples of 5 g of fecal material collected at different places in the stables. Control locations were selected by identifying habitats comparable to the other farms under investigation and ensuring that no animal production (i.e. pig, cattle or poultry) had taken place at that location during the previous year.

Collection of samples

Four sampling rounds were carried out in approximately 6-month intervals over 2 years (i.e. January to February and August to September). At each round, two farms at a time were sampled for 1 week. During each round, samples were collected from production animals, pets, birds, rodents and insects living at or near the farms. Controls were sampled only once. On the same day they were collected, the samples were transported at ambient temperature to the laboratory, refrigerated overnight, and cultured the following day.

Production and pet animals

Pooled fecal samples were collected each round from production animals and other domestic animals (if present) to determine their *Salmonella* status. Fecal samples were taken from as many places in the stable as possible. A sample was also taken from the slurry tank at the farm when possible.

Table 1 *Salmonella* isolation* from 13 production animal herds and wildlife in the winters and summers of 2001 and 2002

<i>Salmonella</i> status†	Animal	Winter 2001 n/pos‡	Summer 2001 n/pos‡	Winter 2002 n/pos‡	Summer 2002 n/pos‡
Noninfected	Pig Wildlife§	21/0 126/0	21/0 157/0		
Noninfected	Pig Wildlife	21/0 87/0	21/0 72/0	21/0 128/0	21/0 122/0
Noninfected	Cattle Wildlife	21/0 75/0	21/0 77/0		21/1 91/0
Noninfected	Cattle Wildlife	21/0 81/0	21/0 60/0	21/0 60/0	
Infected	Pig Wildlife	20/11** 70/1††	20/17** 70/1**	21/18** 89/2**	20/4 57/0
Infected	Pig Wildlife	21/1‡‡ 38/1	21/3 92/1	21/19 94/6	21/9†† 104/2††
Infected	Pig Wildlife			21/18 25/0	20/15 65/2
Infected	Cattle Wildlife	21/1 87/0	21/0 79/0		
Infected	Cattle Wildlife	21/2 70/1	21/0 76/0		
Infected	Cattle Wildlife			20/13§§ 107/5	20/0 101/0
Infected	Cattle Wildlife			21/2 82/1	21/3 71/0
Infected	Cattle Wildlife			21/4 45/1	
Infected	Cattle Wildlife				29/14 82/13

*Locations where *Salmonella* was not detected are marked with dashed borders, locations where only the production animals were *Salmonella*-positive are marked with thin black borders, and locations where *Salmonella* was found in both production and nonproduction animals are marked with thick black borders.

†*Salmonella* status when included in the study.

‡The number of pooled samples taken from production animals and the number of single or pooled samples taken from wildlife, and the number of these found to be *Salmonella*-positive. If unmarked, the serotype is *S. Typhimurium*, including samples (§) from birds, insects, rodents, dogs, cats, horses and sheep.

***S. Typhimurium* and *S. Derby*.

††*S. Derby*.

‡‡One sampling occasion where the *Salmonella* type identified in the nonproduction animals was not the same as the type identified in the production animals (see Table 2).

§§ *S. Typhimurium* and *S. Newport*.

Rodents

Rats, mice and voles were trapped during the first sampling round at all farms. The highest priority was given to commensal rodents that commonly reside inside buildings. In general, 20 traps for rats and 50 traps for mice were set, both inside and outside, at each farm. A single-capture wire-mesh trap was used for rats and Longworth small mammal live traps were used for mice and voles. Both types of traps were made of metal. Trapped animals were killed with CO₂ and placed in a plastic bag.

After the first round, the laborious sampling procedure was modified for subsequent rounds. Animals were trapped only on selected farms or were trapped occasionally by hand during the inspection of buildings. During

some sampling periods, rodent feces were collected inside and around buildings, especially in places where traps were not set and also at control farms. Fecal samples were classified as originating from rats or mice based on morphology and size before referral to the laboratory.

Birds

Birds were caught by licensed ringers with mist-nets, Manson traps (a plastic-coated wire-metal maze trap with walk-in funnels), and by hand, as close to the stables as possible. The highest priority was given to species that live close to and in constant contact with production animals. The majority of birds were captured within the stables or within a 100 m radius from the stables. A few

individuals were captured as far away as 500 m from stables.

Cloacal swab samples were collected on each farm over a 4- to 5-day period. All birds were released after samples were taken. To ensure that a sufficient number of birds were caught during the winter months, several feeding places were established at each farm using sterilized bird seeds (sunflower, millet and hemp). Birds at control locations were caught within a radius of 50–100 m. At these locations, the birds collected were chosen from a list of 13 species that are most closely associated with production animals. Cloacal swab samples were collected from all birds caught. However, birds that were recaptured within the same sampling round were only sampled once. The swabs were placed in a plastic tube containing 1 ml sterile water.

Insects

Insect species frequently found in close contact with production animals were collected from all production herds. Insects were not collected at control sites due to the limited occurrence of insects at these sites. Samples were collected from stables and, in the summer, among cattle on pastures. Insects were collected with a net placed on or around the production animals. They were then anesthetized with CO₂ and kept in small plastic boxes. A pooled sample of insects typically consisted of several species, which were identified on the spot and noted. In the pasture, one or more cattle were tethered to enable a sufficient number of insects to be sampled.

Bacteriological examination and genotype characterization

Bacteriological examination was carried out by two laboratories at the Danish Food and Veterinary Research. Using accredited methods (The Danish Accreditation and Metrology Fund – DANAK no. 412 and 413), the *Salmonella* bacteria were isolated, identified, serotyped and phage-typed. Two slightly different standard culturing procedures were used at each of the two laboratories (Anon 1993; Baggesen *et al.* 1999; Feld *et al.* 2000). Both procedures are based on nonselective pre-enrichment (BPW, Merck 7228, Darmstadt, Germany) at 37°C for 16–20 h, followed by selective enrichment either in a Rappaport-Vassiliadis based medium (modified semi-solid Rappaport-Vassiliadis (MSRV, Oxoid CM910, Basingstoke, UK) or a Rappaport-Vassiliadis-soya peptone broth (Oxoid CM866), and in cysteine selenite broth (Merck 7709). All selective media were incubated overnight at 41.5–42.0°C. *Salmonella* bacteria were isolated on either Brilliant Green Agar (Oxoid CM329) or Rambach agar (Merck 73387) after incubation overnight at 37°C.

Fecal material (22 g) from production as well as pet animals was suspended in BPW to a total volume of 200 ml. Swab samples from birds and small pet animals were transferred to 1 ml of sterile water and mixed with BPW (to a total volume of 10 ml) prior to incubation. Rodents were necropsied, and both livers and 2–5 cm of intestines were transferred to 9 ml BPW. Approximately 1 g of the fecal samples from these animals was transferred to 9 ml BPW. Upon arrival to the laboratory, insects were killed at –20°C for 1-h prior to further processing. One gram of insects from each field sample was weighed in a test tube and then macerated. BPW was added to the sample to a final volume of 10 ml.

Ten single, presumptive, *Salmonella*-like colonies were collected from each plate and serotyped according to the Kauffmann–White classification scheme (Popoff and Le Minor 1997). All isolates of *Salmonella* Typhimurium and *S. enteritidis* were phage-typed. Phage-typing of isolates of *S. Typhimurium* was performed according to Callow (1959) as modified by Anderson *et al.* (1977) while phage-typing of isolates of *S. enteritidis* was performed as described by Ward *et al.* (1987).

PFGE was performed on three isolates per positive sample. Bacterial DNA for PFGE was prepared as prescribed by Olsen *et al.* (1994). Agarose plugs (chromosomal grade agarose from Biorad) containing DNA were digested with the restriction enzymes *BlnI* and *XbaI* (Amersham Life Science) for 4 h each. Electrophoresis was performed as described by the Centers for Disease Control (CDC) PulseNet (Swaminathan *et al.* 2001). The CDC standard H9812 was digested with *XbaI* and used as the molecular size marker. For *S. Newport* isolates, 200 µmol l⁻¹ thiourea was added to the TBE running buffer, as described by Fawley and Wilcox (2002). Gels were stained in aqueous ethidium bromide (Sigma, 2 µg ml⁻¹) and destained in distilled water for 15 min. Gels were then photographed (using 300-nm UV light), analysed, and interpreted by the use of BioNumerics (Applied Math, Siut-Martens-Latem, Belgium). All visible bands were included in the interpretation of PFGE patterns and in the creation of a dendrogram. Isolates were classified as different PFGE types if their patterns differed by more than one band.

Statistical analyses

Due to the limited number of positive samples, only descriptive uni- and bivariate (chi-square) statistical analyses were performed (Dohoo *et al.* 2003) on *S. Typhimurium*-positive samples from wild birds. Behavioural patterns (e.g. distance to the herd, migration pattern, contact with slurry, foraging behaviour and foraging location) were analysed for biological association with

S. Typhimurium using the odds ratio (OR) (Dohoo *et al.* 2003).

Results

During the study, 2933 samples were collected from non-production animals and 689 samples were collected from production animals. *Salmonella* was not isolated from any of the nonproduction animals living at or near control locations, nor in herds without *Salmonella* infections. *Salmonella* was only detected in wildlife living at or near farms where *Salmonella* had been previously detected in the production animals and only during the periods when *Salmonella* was detected in the production animals (Table 1). In 72% (13 out of 18) of the sampling occasions where *Salmonella* was found in the production animals, one or more samples from the associated non-production animals were *Salmonella*-positive. At any given farm, the *Salmonella* type isolated from the nonproduction animals was, with only one exception, identical to the type in the production animals within the same sampling round. The exception was the *S. Typhimurium* phage type RDNC (routine dilution no conformity), isolated from a wild bird in the first sampling round. The type isolated from the production animals in the same sampling round was *S. Typhimurium* phage type U288 (Tables 1 and 2). PFGE typing showed that these isolates were not identical (*Xba*I PFGE type X5 and X6 in Fig. 1). However, during the next sampling round (6½ months later), *S. Typhimurium* phage type RDNC (phenotypically and genotypically identical to the type isolated previously from the wild bird) was isolated from production animals, along with phage types DT12 and U288 (Table 2).

Salmonella Typhimurium was the most frequently detected serotype isolated in all *Salmonella*-infected herds. Phage types such as DT12, DT66, DT120, DT135, U288, RDNC and NT (not typable) were present in the form of either single or mixed infections. 20 *Bln*I PFGE types (data not shown) and 20 *Xba*I PFGE types (Fig. 1) were identified. In Fig. 1, both the CDC-standardized *Xba*I PFGE profiles and the dendrogram for the 20 types are given.

In the pig herds, *S. Typhimurium* was found either alone, or together with either *S. Newport* or *S. Derby*. *Salmonella* infections in all pig herds appeared to be persistent as the bacteria were isolated from fecal samples (pooled fecal samples or slurry samples) in all sampling rounds (Table 1). *S. Typhimurium* was the only serotype detected in cattle herds. The cattle infections appeared to be self-limiting, as *Salmonella* was not detected more than once in a given herd, with one exception; *S. Typhimurium* was detected in both winter 2002 and summer 2002 in one of the cattle herds (Table 1).

Samples were collected from numerous species of wildlife. A total of 2567 cloacal samples were collected from fifty-five different species of birds (Table 3). The house sparrow (*Passer domesticus*), tree sparrow (*Passer montanus*), barn swallow (*Hirundo rustica*), and blackbird (*Turdus merula*) were the most dominant species (Table 3). The dominant species among rodents ($n = 225$) were the Norway rat (*Rattus norvegicus*) and the house mouse (*Mus musculus*). The yellow-necked field mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*), and bank vole (*Clethrionomys glareolus*) were trapped in lower numbers. Among the insect samples collected ($n = 46$) from the stables, houseflies (*Musca domestica* (L.)) and stable flies

Table 2 A summary of *S. Typhimurium* isolation and PFGE genotypes* from a pig herd over a 2-year period

Winter 2001	Summer 2001	Winter 2002	Summer 2002
<i>S. Typh.</i> † phage type U288 (<i>Xba</i> I PFGE X6‡) in: 1 slurry sample (1)	<i>S. Typh.</i> phage type U288 (<i>Xba</i> I PFGE X6) in: 1 pig sample (20)	<i>S. Typh.</i> phage type U288 (<i>Xba</i> I PFGE X6, X11, and X12**) in: 18 pig samples (20)**	<i>S. Typh.</i> phage type U288 (<i>Xba</i> I PFGE X6 and X12**) in: 8 pig samples (20) **
<i>S. Typh.</i> phage type RDNC§ (<i>Xba</i> I PFGE X5) in: 1 bird sample (29)	<i>S. Typh.</i> phage type RDNC (<i>Xba</i> I PFGE X5) in: 1 pig sample (20) 1 rodent sample (5)	1 slurry sample (1) 4 rodent samples (38) 1 insect sample (1) 1 bird sample (53)	1 slurry sample (1) 2 dog samples (2)
	<i>S. Typh.</i> DT12¶ (<i>Xba</i> I PFGE X5) in: 1 pig sample (20)		

*The *Xba*I PFGE types and total number of samples are given in brackets.

†*S. Typhimurium* (*S. Typh.*).

‡For PFGE profile see Fig. 1.

§Routine dilution no conformity (RDNC).

¶The phage type (definitive type (DT)) of *S. Typhimurium*.

**All the variations of *Xba*I PFGE types were found in the pig samples.

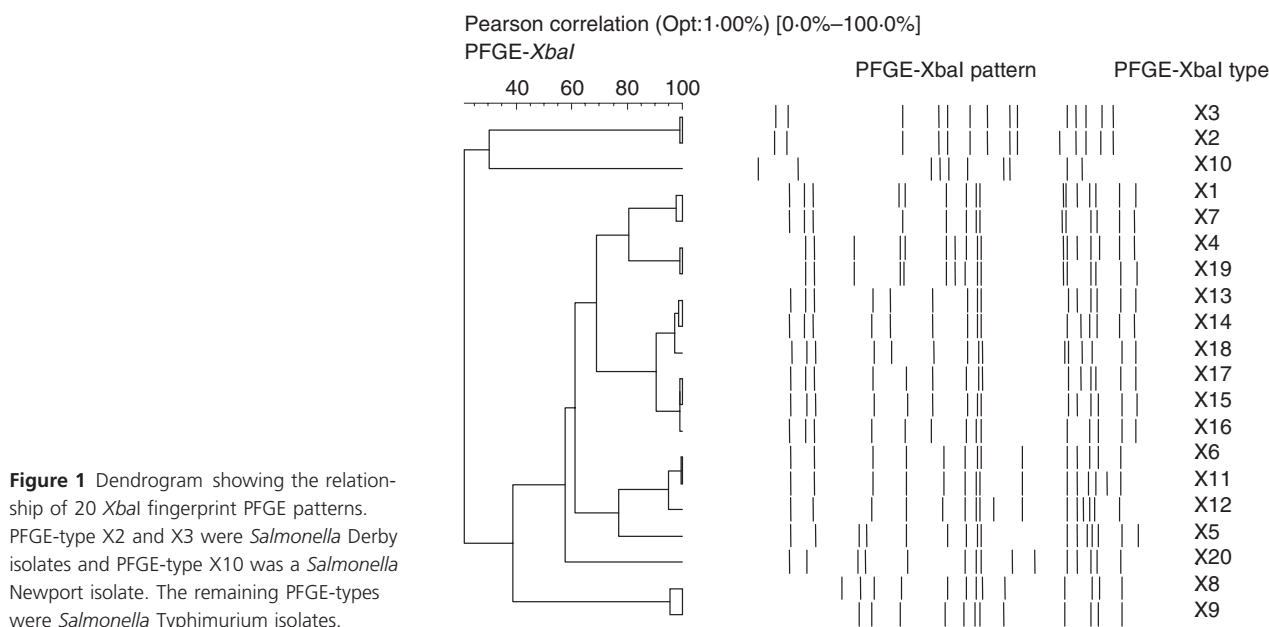


Figure 1 Dendrogram showing the relationship of 20 *Xba*I fingerprint PFGE patterns. PFGE-type X2 and X3 were *Salmonella* Derby isolates and PFGE-type X10 was a *Salmonella* Newport isolate. The remaining PFGE-types were *Salmonella* Typhimurium isolates.

Table 3 *Salmonella* isolation from wild birds* sampled from the different locations

English names	Latin names	Ecological guild	2001		2002		Total
			Winter	Summer	Winter	Summer†	
			(n/pos)	(n/pos)	(n/pos)	(n/pos)	(n/pos)
Barn Swallow	<i>Hirundo rustica</i>	Aerial insectivorous	0/0	132/0	0/0	134/1	266/1
Blackbird	<i>Turdus merula</i>	Thrushes	93/2	37/0	70/4	58/2	258/8
Blackcap	<i>Sylvia atricapilla</i>	Foliage-gleaners	0/0	6/0	0/0	15/0	21/0
Blue Tit	<i>Parus caeruleus</i>	Parus	21/0	9/0	21/0	12/0	63/0
Chaffinch	<i>Fringilla coelebs</i>	Foliage-gleaners	14/0	1/0	22/1	12/0	49/1
Dunnock	<i>Prunella modularis</i>	Terrestrial and low flycatching feeders	6/0	5/0	2/0	33/0	46/0
Great Tit	<i>Parus major</i>	Parus	63/0	24/0	44/0	27/1	158/1
Greenfinch	<i>Carduelis chloris</i>	Passerine Seed eaters	43/0	2/0	120/0	57/0	222/0
House Martin	<i>Delichon urbica</i>	Aerial insectivorous	0/0	78/0	0/0	41/0	119/0
House Sparrow	<i>Passer domesticus</i>	Passer	202/0	154/0	100/2	127/2	583/4
Pied Wagtail	<i>Motacilla alba</i>	Open-land insectivorous	0/0	8/0	0/0	22/3	30/3
Redpoll	<i>Carduelis flammea</i>	Passerine Seed eaters	3/0	0/0	18/0	0/0	21/0
Robin	<i>Erithacus rubecula</i>	Terrestrial and low flycatching feeders	16/0	3/0	11/0	53/0	83/0
Starling	<i>Sturnus vulgaris</i>	No guild	0/0	3/0	0/0	37/1	40/1
Tree Sparrow	<i>Passer montanus</i>	Passer	59/0	45/0	77/0	167/0	348/0
Whitethroat	<i>Sylvia communis</i>	Foliage-gleaners	0/0	15/0	0/0	22/0	37/0
Willow Warbler	<i>Phylloscopus trochilus</i>	Foliage-gleaners	0/0	4/0	0/0	3/1	7/1
Wren	<i>Troglodytes troglodytes</i>	Terrestrial and low flycatching feeders	11/0	14/0	5/0	13/0	43/0
37 other species of wild birds‡			23/0	68/0	38/0	44/0	173/0
Total			554/2	608/0	528/7	877/11§	2567/20

*Only bird species sampled 20 or more times, as well as species that tested positive for *S. Typhimurium* are listed.

†Figures for the summer of 2002 include samples taken at locations without production animals.

‡Including Brambling ($n = 10$), Bullfinch (9), Buzzard (2), Chiffchaff (19), Collared Dove (5), Common Gull (2), Domestic/feral pigeon (6), Fieldfare (3), Garden Warbler (14), Goldfinch (2), Green Sandpiper (1), Grey Wagtail (1), Icterine Warbler (3), Lesser Whitethroat (15), Linnet (2), Long-tailed Tit (15), Marsh Tit (19), Marsh Warbler (6), Mistle Thrush (1), Nuthatch (1), Pheasant (1), Pied Flycatcher (1), Redstart (1), Reed Warbler (1), Rook (2), Short-toed Treecreeper (1), Skylark (1), Song Thrush (1), Sparrowhawk (3), Spotted Flycatcher (2), Thrush Nightingale (1), Tree Pipet (1), Tree-creeper (1), Waxwing (1), Wood Warbler (1), Yellow Wagtail (1), Yellowhammer (17).

§All 11 *Salmonella*-positive birds were caught at the same herd.

Table 4 Prevalence of *Salmonella* in wildlife in control locations and in locations associated with infected and noninfected herds

	Infected herds	Noninfected herds	Locations without production animals	Total
	n/pos (% pos)	n/pos	n/pos	(n)
Birds (single animal samples)	1285/20 (1.5%)	1004/0	278/0	2567
Rodents (pooled or single animal samples)	135/7 (5.2%)	68/0	22/0	225
Insects (pooled samples)	31/7 (22.6%)	15/0	Not sampled	46
Dogs and cats (pooled and single animal samples)	46/3 (6.5%)	34/0	Not sampled	80
Other animals* (pooled samples)	8/1 (12.5%)	5/0	2/0	15
Total	1505/38 (2.5%)	1126/0	302/0	2933

*Samples from horses and sheep.

(*Stomoxys calcitrans* (L.)) were the predominant species. The sheep head fly (*Hydrotaea irritans*), the horn fly (*Haematobia irritans* (L.)), and the biting fly (*Haematobosca stimulans* (Mg.)) were the predominant species associated with cattle on pasture.

Pooled samples from insects living with, or in close proximity, to the infected herds were 22.6% positive for *Salmonella*. Samples (some of which were pooled) from rodents were 5.2% positive, cats and dogs were 6.5% positive and from wild birds were 1.5% positive (Table 4).

Salmonella (*S. Typhimurium*) was detected more often in wild birds exposed to cattle herds suffering clinical outbreaks, than in wild birds exposed to pig herds with persistent infections. Samples from wild birds located near infected pigs were found to be *Salmonella*-positive in only 2 of 10 sampling occasions. *Salmonella*-positive birds were detected in 4 of the 10 sampling occasions where *Salmonella* was detected in cattle at the same time. The *Salmonella*-positive birds near infected pig herds were exclusively blackbirds caught at the same farm, one during each winter sampling (Table 2). The *Salmonella*-positive birds near cattle herds were composed of various species. Near one herd, there was a single blackbird. Near another herd, there were three blackbirds, one house sparrow, and one chaffinch (*Fringilla coelebs*). A single house sparrow was near a third herd. Finally, there were three pied wagtails (*Motacilla alba*), two blackbirds, two house sparrows, one willow warbler (*Phylloscopus trochilus*), one great tit (*Parus major*), one starling (*Sturnus vulgaris*), and one barn swallow near the last herd. All *Salmonella*-positive birds were caught during the two winter sampling rounds (Table 3), with only one exception. *Salmonella Typhimurium* was isolated from 11 wild birds in summer 2002 (Table 3) in one of the cattle herds.

Statistical analysis of factors correlated with occurrence of *S. Typhimurium* in birds (Table 3) revealed that both the behaviour and the migration pattern were correlated with the occurrence of *S. Typhimurium*. Thus, partially migratory (OR = 3.0) or short-to-medium distance

migratory (OR = 5.8) birds were at higher risk of contracting *Salmonella* infections than nonmigrating (resident) birds. Long-distance migrants (OR = 0.9) were at a somewhat lower risk compared to nonmigrating birds.

The detection of *S. Typhimurium* in birds was significantly associated with the food preferences of the birds. In the summer, birds feeding on insects and invertebrates were at a higher risk of infection compared to birds feeding on seeds and grains (OR = 3.6). There were no statistical differences in the detection of *S. Typhimurium* ($P = 0.08$) among birds foraging predominately on the ground, in vegetation, or in the air. However, a slightly higher risk of *S. Typhimurium* detection was detected for birds foraging on the ground in comparison to aerial foraging or foraging in the vegetation (OR = 3.2). Wild birds that were at some point in contact with slurry were not significantly associated with positive samples ($P = 0.09$). No significant difference was observed between species living close to or inside stables compared to species living peripherally to the buildings (summer: $P = 0.18$; winter: $P = 0.22$).

Discussion

Salmonella was isolated from wildlife at farms where *Salmonella* was also detected in the production animals and only during the periods when *Salmonella* was detected in the production animals. *Salmonella* was isolated from insects (22.6%), rodents (5.2%), cats and dogs (6.5%), and wild birds (1.5%) living close to the infected herds. The percentage of infected animals found in each herd in this study is not directly comparable with results from other studies (Barber et al. 2002; Hilton et al. 2002; Refsum et al. 2002a; Veling et al. 2002) because the actual prevalence depends on the sampling location and methods applied. Thus, the frequency of *Salmonella* isolated from 22 g of feces from production animals was compared to the isolation frequencies from 1 g of rodents and from cloacal swab samples from birds. This may result in an unrealistically low prevalence of *Salmonella*,

e.g. in birds. However, previous Danish investigations based on bird carcasses have also revealed a low prevalence of *Salmonella* in wild birds (Jørgensen 2002). Barber *et al.* (2002) showed that only 6% of samples from flies were *Salmonella*-positive, whereas Bailey *et al.* (2001) found a prevalence (18.7%) more closely resembling the results of this study (22.6%). *Salmonella* infections in rodents were investigated in the United Kingdom by Healing (1991), who found that rarely more than 10% of the animals carried *Salmonella*. Other investigations of *Salmonella* in birds (Fenlon 1981; Palmgren *et al.* 1997; Edel *et al.* 2002; Refsum *et al.* 2002a) reported a higher prevalence of *Salmonella*-positive birds than reported in this study. Blackbirds, pied wagtails, and house sparrows are the species most frequently infected with *Salmonella* according to this study. Other studies showed that (Fenlon 1981; Kapperud and Rosef 1983; Palmgren *et al.* 1997; Morishita *et al.* 1999; Bonnedahl *et al.* 2002; Palmgren *et al.* 2002; Refsum *et al.* 2002a) *Salmonella* was predominantly found in other bird species such as starling (Morishita *et al.* 1999), gulls (*Larus* spp.) (Fenlon 1981; Edel *et al.* 2002) and bullfinch (*Pyrrhula pyrrhula*) (Refsum *et al.* 2002a) with a 7.1%, 12.9% and 54% prevalence, respectively. However, this investigation included post-mortem records (Refsum *et al.* 2002a), which is not comparable to the methods we used. Kapperud and Rosef (1983) discovered a prevalence (0.8%) of *Salmonella*-positive birds among birds lacking clinical disease, a value comparable to that presented in the present study. In contrast to our study, Cizek *et al.* (1994) discovered a far higher prevalence of *Salmonella*-positive house sparrows in the vicinity of *Salmonella*-infected cattle farms (23%, as opposed to 1.5%); however, they did not find any *Salmonella*-positive blackbirds or pied wagtails (Cizek *et al.* 1994). Kirk *et al.* (2002) found the prevalence of *Salmonella*-positive cowbirds (*Molothus ater*) to be 1.2% and house sparrows to be 3.2%. In conclusion, the prevalence of *Salmonella* in wildlife varies a great deal between different studies, and it is not possible to determine whether the variation is a result of different sampling methods and study designs applied, or whether it reflects a significant difference in disease prevalence among countries.

Both phenotypical and genotypical typing of the isolates show that the *Salmonella* types isolated from wildlife were identical (with the exception of one case) to at least one of the types isolated from production animals on the same sampling occasion. This suggests that the population of *Salmonella* may be shared among different animal species at each farm. In cases where the phenotype did not allow us to characterize the clonal relationship between isolates (e.g. identification of inconclusive phage types such as RDNC or NT), we used genotypic typing.

The use of both typing methods has previously been used to strengthen conclusions regarding the transmission of isolates between different sources (Bonnedahl *et al.* 2002; Refsum *et al.* 2002b; Liebana *et al.* 2003). *Salmonella*-positive birds near pig herds were found only during the winter. Five *Salmonella*-positive rodent samples were found during the winter and only two positive samples during the summer. Although the numbers are small, our results suggest that in herds where *Salmonella* infection has a more persistent character, the transmission between wildlife and production animals is more likely to take place during the humid winter season than during the dry summer. This may be due to a change in behaviour in the winter as wildlife may move closer to farms in search of food and shelter. In Denmark, house mice commonly move in the spring from human settlements, such as farms, to the open countryside and back again in the autumn (Carlsen 1993).

In cattle herds, both the presence of clinical disease and the high prevalence of *Salmonella* among the production animals (Table 1), appear to be the most important factors for the transmission of *Salmonella* to nonproduction animals. Refsum *et al.* (2002a) found that outbreaks of salmonellosis among birds peak in February and March, although their study did not investigate the potential contact between wildlife and production animals.

Both migratory behaviour and food preference of birds were factors significantly associated with the distribution of *S. Typhimurium*. This association was, however, not evident with respect to their foraging activities relative to the location of food sources (i.e. on the ground, in air or in vegetation). In accordance with our study, other investigations identified the source of food as a possible risk factor for bacterial infections in birds. Accordingly, Brittingham *et al.* (1998) investigated whether the prevalence of six different genera of bacteria was related to diet. None of the samples were *Salmonella*-positive. However, the results showed that omnivorous species had a higher prevalence of *Streptococcus* spp. than herbivorous species. In addition, Waldenström *et al.* (2002) showed that both birds feeding on invertebrates and opportunistic feeders were more commonly infected with *Campylobacter* spp. than other species. In agreement with our *Salmonella* results, Waldenström *et al.* (2002) also showed that the prevalence of *Campylobacter* spp. was higher in short-distance migrants than in long-distance migrants.

In conclusion, our results indicate that there is a transmission of *Salmonella* between production animals and nonproduction animals. Similar results have been reported by other workers (Kapperud *et al.* 1998; Warnick *et al.* 2001). However, in the present study the interaction seems to be most pronounced in relation to

outbreaks of clinical disease among the production animals. Both the design of this study, as well as the low number of *Salmonella*-positive samples, does not allow us to draw firm conclusions about the direction of the transmission. Nonetheless, the results strongly indicate that *Salmonella* is most likely transmitted from production animals to nonproduction animals living near to infected herds, especially during acute outbreaks with concomitant high concentrations of bacteria in the surroundings. Our results do not support the hypothesis that nonproduction animals are the source of infection in production animals. However, it must be pointed out that nonproduction animals may act as a reservoir for pathogens, and/or as passive carriers, and may pose a potential risk of transmission to other herds, or reintroduction to the original herd.

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