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Is *Salmonella enterica* shared between wildlife and cattle in cattle farming areas? An 11-year retrospective study in Tokachi district, Hokkaido, Japan

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Abstract

Background: *Salmonella enterica* in cattle has long been problematic and suspected to be transmitted by wildlife in Tokachi, Hokkaido, a major cattle farming area in Japan. Understanding the role of wildlife in *S. enterica* transmission would be helpful for developing control strategies of bovine salmonellosis.

Objectives: We aimed to elucidate the possibility of *S. enterica* transmission between sympatric wildlife, including raccoons and crows and cattle, in Tokachi from 2008 to 2018 by analysing *S. enterica* detection records, and the genetic relatedness of serotypes shared between wildlife and cattle.

Methods: *S. enterica* detection records were based on the results of a field survey and existing cattle records at relevant organisations, including clinical reports, a monitoring survey and quarantine for introduced calves at growing farms and public calving farms. *S. enterica* was identified by polymerase chain reaction assay and serotyped by agglutination assay. The detection records were organised chronologically to investigate whether common serotypes in wildlife and cattle were detected in the same year. The isolates corresponding to detection records were assessed for their genetic patterns by pulsed-field gel electrophoresis.

Results: The prevalence of *S. enterica* in raccoons and crows was 10.7% (17/159) and 5.7% (55/967), respectively. The following serotypes were detected from both wildlife and cattle: Braenderup, Dublin, Infantis, Mbandaka, Montevideo, 4,[5],12:i:- and Typhimurium. Genetically similar isolates for *S.* Braenderup, *S.* Dublin, *S.* Montevideo and *S.* 4,[5],12:i:- were detected from both species in the same year.

Conclusions: Our long-term retrospective observations supported that *S. enterica* was shared between wildlife and cattle. Wildlife invasions should be controlled at farms to prevent inter-species transmission of *S. enterica* from livestock farms.

KEYWORDS
cattle, pulsed-field gel electrophoresis, *Salmonella enterica*, wildlife

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1 | INTRODUCTION

Salmonella enterica is one of the most common and burdensome food-borne pathogens worldwide (EFSA, 2019a; Lee & Yoon, 2021). It is carried by various vertebrates, including livestock (Markey et al., 2013). The global yearly estimate of *Salmonella* gastroenteritis is 95.1 million cases, with 50,771 deaths in 2017 (Stanaway et al., 2019), which may come from livestock products including poultry, eggs, pork, beef and dairy products (Pires et al., 2014; Scallan et al., 2011). Therefore, understanding *S. enterica* ecology around livestock will help in controlling human salmonellosis.

Various surveys have shown the prevalence of *S. enterica* in wildlife (Bondo et al., 2016; Janecko et al., 2015; Skov et al., 2008), and genetic analyses have implied the association of *S. enterica* between wildlife and livestock (Carlson et al., 2015; Horton et al., 2013; Mentaberre et al., 2013). Accordingly, it has been postulated that wildlife carriers might transmit *S. enterica* from contaminated farms to other farms, which makes *S. enterica* prevention more difficult on farms in a large area. However, livestock farms strongly and unwittingly attract wildlife by providing them with feed and resting spaces (Tsukada et al., 2010); therefore, the protection measures demand great efforts, for which they may not be motivated. Further investigation of the transmission of *S. enterica* between wildlife and cattle will aid the decision-making in applying protection measures.

Tokachi district, a major cattle farming area in Hokkaido, Japan, has 1400 dairy farms and 800 beef farms rearing 450,000 cattle (Ministry of Agriculture, Forestry and Fisheries, 2018); cattle salmonellosis has occurred here every year (Hokkaido Prefectural Government, 2020). Livestock products are notable food sources for human foodborne salmonellosis (Pires et al., 2014); thus, *S. enterica* prevention in cattle farms in Tokachi is critical for both farmers' health in this district, and for food safety nationwide. A previous study (Fujii et al., 2012) revealed that wildlife was infected with the same serotypes of *S. enterica* as cattle in Tokachi, which suggested that the wildlife was potentially a source of bovine salmonellosis. Due to this background, farmers are concerned about *S. enterica* in both wildlife and cattle. *S. enterica* surveys such as monitoring surveys using wildlife carcasses and environmental samples at cattle farms have been performed in some municipalities in Tokachi. However, the survey results have not been sufficiently well-organised to investigate inter-species transmission of *S. enterica*. Comprehensive knowledge of *S. enterica* from wildlife and cattle in Tokachi, including genetic characteristics of isolates, will be helpful to evaluate the possibility of transmission of *S. enterica* between wildlife and cattle.

The objective of this study was to elucidate whether *S. enterica* has been shared between wildlife and cattle. Then, we aimed to organise retrospectively *S. enterica* detection information derived from survey results and cattle clinical records by serotype between 2008 and 2018 in Tokachi, and assess the trends of detection of each serotype in each species, and similarity of the genetic characteristics in the corresponding *S. enterica* serotypes from wildlife and cattle. In this study, we tested the following working hypotheses: (1) wildlife and cattle inhabiting the same area were infected with the same *S. enterica* serotypes in the same time period and (2) *S. enterica* serotypes from wildlife and cattle had similar genetic patterns [pulsed-field gel electrophoresis (PFGE) patterns]. In Tokachi, the previous study showed that jungle crows (*Corvus macrophalynchos*), carrion crows (*Corvus corone*) and raccoons (*Procyon lotor*) were infected with *S. enterica* (Fujii et al., 2012). These species are known to often invade livestock farms in search of feed and resting sites (Takeda et al., 2015; Yamaguchi et al., 2020), which probably results in *S. enterica* transmission between wildlife and cattle via faeces or direct contact. Accordingly, we surveyed these wildlife species as high-risk species for *S. enterica* transmission between cattle farms in Tokachi.

2 | MATERIALS AND METHODS

2.1 | Study area

We investigated *S. enterica* in wildlife, consisting of raccoons and crows, and cattle in three municipalities in Tokachi retrospectively between 2008 and 2018, using the past monitoring survey results for wildlife and cattle environments, diagnosis records for individual cattle and testing records for introduced calves. Three adjacent municipalities, towns A, B and C, were selected as study areas to survey wildlife and cattle sympatrically inhabiting Tokachi. The number of cattle farms in these municipalities ranged from 50 to 200, and tended to decrease as the study period progressed. The average herd size ranged from 150 to 200 in dairy farms and from 700 to 2300 in beef farms in 2015, and has recently tended to increase.

2.2 | Collection of wildlife samples

To identify *S. enterica* serotypes in wildlife, first of all, we collected the individuals exterminated in towns A, B and C between 2008 and 2018: the details for each species are shown in Table 1. Crows were collected only in town A. The individuals were caught by traps and sacrificed by CO_2 gas or gun shots under pest control programs. Because samples from wildlife were dependent on the program, samples could not be collected evenly across years, species and towns. Among the tested individuals, approximately 60% of raccoons and 90% of crows were captured at cattle farms. The other capture sites included riverside, cropfields and yards of human residences for raccoons and landfill for crows. Rectal/cloacal swabs were collected from all individuals. Considering the possibility of mechanical transmission of *S. enterica* by wildlife, footpad swabs were collected from 60% to 90% of individuals in each species. We did not test the footpad swabs provided with other specimens in a single bag, because of the possibility of cross-contamination. After collection, the swab samples were immediately put into pre-enrichment or enrichment broth for culture tests.

'*S. enterica*-positive' in each individual was defined as isolating *S. enterica* from either or both sample types and counted as one

TABLE 1 Number of tested samples from wildlife for *Salmonella enterica* between 2008 and 2018, in Tokachi, Hokkaido, Japan

Species	Town	Year										
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Raccoon	A, B, C	–	39 (39)	11 (11)	15 (15)	14 (7)	6 (2)	–	–	–	70 (14)	4 (4)
Jungle Crow	A	–	41 (41)	37 (37)	73 (73)	54 (11)	23 (0)	–	9 (9)	176 (176)	151 (151)	41 (34)
Carrion Crow	A	14 (12)	74 (74)	19 (19)	37 (37)	50 (20)	25 (0)	–	1 (1)	100 (100)	32 (32)	10 (8)

Note: The number outside of parenthesis refers to the number of individuals from which rectal/cloacal swabs were taken. The number in parenthesis refers to the number of individuals from which footpad swabs were also taken.

detection by serotype. The prevalence of *S. enterica* in wildlife was calculated by species and sample type. We tested the difference in prevalence by species and sample type using Fisher's exact test in R version 4.0.2 (R Core Team, 2020).

In organising the prevalence and serotypes of *S. enterica* in each species, the data in wildlife between 2008 and 2010 referred to the previous report (Fujii et al., 2012). Ten isolates, including *S.* Typhimurium, *S.* Braenderup and *S.* Infantis, reported by Fujii et al. (2012) were assessed genetically by PFGE analysis for the first time in this study.

2.3 | Collection of cattle samples

To determine *S. enterica* serotypes in cattle, we collected *S. enterica* detection records between 2008 and 2018 in the following four collection schemes. A brief explanation of the records collected in each scheme follows: (1) clinical signs: *S. enterica* detection using faeces or blood samples from cattle presenting clinical signs suspected to be salmonellosis in dairy and beef farms in towns A, B and C; (2) introduction test: *S. enterica* detections in quarantine in faeces samples from calves introduced into beef growing farms or public calving farms in towns A, B and C; (3) annual monitoring: results of monitoring for *S. enterica* dissemination using environmental samples taken from manure storage and alleys for lactating cattle at all dairy farms in town A once or twice a year and (4) monthly monitoring: results of monthly monitoring for *S. enterica* dissemination using environmental samples by the same method as with annual monitoring at nine dairy farms sending calves to public calving farms in town A between 2017 and 2018. In the clinical signs and introduction tests, the number of detections of *S. enterica* serotypes shared between wildlife and cattle was available in each town, but the total number of tests was not available. The details of each scheme are described in the Appendix.

S. enterica detection records were collected from the Union for Livestock Infectious Diseases Prevention (ULIDP), comprised local livestock farming-related organisations, and from Tokachi Livestock Hygiene Service Center (LHSC), a public organisation for diagnosis of livestock diseases. Collection of culture test samples and *S. enterica* testing for each sampling scheme were performed by multiple organisations including ULIDP, LHSC and the Animal Research Center (see Table S1).

2.4 | *Salmonella enterica* culture test and serotyping of isolates

S. enterica culture tests were performed according to the previous study (Fujii et al., 2012). The footpad swabs from wildlife and environmental samples were incubated at 37°C for 18–24 h in pre-enrichment broth. The cloacal/rectal swabs from wildlife were inoculated in enrichment broth, skipping the pre-enrichment stage. For pre-enrichment, 10 ml of buffered peptone water (BPW) (Oxoid) and 9 ml of Enterobacteriaceae Enrichment Mannitol (EEM) (Eiken) broth per sample were used in 2008 and in 2009 to 2018, respectively. In enrichment, 1 ml of the pre-enrichment culture or rectal/cloacal swab sample was added to 10 ml of enrichment broth and incubated at 42°C for 18–24 h. For enrichment, tetrathionate broth (Eiken) and Rappaport-Vassiliadis broth (Eiken or Atect) were used in 2008 and in 2009 to 2018, respectively. After enrichment, the culture was plated on both desoxycholate hydrogen sulfide lactose (Eiken) agar containing 20 μg novobiocin/ml (n-DHL; Sigma-Aldrich Japan) and ES Salmonella Agar II (Eiken) and incubated at 37°C for 24–48 h. For *S. enterica* detections from cattle in the sampling schemes for clinical signs and introduction tests, only the detection records with their serotypes were provided by LHSC.

A colony suspected to be *S. enterica*, black-coloured on n-DHL agar or pink-coloured on ES Salmonella II, was picked up from each agar and cultivated on the same kind of agar to obtain a pure culture. The isolates were identified serologically using polyvalent O- and O1-grouping antisera (Denka Seiken) and confirmed by PCR assay by detecting the *inv*A gene of *S. enterica*. The template DNA for PCR was extracted from a purely cultivated colony using Instagene matrix (BioRad Inc.). The primers are as follows, referring to Chiu and Ou (1996): INVA-1 (5′-ACAGTGCTCGTTTACGACCTGAAT-3′) and INVA-2 (5′-AGACGACTGGTACTGATCGATAAT-3′). The total 25 μl volume of PCR reaction mixture constituted 12.5 μl of KAPA2G™ Fast Hot-Start ReadyMix (Kapa Biosystems) and 1.25 μl of each 10μM primer and 2.5 μl template. The thermal protocol was modified according to manufacturer recommendations and involved 3 min of initial denaturation at 95°C, followed by 35 cycles of denaturation (95°C, 15 s), primer annealing (60°C, 15 s), extension (72°C, 5 s) and a final extension (72°C, 1 min) using i Cycler Thermal cycler (BioRad Inc.). DNA extracted from a known *S. enterica* Typhimurium colony by the same method as the samples was used as a positive control. Amplified PCR products were electrophoresed on 1.5% agarose gel to verify the

presence of amplicons of the expected size (224 bp). *S. enterica* isolates were serotyped using commercial O and H antisera (Denka Seiken) based on the Kauffmann-White scheme (Grimont & Weill, 2007). Isolates determined to be monophasic variants of *S.* Typhimurium, that is *S.* 4,[5],12:i:-, were considered separate from *S.* Typhimurium.

2.5 | Organising *Salmonella enterica* detection records

All *S. enterica* detection records were arranged chronologically by species and serotype to investigate whether the same serotype was detected from both wildlife and cattle in the same year. The month-level data were also investigated for the relevant detection events caused by genetically similar isolates between wildlife and cattle. *S. enterica* detection was recorded individually in wildlife and at farm level in cattle. Multiple detections of the same serotype in cattle in the same farm in a year were considered to be a sequential outbreak and counted as one detection. Because cattle sample collection methods and the subject serotype in town A were different from those in towns B and C, the detection records from cattle in town A were also arranged by sampling schemes independently. This study retrospectively analysed the existing sampling records, which were not initially recorded with the aim of comparing the prevalence between species in the long term; therefore, the numbers of collected records were irregular and uneven among species. Then, we focused on the trends of detection of each serotype among detected *S. enterica*.

2.6 | Pulsed-field gel electrophoresis (PFGE) analysis

The serotypes detected from both wildlife and cattle were assessed by PFGE analysis to determine the genetic diversity and relatedness among isolates and their corresponding detection records. One isolate recovered at each *S. enterica* detection event was subjected to PFGE analysis. Multiple detections of the same serotype from the same species caught at the same place in a year were considered to be derived from a single epidemic strain with the same genetic characteristics; isolates from the first individual identified to be positive for *S. enterica* in each serotype and species in a year were subjected to PFGE analysis. Without a significant difference in *S. enterica* prevalence between the crow species, they were regarded as the same species 'crow' in selecting the isolate for PFGE analysis.

PFGE analysis was performed to determine the genetic patterns of *S. enterica* isolates referring to the following protocol (Ribot et al., 2006). Bacterial DNA prepared in agar plug was digested with 30 units of *Xba*I (Nippon gene). The plugs were run off in 22 h using a CHEF Mapper XA system (Bio-Rad Laboratories) in 0.5 × Tris-borate-EDTA buffer (Nippon gene) supplemented with 20 μM thiourea at 14°C and 6 V/cm, while the pulse switch time was increased from 2.2 to 63.8 s. Lambda PFG Ladder N0341 (New England Biolabs Japan Inc.) was used as a molecular reference marker. The fragment patterns were automatically analysed, comparing them with the pattern of the reference marker using GelJ software (Heras et al., 2015). Dendrograms for each serotype were constructed using the unweighted pair group matching algorithm (UPGMA) using Dice coefficients with 2% band position tolerance. Based on the criteria for interpreting PFGE patterns (Tenover et al., 1995), the same Clade ID was given to isolates with PFGE band differences $\leq$ 3, designating them as closely related strains.

3 | RESULTS

3.1 | *Salmonella enterica* detections in wildlife

Prevalence of *S. enterica* at the individual level between 2008 and 2018 was 10.7% (17/159, 95% CI: 6.4–16.5%) in raccoons, 5.8% (35/605, 95% CI: 4.1–8.0%) in jungle crows and 5.5% (20/362, 95% CI: 3.4–8.4%) in carrion crows (Table 2). In raccoons and jungle crows, *S. enterica* was detected more frequently from rectal/cloacal swabs than from foot-pad swabs ($p = 0.02$, $p < 0.01$, respectively). In carrion crows, the frequency of *S. enterica* in cloacal swabs did not significantly differ from that in footpad swabs ($p = 0.2$). While the prevalence did not significantly differ between the crow species ($p = 1$), *S. enterica* was more prevalent in raccoons than in crows ($p = 0.02$). Isolated serotypes were Agona, Braenderup, Dublin, Infantis, Manhattan, Mbandaka, Montevideo, Nigeria, 4,[5],12:i:-, 4,[5],12:HUT (untypable H antigen), Stanley, Thompson and Typhimurium (Table 2). The most frequently isolated serotypes were Braenderup and Thompson in raccoons, and Infantis and Mbandaka in both crow species (Table 2).

3.2 | *Salmonella enterica* detections in cattle

In town A, 29 detections of *S. enterica* of nine serotypes, Anatum, Braenderup, Dublin, Muenster, 4,[5],12:d:-, 4,[5],12:i:-, Reading, Typhimurium and Wangata, were recorded in dairy farms and one detection of *S.* Dublin was recorded in a beef farm (Table 3). Twenty-one detections of the same serotype as in the wildlife isolates were recorded in dairy cattle in Town A throughout the study period: Braenderup, Dublin, 4,[5],12:i:- and Typhimurium. Table S2 shows the detected serotypes in town A by year and each scheme. In towns B and C, 60 detections of the same serotype as the wildlife isolates were recorded throughout the study period: Braenderup, Dublin, Infantis, Mbandaka, Montevideo, 4,[5],12:i:- and Typhimurium (Table 3).

3.3 | Common serotypes in wildlife and cattle

Seven serotypes were detected from both wildlife and cattle in towns A, B and C: Braenderup, Dublin, Infantis, Mbandaka, Montevideo, 4,[5],12:i:- and Typhimurium (Table 3). Comparing the number of detections of each serotype by species, *S.* Dublin (wildlife: 2, cattle: 11) and *S.* 4,[5],12:i:- (wildlife: 3, cattle: 51) were frequently found in cattle. In

TABLE 2 Prevalence and serotypes of Salmonella isolated from swab samples from wildlife in Tokachi, Hokkaido, Japan, between 2008 and 2018

Species	Rectum/cloaca		Footpad		Individual[b]
Raccoon	16/159 (10.1%)	Braenderup (5) Infantis (1) Stanley (2) Thompson (4) 4,[5],12:i:- (2) Typhimurium (2)	2/92 (2.2%)	Braenderup (1) Stanley (1)	17/159 (10.7%)
Jungle crow	32/605 (5.3%)	Agona (2) Dublin (2) Infantis (12) Manhattan (1) Mbandaka (10) 4,[5],12:i:- (1) Typhimurium (4)	7/532 (1.3%)	Infantis (4) Montevideo (2) Typhimurium (1)	35/605 (5.8%)
Carrion crow	16/362 (4.4%)	Agona (2) Infantis (5) Mbandaka (6) Montevideo (1) Nigeria (1) 4,[5],12:HUT[b] (1)	7/303 (2.3%)	Braenderup (1) Infantis (2) Mbandaka (1) Montevideo (3)	20/362 (5.5%)

[a]*Salmonella*-positive individual was defined as isolating *Salmonella* from either or both sample types.
[b]HUT: untypable H antigen.

contrast, *S.* Infantis (wildlife: 19, cattle: 3) and *S.* Mbandaka (wildlife: 16, cattle: 3) were more frequently isolated from wildlife. *S.* Typhimurium (wildlife: 7, cattle: 7) and *S.* Braenderup (wildlife: 7, cattle: 6) were isolated from all species at a similar frequency. *S.* Montevideo was detected in a cattle farm and from five crows caught in the same trap on the same day in 2011. Out of the five crows, one crow was positive for *S.* Montevideo in both cloacal and footpad samples, while the others were positive only in footpad samples; therefore, these crows were probably contaminated with *S.* Montevideo in the trap mechanically. The seven serotypes were detected from wildlife and cattle in the same year at least once during the study period.

3.4 | Similarity of PFGE patterns

Similarity of PFGE patterns between wildlife and cattle isolates varied by serotype (Figures 1–7). For *S.* Braenderup, *S.* Dublin, *S.* Montevideo, *S.* 4,[5],12:i:- and *S.* Typhimurium, a part of the PFGE clade included both wildlife and cattle isolates. For *S.* Braenderup, *S.* Dublin, *S.* Montevideo and *S.* 4,[5],12:i:-, some of the wildlife and cattle isolates grouped into the same PFGE clade were recovered in the same year. For *S.* Braenderup, raccoon isolates were recovered 4 months before and 4 months after the cattle isolates in 2009, and 1 month after the cattle isolates in 2012. Month data were missing for cattle isolates in 2011. For *S.* Dublin, the crow isolate was recovered 1 month after the recovery of the cattle isolate in 2015, and the crow and cattle isolates were recovered in the same month, and cattle isolates were also recovered 2 months before and 1 month after in 2016. For *S.* Montevideo, the cattle isolate data at the level of month were not available. For *S.* 4,[5],12:i:-, cattle isolates were intermittently recovered throughout the year in

2016 and 2017, in which wildlife isolates were recovered. For *S.* Infantis and *S.* Mbandaka, PFGE clades for wildlife and cattle isolates were different from each other. Four isolates were not able to be assessed for PFGE analysis: one isolate each of *S.* Braenderup, *S.* Typhimurium, *S.* 4,[5],12:i:- and *S.* Dublin.

4 | DISCUSSION

Our retrospective study organised the *S. enterica* detection records in Tokachi stored in relevant organisations in order to elucidate the distribution and genetic relatedness of *S. enterica* serotypes in wildlife and cattle over the long term (10 years). We observed detections of the same serotype of *S. enterica* from wildlife and cattle in the same year. For *S.* Braenderup, *S.* Dublin, *S.* Montevideo and *S.* 4,[5],12:i:-, the isolates from both species in the same year were genetically similar to each other (Figures 1, 2, 5 and 6), which demonstrated that *S. enterica* was shared between wildlife and cattle. These serotypes included critical ones for livestock hygiene and public health: *S.* Dublin, highly pathogenic in cattle (Harvey et al., 2018), and *S.* 4,[5],12:i:-, which has recently been widespread worldwide in livestock and humans (Arai et al., 2018; EFSA, 2019b). All isolates of *S.* Dublin and *S.* 4,[5],12:i:- from wildlife and one *S.* Braenderup isolate from a raccoon were recovered in the same month or, 1 month before or after the cattle isolates of the same serotype. Such detection patterns indicate that *S. enterica* probably transmitted between species directly or indirectly in the detection period. Prevalence in jungle crows and carrion crows was not significantly different and the typical serotypes were *S.* Mbandaka and *S.* Infantis in both types of crows (Table 2). Such similarity in prevalence and typical serotypes in the crows may be caused by horizontal

TABLE 3 Serotypes of Salmonella isolated from cattle and wildlife animals in Tokachi, Hokkaido, Japan, between 2008 and 2018

	Cattle			Wildlife		
	Dairy cattle	Beef cattle	Dairy and Beef cattle[a]	Raccoon	Jungle crow	Carrion crow
Year	Town A	Town A	Town B and C	Town A, B, C	Town A	Town A
2008	Anatum(2) 4,[5],12:d:-(1) **Typhimurium(2)**	None	**Braenderup(1)** Infantis(1) **Typhimurium(1)**	ND[c]	ND	**Braenderup(1)**
2009	**Typhimurium(1)**	None	**Braenderup(2)** **Typhimurium(1)**	**Braenderup(1)** Thompson(2)	Infantis(4) **Typhimurium(1)**	Infantis(4)
2010	None[b]	None	**Infantis(1)** **Typhimurium(1)**	Braenderup(1) Thompson(1) **Typhimurium(2)**	**Infantis(3)**	**Infantis(1)**
2011	4,[5],12:d:-(1)	None	**Braenderup(1)** **Infantis(1) Montevideo(1)**	**Braenderup(2)** Thompson(1)	**Montevideo(2)**	**Infantis(1)** **Montevideo(3)**
2012	**Braenderup(1)**	None	Mbandaka(1) 4,[5],12:i:-(1)	**Braenderup(2)**	**Infantis(1)** Manhattan(1) Typhimurium(1)	4,[5],12:HUT(1)
2013	4,[5],12:i:-(1)	None	4,[5],12:i:-(4)	None	Infantis(2)	None
2014	Braenderup(1) 4,[5],12:i:-(1)	None	4,[5],12:i:-(2)	ND	ND	ND
2015	**Dublin(1)** 4,[5],12:i:-(1)	**Dublin(1)**	4,[5],12:i:-(10)	ND	Infantis(2) **Dublin(1)**	None
2016	**Dublin(3)** Muenster(1) **4,[5],12:i:-(1)**, Wangata(1)	None	**Dublin(1)** **4,[5],12:i:-(12)**	ND	Agona(1) **Dublin(1)** Mbandaka(3) **4,[5],12:i:-(1)** Typhimurium(3)	Agona(2) **Mbandaka(6)**
2017	Dublin(1) Muenster(1) **4,[5],12:i:-(3)**	None	Dublin(1) Mbandaka(1) **4,[5],12:i:-(7)**	Infantis(1) **4,[5],12:i:-(2)** Stanley(2)	**Mbandaka(6)**	None
2018	**Dublin(2)** 4,[5],12:i:-(1) Reading(1) Typhimurium(1)	None	**Dublin(1)** **Mbandaka(1)** 4,[5],12:i:-(7)	None	Agona(1) **Mbandaka(1)**	Nigeria(1)

Note: The number in parenthesis in cattle refers to the number of farms in which each serotype was detected. The number in parenthesis in wildlife refers to the number of individuals in which each serotype was detected. Serotypes in bold characters were common to both wildlife and cattle. Underlined serotypes were common to both wildlife and cattle in each year.

[a]Only detections of the same serotype with wildlife isolates are described.

[b]ND means *Salmonella* test was not done.

[c]None means no *Salmonella* was detected.

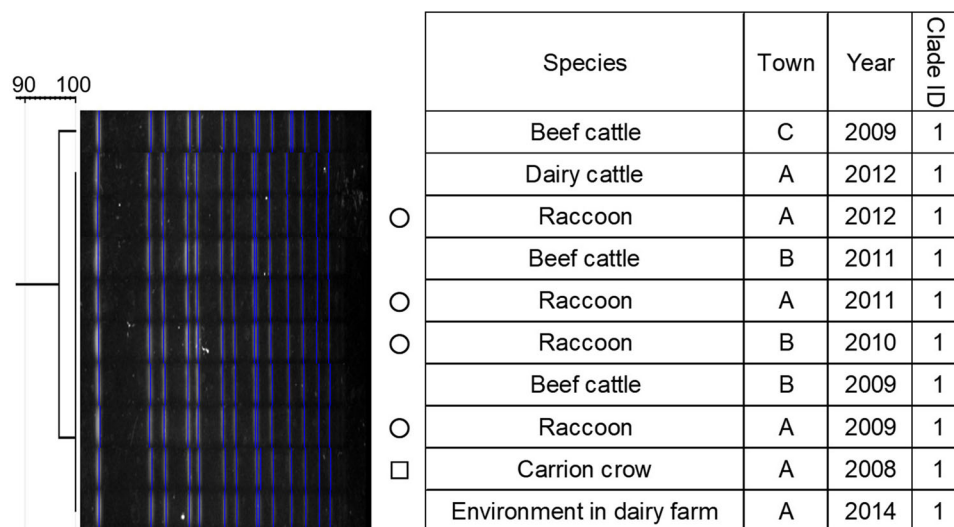| Species	Town	Year	Clade ID
Beef cattle	C	2009	1
Dairy cattle	A	2012	1
Raccoon	A	2012	1
Beef cattle	B	2011	1
Raccoon	A	2011	1
Raccoon	B	2010	1
Beef cattle	B	2009	1
Raccoon	A	2009	1
Carrion crow	A	2008	1
Environment in dairy farm	A	2014	1

FIGURE 1 Dendrogram of pulsed-field gel electrophoresis (PFGE) gel band patterns for *Salmonella* Braenderup isolates. Isolates from raccoons are marked with circles. The isolate from the crow is marked with a square
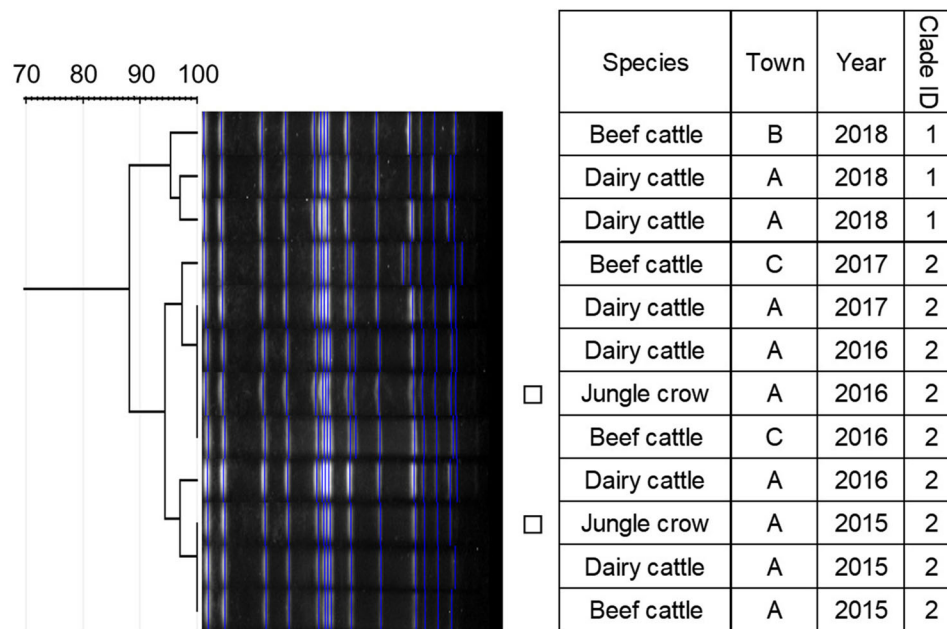
FIGURE 2 Dendrogram of pulsed-field gel electrophoresis (PFGE) gel band patterns for *Salmonella* Dublin isolates. Isolates from crows are marked with squares
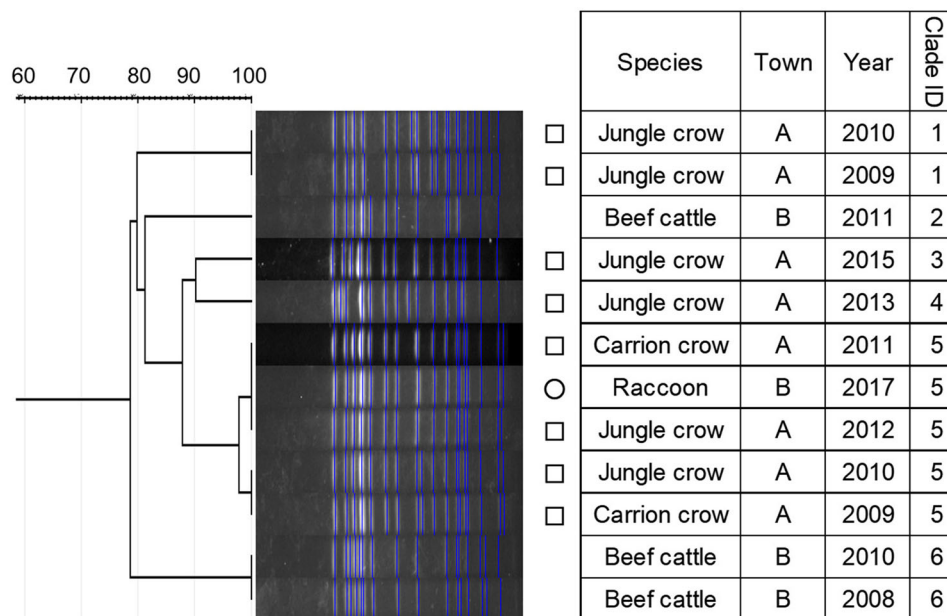

FIGURE 3 Dendrogram of pulsed-field gel electrophoresis (PFGE) gel band patterns for *Salmonella* Infantis isolates. The isolate from the raccoon is marked with a circle. Isolates from crows are marked with squares

transmission between crows due to shared feeding grounds and mixed-species flocks.

Although it was difficult to compare the frequency using test data based on uneven sampling methods, *S.* Dublin and *S.* 4,[5],12:i:- were infrequently detected from wildlife, unlike from cattle (Table 3). Additionally, these serotypes were detected from wildlife only in years with outbreaks of each serotype in cattle (Table 3). In contrast, *S.* Infantis

and *S.* Mbandaka isolates that were not genetically similar between each species were detected frequently from wildlife, unlike from cattle (Figures 3 and 4 and Table 3). Considering these observations of *S. enterica* detection, the shared isolates for *S.* Dublin and *S.* 4,[5],12:i:- likely originated from cattle or other unsurveyed species; wildlife did not appear to play a role in the source of infection for transmission. Inherent characteristics of *S.* Dublin and *S.* 4,[5],12:i:- also corroborate
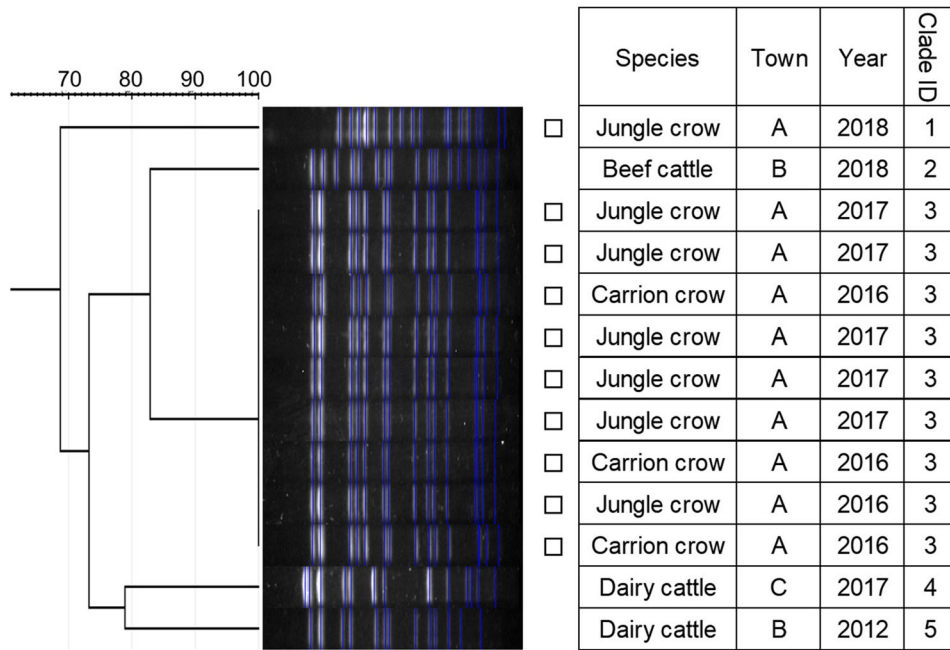
FIGURE 4 Dendrogram of pulsed-field gel electrophoresis (PFGE) gel band patterns for *Salmonella* Mbandaka isolates. Isolates from crows are marked with squares

FIGURE 5 Dendrogram of pulsed-field gel electrophoresis (PFGE) gel band patterns for *Salmonella* Montevideo isolates. The isolate from the crow is marked with a square
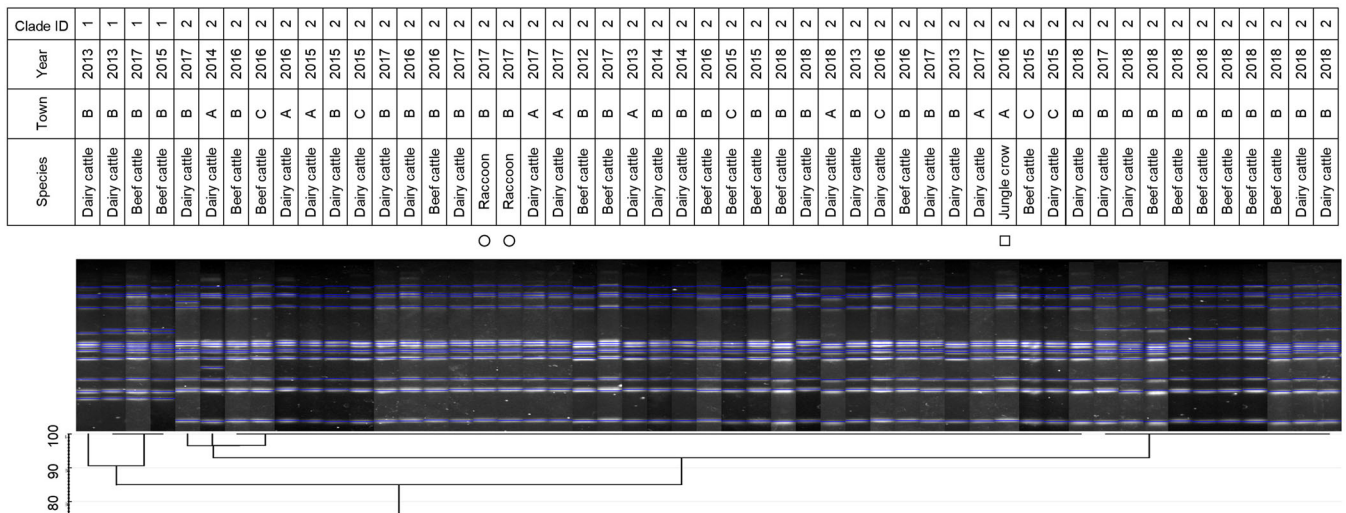

FIGURE 6 Dendrogram of pulsed-field gel electrophoresis (PFGE) gel band patterns for *Salmonella* 4,[5],12:i:- isolates. Isolates from raccoons are marked with circles. The isolate from the crow is marked with a square
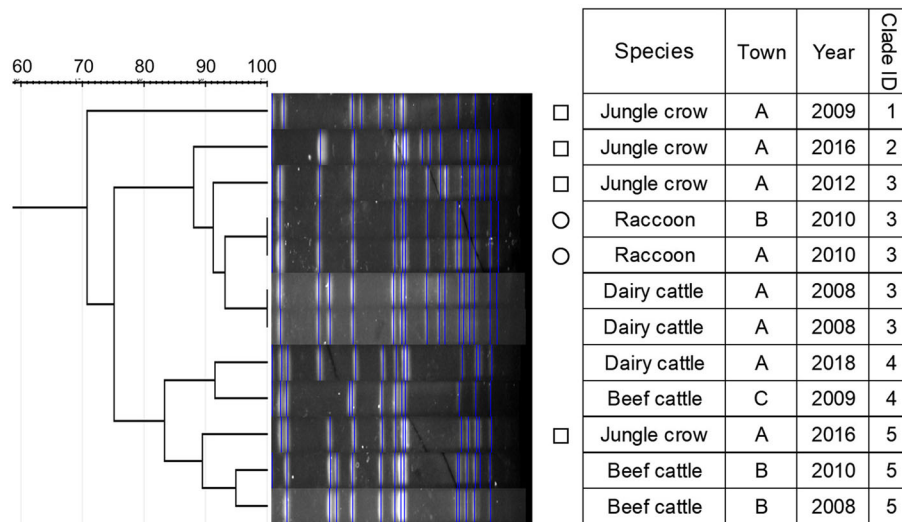
	Species	Town	Year	Clade ID
☐	Jungle crow	A	2009	1
☐	Jungle crow	A	2016	2
☐	Jungle crow	A	2012	3
○	Raccoon	B	2010	3
○	Raccoon	A	2010	3
	Dairy cattle	A	2008	3
	Dairy cattle	A	2008	3
	Dairy cattle	A	2018	4
	Beef cattle	C	2009	4
☐	Jungle crow	A	2016	5
	Beef cattle	B	2010	5
	Beef cattle	B	2008	5

FIGURE 7 Dendrogram of pulsed-field gel electrophoresis (PFGE) gel band patterns for *Salmonella* Typhimurium isolates. Isolates from raccoons are marked with circles. Isolates from crows are marked with squares

this suggestion. *S.* Dublin is a cattle specific serotype (Harvey et al., 2018); detection of this serotype from wildlife is limited to a few studies in wild mammals including mice and foxes (Glawischnig et al., 2017; Jones & Twigg, 1976; Tablante & Lane, 1989), and the present study shows the first detection from wild birds. In the previous reports, *S.* Dublin was detected from wildlife in the vicinity of *S.* Dublin epidemics in cattle as in this study, which implied that *S.* Dublin seemed to be transmitted from cattle to wildlife (Glawischnig et al., 2017; Jones & Twigg, 1976). A monophasic variant of *S.* Typhimurium, *S.* 4,[5],12:i:-, has been increasingly reported as the causative agent of salmonellosis in livestock and humans around the world, including Japan (EFSA, 2018; Kijima et al., 2019; Soyer et al., 2009). Previous studies suggested that a certain epidemic clone was dominant in recent *S.* 4,[5],12:i:- isolates in Europe and Japan (Arai et al., 2018; Petrovska et al., 2016). In this study, detections of *S.* 4,[5],12:i:- isolates with the same genetic patterns have rapidly increased since 2012, which probably implies that the *S.* 4,[5],12:i:- strains originated in the same epidemic clone spreading globally in humans and domestic animals. Therefore, the *S.* 4,[5],12:i:- strains in Tokachi might not be mainly sourced from wildlife, but rather from cattle or other livestock animals and humans, which were not surveyed here. The genetically similar *S.* Typhimurium strains were shared between wildlife and cattle, but not detected in the same year. *S.* Typhimurium is a common serotype in both wildlife and cattle (Kijima et al., 2019 Simpson et al., 2018; Tizard, 2004). Considering the year of detection of isolates in each species for clade IDs 3 and 5, to which both wildlife and cattle isolates belong (Figure 7), cattle strains were possibly transmitted to wildlife and then maintained in the population.

Many pathogens including *S. enterica* have been shown to be potentially transmitted between wildlife and livestock at their interface (Gortázar et al., 2016; Miller et al., 2013). In the case of *S. enterica*, pathogens seem to be transmitted between wildlife and livestock via feeding (Carlson et al., 2011a; Davies & Wales, 2013) and the number of wild birds invading cattle farms could be a risk factor for *S. enterica* contamination of the farms (Carlson et al., 2011b). On the other hand, *S. enterica* transmission from livestock to wildlife has also been implicated (Mentaberre et al., 2013; Skov et al., 2008); our obtained results for *S.* Dublin and *S.* 4,[5],12:i:- seemed to be similar to the latter. Raccoons and crows use various types of habitat including livestock farms, cities and natural environments (Ikeda et al., 2004; Morishita et al., 2003; Takeda et al., 2015; Zeveloff, 2002), which may allow them to spread pathogens into various types of environments, resulting in a hub of transmission between livestock and other environments. The serotypes detected from wildlife, comprised Infantis, Stanley, Typhimurium, Thompson and 4,[5],12:i:-, were also identified as common serotypes of foodborne *S. enterica* in Japan (National Institute of Infectious Diseases Japan, 2018). Accordingly, the importance of preventing *S. enterica* spreading by wildlife in Tokachi is heightened not only for livestock hygiene but also for food safety of farm products. Recently, antimicrobial resistant (AMR) *S. enterica* has become a growing concern in human and animal health worldwide (Hernando-Amado et al., 2019); wildlife is also infected with AMR organisms (Torres et al., 2020; Vittecoq et al., 2016). Livestock farms have been implicated in playing an important role in producing AMR organisms because of their frequent antimicrobial use (Silbergeld et al., 2008). Because wildlife around cattle farms might contribute to spreading AMR. *S. enterica* sourced from cattle, the degree of AMR of the isolates from wildlife would be critical for considering the risk of *S. enterica* infection in wildlife in the study site in future research.

This study had the following limitations. First, because our study aimed to investigate the *S. enterica* distribution retrospectively using existing detection records, which were based on unique sampling schemes in each municipality, detection records were uneven and irregular in each species. Furthermore, only one isolate per serotype per year in each species was chosen for PFGE analysis, which might lead to underestimation of the genetic diversity of *S. enterica*. Because

previous studies suggested that *S. enterica* distribution in wildlife, cattle and the natural environment was seasonal (Bondo et al., 2016; Nielsen et al., 2004; Thomas et al., 2013), *S. enterica* potentially failed to be detected because some sampling in this study was done infrequently (e.g. annual monitoring in the third scheme). Additionally, because *S. enterica* can survive in farm environments for months (Alegbeleye et al., 2018), we should consider the possibility that the cattle farm had been contaminated for several months prior to the detection by the monitoring survey when assessing the relationship of the timing of *S. enterica* detection in wildlife and cattle on a monthly basis. Accordingly, investigation based on an appropriate strategy with regular and even sampling and genetic analysis is essential for evaluating the possibility of *S. enterica* transmission quantitatively and precisely in the future. Second, PFGE analysis might be inappropriate for distinguishing the isolates genetically because the patterns of some isolates for *S.* Braenderup and *S.* Infantis were stable over 7 years (Figures 1 and 3), which made it difficult to envision an epidemiological association between the isolates after such a long time. For better understanding of genetic relationships between *S. enterica* isolates, genetic analyses with high discriminatory power like whole genome analysis (Tang et al., 2019) would be required. Third, it was possible that *S. enterica* was transmitted from a common source to both wildlife and cattle; however, the present study focused on wildlife and cattle and environment contaminated with cattle dung, which made it difficult to assess it. There are some important sources of *S. enterica*, including cattle feed (Van Metre et al., 2008), which were not surveyed in this study. *S. enterica* surveillance in other possible sources would also be helpful to reveal *S. enterica* transmission dynamics.

5 | CONCLUSION

Our study demonstrated that *S.* Braenderup, *S.* Dublin, *S.* Montevideo and *S.* 4,[5],12:i:- with similar genetic patterns were shared between wildlife and cattle in Tokachi. In particular, for *S.* Dublin and *S.* 4,[5],12:i:-, the trends of detection and the epidemiological characteristics of each species suggested that wildlife was not the initial host of the shared isolates, but rather that the source was probably other species including cattle. Therefore, limiting invasions of these wildlife into case farms, possibly the source of infection for wildlife, seems to be particularly critical for preventing dissemination of *S. enterica* at a regional level. Control strategies should be developed based on the role of wildlife in *S. enterica* ecology in livestock areas, which would contribute not only to livestock hygiene but also to public health, in accordance with a One Health concept.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICS STATEMENT

This study was approved by the Animal Ethical Committee and the Animal Care and Use Committee of Obihiro University, and Hokkaido Animal Research Center.

DATA AVAILABILITY STATEMENT

Research data are not shared.

PEER REVIEW

The peer review history for this article is available at https://publons. com/publon/10.1002/vms3.685.

AUTHOR CONTRIBUTIONS

Emi Yamaguchi: Conceptualisation; data curation; formal analysis; investigation; methodology; project administration; visualisation; writing-original draft. **Kei Fujii**: Conceptualisation; data curation; funding acquisition; investigation; methodology. **Mitsunori Kayano**: Conceptualisation; writing-review & editing. **Yoshie Sakurai**: Data curation; investigation; methodology. **Atsuko Nakatani**: Data curation; formal analysis; investigation; methodology. **Motoki Sasaki**: Investigation. **Julia A. Hertl**: Writing-review & editing. **Yrjo Grohn**: Conceptualisation; supervision; writing-review & editing.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

APPENDIX: DETAILS OF FOUR SCHEMES FOR *SALMONELLA ENTERICA* DETECTION FROM CATTLE

1. Clinical signs: Cattle presenting clinical signs such as diarrhoea and fever suspected to be salmonellosis by farmers and veterinarians were diagnosed by culture tests using cattle faeces or blood samples. *Salmonella enterica* (Salmonella) detection in clinical signs was recorded by serotype at farm level in dairy and beef farms in towns A, B and C. Records of Salmonella detection in clinical signs in town A were collected regardless of serotype from ULIDP in town A. In contrast, the records in towns B and C were based on the collection data of Salmonella isolates at LHSC, limited to the same serotypes detected from wildlife. We were only able to collect detection records, and the total number of Salmonella tests for cattle with clinical signs was unknown.

2. Introduction test: Cattle introduced into beef growing farms or public calving farms in towns A, B and C were tested for Salmonella individually with faeces samples when they were introduced into each farm. Samples were sent to private laboratories for Salmonella detection, and LHSC identified the serotype of Salmonella isolates. In each municipality, one public calving farm and fewer than 40 beef growing farms operated. Public calving farms had contracted for taking in and looking after milking calves from dairy farms in the same town until the first parturition or earlier. Salmonella detection in the introduction test was recorded by serotype at supplier farm level. Though some cattle were introduced from sites not included in the study, we also counted Salmonella detection from such cattle because we regarded them as Salmonella introductions into the study site by positive introduced cattle. In cases where the same serotype was detected from multiple supplier farms in the same beef growing farms or public calving farms in a year, the first

isolates from introduced cattle by each municipality were selected for genetic analyses. Records of introduction tests were collected as well as clinical signs; that is, the total number of introduction tests was unknown and Salmonella detection records in towns B and C were limited to the same serotypes detected from wildlife.

3. Annual monitoring: Salmonella dissemination was monitored using environmental samples at all dairy farms in town A, approximately 40 farms, once in summer (July to August) between 2008 and 2013, and once in each summer and autumn (October to November) between 2014 and 2018. We cannot show the specific number of monitored farms, which is equal to the number of dairy farms in town A and would lead to identification of the municipality. Salmonella detection in annual monitoring was recorded by serotype at farm level. The environmental samples were obtained from – two to three locations per farm at manure storage and alleys for lactating cattle, including dung

from many cattle. The samples were taken using sterilised swabs and kept in zipped plastic bags at 4°C before culture tests were performed, detailed in Section 2. Annual monitoring had been conducted by the ULIDP and we undertook the culture tests for samples. At positive farms, all individuals were tested to detect the carriers regardless of serotype. Isolates from individuals were used for genetic analyses. Even if the subsequent individual tests could not detect positive cattle, isolates from environmental samples were assessed.

4. Monthly monitoring: At all nine dairy farms sending calves to public calving farms in town A, Salmonella dissemination was monitored every month between 2017 and 2018 by the same method as with annual monitoring. The tested farms were a part of the tested farms in annual monitoring; therefore, only the results in annual monitoring were adopted in months for annual monitoring to avoid duplication of Salmonella detection.