



# Attributing salmonellosis cases to foodborne, animal contact and waterborne routes using the microbial subtyping approach and exposure weights

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## ABSTRACT

*Salmonella* is a major cause of enteric disease in Canada. Cases of salmonellosis were attributed to retail meats, food animal manure contact, and surface water sources using a microbial subtyping approach coupled with adjustments for exposure. Results indicated that 64.7% of cases were attributed to chicken breast meat, followed by frozen raw breaded chicken products (12.9%), ground chicken (9.1%), water (3.0%), pork chops and sausage (1.3%), ground beef and veal (0.7%), turkey parts (0.5%), and molluscs (0.0%). The salmonellosis incidence rate in the FoodNet Canada sentinel sites fell by one third with a parallel drop of one third in the percent of cases attributed to chicken breast meat between 2015 and 2019. Decreases in the contribution of many of the top serovars to the percentage of cases attributed to chicken breast meat indicates some emerging success with broiler breeder chicken vaccination programs. In addition, preliminary prevalence results for frozen raw chicken products in late 2019 suggests the Canadian Food Inspection Agency intervention in 2019 requiring any *Salmonella* on these products to be below a detectable amount may be having an impact, though more data post intervention is needed to be more conclusive.

## 1. Introduction

Non-typhoidal *Salmonella enterica* ssp. is globally the fourth most common foodborne enteric disease behind enterotoxigenic *E. coli*, *Campylobacter* and Norovirus (Kirk et al., 2015). In Canada, it has a high burden of illness, causing a higher number of hospitalisations related to domestically acquired foodborne illness than any other enteric bacteria annually and is among the top three enteric pathogens causing the greatest number of deaths (Thomas et al., 2015). Although *Salmonella* is a frequent cause of foodborne outbreaks, the majority of cases are sporadic (Public Health Agency of Canada, 2019). *Salmonella* is found in many animal reservoirs and there are many ways by which humans can acquire the infection. The most important way in Canada being contact with, or handling of, raw chicken meat, or consumption of undercooked chicken meat (Christidis, Hurst, Rudnick, Pintar, & Pollari, 2019). The top three most prevalent serovars in 2019 in Canada were *S. Enteritidis*, *S. Typhimurium* and *S. ssp* I 4,[5],12:i-, making up 35%, 9% and 5% of

isolates, respectively (Public Health Agency of Canada, 2020d). Foodborne disease surveillance in Canada reported in 2018 that over 85% of *S. Enteritidis* isolates recovered from chicken manure and chicken meat sources were genetically related to human clusters of *S. Enteritidis* infection (Public Health Agency of Canada, 2019).

A Canadian surveillance program that monitors enteric disease trends, the National Enteric Surveillance Program, reported an annual incidence rate of 19.7 *Salmonella* laboratory confirmed isolations per 100,000 population in 2018, with similar levels reaching back almost two decades (Public Health Agency of Canada, 2011; Public Health Agency of Canada, 2020a). Though in 2019, the incidence rate decreased to 16.9 (Public Health Agency of Canada, 2020d). Rises in the incidence rate of *S. Enteritidis* from 3.9 per 100,000 population in 2000 to 8.3 in 2010 in particular (Public Health Agency of Canada, 2021b), precipitated a national strategy to reduce human illness due to *S. Enteritidis* in poultry sources (Health Canada, 2015). More generally, temporal and spatial changes in the distribution of pathogenic serovars

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found in humans have been observed (Public Health Agency of Canada, 2019). These changes are driven by the serovar distribution found in the major sources of the pathogen and the effectiveness of reduction strategies applied to them. Because of this, spatiotemporal source attribution models identifying the important sources of salmonellosis at the point of exposure are fundamental to understanding the dynamics of the disease and the impact of any intervention along the food chain (Snary, Swart, & Hald, 2016). In addition, the food safety inspection system in Canada is moving toward a risk-based approach at the sub-product level, requiring better data of the risk for human health for each sub-product (e.g. raw non ready-to-eat non intact, raw non ready-to-eat comminuted) at the point of processing and/or consumption (Zanabria et al., 2019).

This study uses such models to attribute sporadic salmonellosis cases in Canada at the point of exposure to potential sources over time and geographical area, using data collected by FoodNet Canada (Public Health Agency of Canada, 2013), the country's integrated enteric surveillance program. This work falls directly within the purview of this program, as one of its primary objectives from program inception is determining the sources of enteric illness. FoodNet Canada is well equipped for this task as it systematically and uniformly collects and analyses data and isolates from human cases and non-human sources, including retail chicken meat, beef and pork meat, poultry, cattle and swine manure, and recreational, surface and irrigation water samples, in sentinel sites across Canada. The broad and relatively consistent data collection is ideal for modelling (Mughini-Gras, Kooh, et al., 2018), thus avoiding challenges encountered when using data from surveillance systems that are voluntary or that have components with differing objectives and sources sampled (de Knecht, Pires, & Hald, 2015b). Outputs from these models provide information on the relative importance of *Salmonella* infection sources, which is essential for setting public health goals and prioritizing food safety interventions.

Examining yearly trends is of primary interest, and will allow decision makers to assess the effectiveness of changes in food safety policy, both those enacted by government and those driven by industry (Snary et al., 2016). In particular, we expect a decreasing trend in the percent of cases attributed to different retail chicken meats, given the interventions put in place over time to control for *Salmonella* on these products. Frozen raw breaded chicken products (FRBCP) are also of interest because the product can appear cooked even though it is raw (Canadian Food Inspection Agency, 2018). In fact, between 2015 and 2019, 12 outbreaks and 285 salmonellosis cases were associated with these products (Morton et al., 2019). In 2018, the Government of Canada issued a new directive to industry to combat this problem, specifying that any *Salmonella* on these products must be below detectable limits by April 1, 2019 (Canadian Food Inspection Agency, 2019).

This study describes the temporal and spatial variation of salmonellosis source attribution at the point of exposure. A secondary analysis was also conducted which focused on retail chicken meat products to document the rise of case incidence rates related to them and their decline following the implementation of specific measures to control *Salmonella* in this commodity.

## 2. Materials and methods

### 2.1. Sources of isolates

Case data were collected up to December 2019 from three FoodNet Canada sentinel sites located in the provinces of Ontario (Middlesex London Health Unit) starting in August 2014, British Columbia (Fraser Health Authority: Burnaby, Chilliwack, and Abbotsford) starting in April 2010, and Alberta (Calgary and Central Zones) starting June 2014. Data from January 2010 to March 2014 from the Ontario pilot site (Region of Waterloo) were also included. Endemic cases were selected for analysis, as well as one case per known outbreak to avoid biasing results towards outbreak sources.

A key objective of FoodNet Canada is conducting source attribution for enteric pathogens. To meet this goal for *Salmonella*, it explicitly selects and collects samples from potential non-human sources where the pathogen could be present. Water samples were collected from the Grand River and Thames River watershed in Ontario, the Fraser River watershed in British Columbia (recreational locations in the watershed and irrigation ditch sources) and irrigation ditches from the Bow River watershed in Alberta (Table 1). Manure samples were collected from swine, broiler chicken, beef cattle, dairy cattle and turkey farms and these commodities varied by site. Retail products were randomly collected from a variety of large and small food retail outlets on a weekly basis. Core commodities sampled annually included skinless chicken breast, a proxy for chicken pieces and parts, and ground beef. Targeted retail sampling included pork (chops, sausage, and ground), veal, turkey, FRBCP, ground chicken and mollusks were sampled episodically.<sup>1</sup>

The FRBCP were primarily chicken nuggets up to 2018, and included chicken burgers in 2019. Some cooked product was sampled in early 2019 with the product comprising a growing proportion towards the end of 2019 as a result of the Canadian Food Inspection Agency (CFIA) directive. Farm food animal and retail food categories were selected according to FoodNet Canada protocols. Individual retail samples were selected randomly in-store. The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) collected the pork chop and turkey parts samples; farm samples were collected by CIPARS in collaboration with FoodNet Canada.

Additional information on the FoodNet Canada program, protocols, testing methods, and the sentinel sites is available elsewhere (Public Health Agency of Canada, 2006; Public Health Agency of Canada, 2020c).

### 2.2. Source attribution modelling

The source attribution estimates of salmonellosis cases at the point of exposure were calculated by combining the comparative exposure assessment and the microbial subtyping methods, following the approach developed in Canada for campylobacteriosis (Ravel et al., 2017), with an added modification to calculate yearly results. This approach uses the Dutch model frequency-based methodology with the added modification of weights that correct for the average population exposure using estimates for Canada from previously published work (Christidis et al., 2019). The model relies on the ecological link between the clinical isolates and the isolates from potential sources collected from retail stores, farms and water sampling locations in the same geographic area. FoodNet Canada's sentinel site design, which collects data at the local public health level, is ideally suited for this purpose.

The Dutch model generates similar results compared to the alternative Hald-type model (Jabin, Correia Carreira, Valentin, & Käsbohrer, 2019). The initial modification of the original Dutch model includes the national consumption level (in tons) of each food studied as a source of *Salmonella* (David, Guillemot, et al., 2013). The inclusion of this parameter has been debated and the conclusion reached was that including both the amount of food consumed and its likelihood of undercooking as factors improved the validity of the modified Dutch model (Mughini-Gras & van Pelt, 2014). The model used here is a natural extension of these historical approaches, since weights from the comparative exposure assessment includes consumption, undercooking, as well as other exposure-related factors (Ravel et al., 2017).

The Dutch model was preferred over Hald models, first, for the simplicity and efficiency of parameter estimation, and second, because the exposure weights are directly calculated with a known model made up of many factors determining exposure versus Hald models that esti-

<sup>1</sup> Isolates and samples collected by year, for each site, can be found in Appendix A, tables A2 to A4, in the supplementary materials.

**Table 1**  
Count of human salmonellosis cases, as well as non-human *Salmonella* isolates, by year and by sentinel site.

Origin	Year										Sentinel site <sup>a</sup>		
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	ON	BC	AB
	Count												
Human endemic	128	141	121	100	223	303	306	288	229	191	518	703	809
Chicken breast	57	109	92	54	64	92	93	75	76	64	290	347	139
Ground chicken	11	155	171	113							242	208	
Turkey parts/ground	1	95	71	58	82	91	63	61	37		242	269	48
Frozen raw breaded chicken products	9	135	112	66	84	112	115	94	100	63	379	341	170
Ground beef and veal beef	1					7		4			3	3	6
Pork chops, sausage, and ground sausage	4	9	5	3	11	11	44	2	21	21	92	23	16
Mollusks													
Broiler chicken manure <sup>b</sup>	75	73	69	138	145	174	146	148	167	153	463	514	311
Turkey manure <sup>b</sup>				39	27	47	120	114	189	218	329	363	62
Beef cattle manure <sup>b</sup>	15	11	10	13	2		3	1	2	13	51		19
Dairy cattle manure <sup>b</sup>	15	16	7	11	7						71	5	1
Swine manure <sup>b</sup>	29	41			67	46	42	34	60	58	338		39
Surface/Irrigation Water	23	35	35	52	11	9	18	15	8	9	140	65	10

Note: a blank cell indicates that *Salmonella* was not isolated.

<sup>a</sup> ON refers to the Ontario site, BC, British Columbia and AB, Alberta.

<sup>b</sup> Multiple pooled manure samples were collected per farm, per year.

mate one overall corrective parameter for each source. The Dutch-Exposure model used in this study is defined as:

$$\lambda_j = \sum_i \frac{p_{ij} E_j}{\sum_j p_{ij} E_j} h_i \quad (1)$$

Where:

$\lambda_j$  = the proportion of cases attributed to source  $j$

$h_i$  = the number of cases to attribute for a given subtype  $i$

$p_{ij}$  = the proportion of *Salmonella* positive samples of subtype  $i$  in source  $j$ , or alternatively, for each subtype  $i$ , it is the number of positive tests for source  $j$  divided by the total number samples tested

$E_j$  = frequency · ingestion · prevalence · concentration, also known as the exposure weight, measured in cells ingested per person per day was taken from previous work (Christidis et al., 2019). Paraphrasing Christidis et al., frequency was the mean count of ingestion events per day, ingestion was the mean mass (or volume) consumed per person per ingestion event, prevalence is the proportion of samples contaminated with the pathogen, and concentration was the mean number of cells per mass (or volume) when contamination occurred. For the chicken meat models, concentration also considered the survival of the bacteria after cooking undercooked or raw portions. The chicken meat models also considered cross contamination. The details of all the factors included in the models for meats, as well as animal contact, and water exposures, can be found in the supplementary materials of Christidis et al.

Prevalence was a component in the formula for  $E_j$  in the Christidis et al. manuscript but has been removed in  $E_j$  for this study because in this study the prevalence factor enters the model directly as  $p_{ij}$ . Modelled in this way,  $p_{ij}$  can vary dynamically by site or year, depending on the analysis. Allowing the concentration component of the weights to vary was considered as well, but there was insufficient sample to do so. The re-calculated values with prevalence removed are found in Appendix A, Table A9. The recreational water exposure estimate is assumed to represent the exposure to the water sources in the sentinel sites.

The analysis performed on a yearly basis imputes missing non-human data with available data from the closest year. If there was a tie, then both pre and post years were averaged. Years with a low sample size (<50) were considered to be inadequate for modelling and were augmented with additional sample that was imputed as described above.

### 2.3. Subtype definition for attribution

FoodNet Canada combines enhanced laboratory testing (culture, serovar and pulsed-field gel electrophoresis (PFGE), etc.) and epidemiological information on human cases of enteric disease with data from pathogen testing of non-human sources (retail, farm and water). The subtyping methods used on the human and non-human data were assessed for their suitability to define a profile on which to perform attribution. Four qualities of each subtype method were considered for the evaluation, namely: percent of data missing, diversity based on Simpson's index of diversity (Oyarzabal & Kathariou, 2014), source specificity, and the percent of cases that are not attributable. The first two are straightforward.

The third, source specificity, is defined as the percent of cases that are attributed to a particular number of sources. A high proportion of cases attributed to a small number of sources implies the subtyping method is more source specific. This is in contrast to a method with a high proportion of the cases attributed to a large number of sources which is less source specific. Source specificity is used as an indicator of subtype discrimination, which is a key factor in source attribution modelling based on subtyping (EFSA Panel on EFSA Biological Hazards, 2013). Specifically, we considered the percentage of cases attributed to three or fewer sources as the measure of source specificity.

The fourth quality of interest is the percent of cases that are not attributable. This quantifies the percent of case subtypes that are not found in any source. A higher percentage indicates the subtype definition is too discriminating.

Thus, considering the third and fourth measure in tandem, if the subtype definition is too granular, then case-source linkages of epidemiological importance may be missed. Here, the percent of cases not attributable will be high. Whereas, if it is not discriminating enough, then it is more difficult to establish a case-source link (de Knecht et al., 2016) as the sources appear similar to each other. In this situation, the percentage of cases attributed to three or fewer sources would be low.

Nearly all *Salmonella* positive samples were tested for their serovar, though only *S. Enteritidis*, *S. Typhimurium*, *S. Heidelberg* and *S. ssp I 4, [5],12:i:-* were potentially phage typed and tested for their PFGE fingerprint. PFGE testing was not performed for a large majority of the top four serovars in the human data, thus, PFGE was not evaluated further. Thus, any subtype profile would require serovar to be included, and the remaining question is if phage type provides better resolution for attribution for these four common serovars.

## 2.4. Source attribution analysis

Differences in immunity affect the risk of exposure and can vary by age group. Employment exposures and eating habits can also vary by age group, geographic location and sex. Because of this, the results were analysed by age group (0–18, 19 to 64, and 65 plus), sex, and geographic location (British Columbia, Alberta and Ontario sites), in order to explore if these factors resulted in differences.

Source attribution point estimates and 95% confidence intervals were first estimated overall, then by sex, age group, year, and geographic location. The non-parametric bootstrap was used to calculate confidence intervals (Ravel et al., 2017). A yearly analysis was performed to explore the temporal variation in the source attribution percentages, as suggested elsewhere (Mikkilä, Ranta, & Tuominen, 2019). The salmonellosis incidence rate was provided by year and is based on the sentinel site human case data as outlined above and thus does not represent the salmonellosis incidence rate for Canada.

Comparisons between age categories, the sexes and years were considered significant if the confidence intervals for the results did not overlap. The power of the subgroup analysis was lower than in the overall source attribution model though it was considered to be more than sufficient to examine epidemiologically important changes in the results. The analysis of FRBCP that compared the last three months of 2019 to the previous nine months computed the statistical significance of the difference in percentages directly. Analysis that considers the contribution of a serovar to the overall percent attributed to a source is descriptive only, as a measure of precision was not available.

Source attribution models were also estimated for each site to document spatial variation; only sources with isolates available for the three sites were used. Because chickens were vaccinated against specific serovars, the contribution of each serovar to the overall attribution percentages for each year are computed to document the impact of chicken vaccination on human cases. This serovar-specific analysis was performed for Ontario and British Columbia because there was evidence available for *Salmonella* vaccination program implementation in poultry in those provinces. Finally, because of the specific focus on *S. Enteritidis* reduction in Canada, a model including only this serovar was estimated.

## 3. Results and discussion

### 3.1. Choice of subtype combination for attribution

There were two subtype definitions to consider: the serovar-phage subtype definition that used both serovar and phage together, and a definition based only on serovar. Phage typing was performed on 61% of human isolates and 73% of non-human isolates (Appendix A, Table A5). Thus, using serovar and phage type together reduced the number of human isolates that were available for attribution. In addition, phage typing was performed at higher rates in the latter half of the study period under analysis, making analysis by year more problematic.

Simpson's index of diversity improved from 0.74 in the human data when using serovar alone to 0.94 when using the serovar-phage type definition, and from 0.90 to 0.94 in the non-human data (Appendix A, Table A6). As expected, using serovar and phage together improved the granularity of the organism's characterisation, though the gain from including phage type in the definition was modest.

The percentage of human isolates that matched three or fewer sources was 4.0% if the serovar definition was used for attribution and 11.6% using the serovar-phage type definition (Appendix A, Tables A7 and A8). This was considered a small improvement at best.

Of the human isolates, 93% matched at least one source using the serovar definition, falling to 83% when the serovar-phage definition was used. Thus, the model using serovar-phage had fewer cases with at least one source. This was considered a moderately negative attribute of the model that used the serovar-phage type definition compared with the alternative.

Overall, the model using the serovar subtype definition was determined to have better data quality and was able to attribute more cases to sources, though at the expense of less diversity and source specificity. However, this approach was preferred as the data quality gain was considered to outweigh the marginal gains in diversity and source specificity. Therefore, the source attribution models used serovar only as the subtype definition. The *S. Enteritidis* model was a modification of the subtype model. It attributes *S. Enteritidis* cases to the sources in which that serovar is found.

### 3.2. Attribution model results

#### 3.2.1. Overview

Overall source attribution subtyping model results (Table 2) indicated that the top sources were chicken breast meat with 64.7% of cases attributed, followed by FRBCP at 12.9%, ground chicken at 9.1%, water at 3.0%, pork chops and sausage at 1.3%, ground beef and veal at 0.7%, turkey parts at 0.5%, and molluscs at 0.0%. Attribution to food animal manure contact was very low (<1%). Although *Salmonella* and its serovars are found in broiler chicken manure, exposure to it by a typical member of the population is quite low compared with chicken meat exposures, which is reflected in its very low attribution percentage. The top three serovars are mostly found in sources in the chicken reservoir (Appendix A, Table A1) which is in alignment with the model results. The percent of unattributed isolates was 7.5%, which suggested the model included a number of important sources.

In general, there were no statistically significant differences in attribution results between age or sex categories. A notable exception was the larger attribution of salmonellosis to chicken breast meat for male cases, 66.2%, CI (65.0%, 67.9%) versus female cases, 63.3%, CI (62.3%, 64.5%). Though men are less likely to follow safe food handling and preparation practices (Murray et al., 2017), it is unclear why this might have affected the attribution result for chicken breast meat specifically versus the other food sources.

Previous Canadian source attribution studies using different methodologies identified the foodborne route as making up roughly 50%–60% of salmonellosis cases (Public Health Agency of Canada, 2017). The top ranked sources within this route were poultry, eggs, dairy and beef meats and produce. In comparison, this study found that the foodborne route made up 89.2% of attributed cases. This difference may be due to this study using more current data and methodological differences.

This study was able to distinguish between consumption of retail meats and contact with food animals—this is rare as most studies worldwide provide results at the animal reservoir only. Comparisons to reservoir level results provides some context on how the Canadian situation may differ from the situation internationally. For instance, results from other countries found that the chicken food-animal route was the most important, though with somewhat smaller magnitudes: 60% of cases attributed to the chicken reservoir from work in Minnesota (Ahlgren et al., 2017), 48% to chicken in the USA (Guo et al., 2011), 71.7% for chicken and eggs from a study in South Australia (Glass et al., 2016), whereas the top reservoir was either poultry (chicken and layer/egg) or pigs in most European countries (David, Sanders, et al., 2013; de Knecht, Pires, & Hald, 2015b; Mughini-Gras et al., 2014; Pires, Vieira, Hald, & Cole, 2014).

Pork chops and pork sausage were attributed a low percentage of cases, 1.3%, which is similar to the results from other work from South Australia that attributed 2.5% (Glass et al., 2016) to the porcine reservoir, which can be viewed as the upper limit of the percent attributed to pig meat. Though results do vary by country, as work from Italy attributed 45% (Mughini-Gras et al., 2014) to pig meat and another from New Zealand attributed 60% to pigs (Mullner et al., 2009). European successes in controlling *S. Enteritidis* in chicken sources and the rise in human cases of *S. Typhimurium*, *S. ssp I 4,[5],12:-* and *S. Derby*, which are most often found in swine, is thought to have driven the exchange in the primary source of *Salmonella* infection from chicken sources to pork



**Table 2**

Percent of salmonellosis cases from three FoodNet Canada sentinel sites, 2010 to 2019, attributed to sources using serovar-based modified Dutch model, weighted by estimates of exposure.

Source	Exposure overall			Male			Female			0 to 18			19 to 64			65+		
	%	LCI	UCI	%	LCI	UCI	%	LCI	UCI	%	LCI	UCI	%	LCI	UCI	%	LCI	UCI
Pork chops, sausage, and ground sausage	1.3	0.8	2.2	1.1	0.6	1.9	1.5	0.9	3.4	0.8	0.4	2.0	1.3	0.9	2.2	2.4	1.0	4.9
Chicken breast	64.7	63.1	66.0	66.2	65.0	67.9	63.3	62.3	64.5	65.1	63.3	67.5	64.6	62.7	66.5	64.6	62.0	66.4
Ground beef, veal	0.7	0.2	1.8	0.5	0.0	1.5	0.8	0.1	2.3	0.6	0.1	1.5	0.8	0.3	2.5	0.6	0.0	2.8
Turkey parts/ground	0.5	0.3	0.6	0.4	0.3	0.6	0.5	0.3	0.9	0.4	0.2	1.1	0.5	0.3	0.7	0.5	0.2	0.9
Frozen raw breaded chicken products	12.9	11.6	14.6	13.3	12.0	14.6	12.5	10.9	13.9	13.6	12.1	14.9	12.6	10.9	13.7	12.4	11.0	14.2
Ground chicken	9.1	8.3	10.8	9.1	8.1	10.1	9.0	8.1	11.4	8.5	7.7	9.5	9.2	7.4	10.5	9.9	8.3	11.8
Swine manure	0.0	0.0	0.6	0.0	0.0	0.1	0.1	0.0	1.0	0.0	0.0	0.1	0.0	0.0	0.0	0.3	0.0	2.6
Chicken manure	0.1	0.0	0.6	0.1	0.0	0.4	0.2	0.0	1.1	0.3	0.0	0.9	0.1	0.0	0.6	0.0	0.0	0.0
Beef manure	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dairy manure	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Turkey manure	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0.5	0.0	0.0	0.0	0.1	0.0	0.4	0.0	0.0	0.0
Surface/Irrigation Water	3.0	1.8	4.3	2.6	1.6	3.8	3.4	1.6	5.4	2.7	1.6	4.4	3.2	2.0	4.6	3.0	0.7	5.5
Mollusks	0.0			0.0			0.0			0.0			0.0			0.0		
Unattributed	7.5	6.5	8.4	6.6	5.0	8.3	8.5	6.8	10.4	8.0	4.8	9.8	7.6	6.5	8.5	6.2	3.9	9.5

Note: a blank cell indicates that a confidence interval could not be calculated. LCI: lower bound of the 95% confidence interval. UCI: upper bound of the 95% confidence interval.

meat (Mughini-Gras et al., 2014).

This study attributed 0.7% to ground beef and veal, whereas 7.4% (Glass et al., 2016) was attributed to the bovine reservoir in South Australia and 28% in the USA (Guo et al., 2011). Turkey meat parts in the present study were attributed 0.5%, though other work from Italy attributed 4–5% (Mughini-Gras et al., 2014) to turkey meat and 17% in the USA (Guo et al., 2011). *Salmonella* was not found on the mollusks analysed in the present study and so, unsurprisingly, were attributed 0% of cases. Since *Salmonella* has been found in Canadian oysters previously (Tamber, Montgomery, Eloranta, & Buenaventura, 2020), it should not be discounted as a possible source.

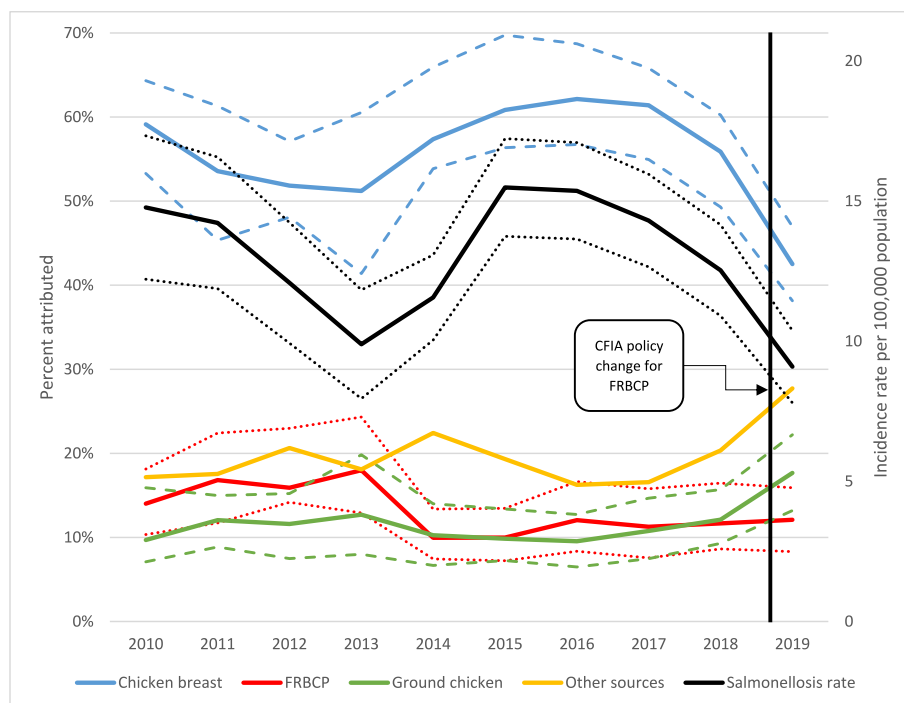
A very low percent of cases, 3.0%, were attributed to water, representing both recreational water exposure, irrigation ditches, and the general environment (impacted by wildlife and agricultural run-off). This value is close to results from other work that used Canadian data sources, which ranged from 2.1% to 8.0% depending on the

methodology (Public Health Agency of Canada, 2017). European Union source attribution work using outbreak data found water was implicated 0.5% of the time (Pires, de Knegt, & Hald, 2011), a substantively similar amount.

3.2.2. Temporal and spatial variations

The proportion of salmonellosis attributed to chicken breast meat fell between 2010 and 2013 from 59.1% to 51.2%, then increased to 62.1% in 2016 and then fell by about one third to its lowest level of 42.5% in 2019 (Fig. 1; Appendix A, Table A10). For FRBCP, the proportion increased from 2010 to 2013 from 14.0% to 18.0%, dropped in 2014 to 10.0% and then was relatively stable to 2019 where it was 12.1%. Ground chicken was roughly stable between 9.5% and 12.7% though it increased in 2019 to 17.7%. It is also worth noting that ground chicken trends increased when chicken breast meat decreased.

Note that concurrently with the proportion of salmonellosis



**Fig. 1.** Salmonellosis incidence rate and percent of salmonellosis cases attributed to three specific retail chicken meats and all other sources in three sentinel sites in Canada, by year. The incidence rate is based on the salmonellosis counts used in the attribution model and the population of the sentinel sites. Note that 95% confidence intervals have been added to the figure (dotted or dashed lines) for each series except other sources. FRBCP represents frozen raw breaded chicken products.

attributed to chicken breast meat falling between 2016 and 2019, a similar decrease was observed for the salmonellosis incidence rate by about one third, from 15.4 to 9.1 cases per 100,000 population. Recent efforts to reduce *Salmonella* on poultry in Canada included a national strategy to reduce human illness due to *S. Enteritidis* that was developed between 2010 and 2015 (Health Canada, 2015), and a subsequent government initiative to reduce *Salmonella* among other pathogens in poultry products (Canadian Food Inspection Agency, 2015), as well as changes to the industry landscape with the introduction of insurance and *Salmonella* vaccination programs for table egg and broiler breeder producers (Health Canada, 2015). These efforts may have had downstream effects that have reduced the prevalence of serovars causing human illness.

In particular, the Ontario broiler breeder producers (also known as hatching egg producers) began mandatory vaccination against *S. Typhimurium*, *S. Enteritidis*, *S. Heidelberg* and *S. Kentucky* in January 2013. The rise in human *S. Infantis* cases prompted the addition of *S. Infantis* in January 2017 (Caffrey et al., 2021; Ouckama, 2017). Table 3 indicates that these serovars contributed to the percentage of cases attributed to chicken breast meat when the sites were analysed together, with the exemption of *S. Kentucky*. The contribution of *S. Typhimurium* and *S. Heidelberg* fell from 2013 to 2019, and *S. Infantis* also decreased between 2015 and 2019. There was a drop in the contribution of *S. Enteritidis* to chicken breast meat in 2019 compared with the previous year. The contribution of *S. Enteritidis* dropped from 48.7 to 28.3 percentage points between 2017 and 2019 when the salmonellosis incidence rate was also falling. This may suggest vaccination interventions had some success with controlling *S. Enteritidis*, at least in the last two years of the data analysed. Table 4 indicates that for the Ontario sentinel site, *S. Typhimurium* and *S. Heidelberg* were more of an issue for chicken breast meat, and *S. Enteritidis* was less of an issue when compared with the other two sentinel sites. This might reflect differences in provincial vaccination programs, and other factors, such as the interprovincial and international exchange of live poultry and meat products.

The poultry industry in British Columbia put in place interventions for the control of *S. Enteritidis* prior to 2012, including measures to manage *S. Enteritidis* positive flocks in the broiler breeder sector. At the end of 2018, all broiler breeder producers were reported to be voluntarily vaccinating their flocks against *S. Enteritidis*. Also, post 2012, processors were implementing upgrades to equipment and chemical dip ingredients to reduce carcass contamination (Centre for Coastal Health, 2019). Table 5 indicates that *S. Enteritidis* contributed the majority of the percentage of cases attributed to chicken breast meat from 2010 to 2019, with no clear trend apparent.

Preliminary data from FoodNet Canada suggests the intervention by the CFIA to control *Salmonella* on FRBCP has been successful, with the overall prevalence on the product dropping from 27% in 2018 to 17% in 2019, and little to no *Salmonella* found in the later months of 2019 and early 2020 (Public Health Agency of Canada, 2021). These results align with the implementation date of the CFIA directive in April 2019 and the reduced availability of raw product in stores for sampling by FoodNet

Canada later in 2019. Despite this, FRBCP was attributed the same proportion of cases in 2019 versus 2018. This is largely explained by the prevalence of *Salmonella* on chicken breast meat also dropping, though not by as much as on FRBCP, between 2018 and 2019, from 19% to 16% (Public Health Agency of Canada, 2021), and by a change in the distribution of serovars which favoured FRBCP. In particular, there were 13 percentage points fewer cases attributed to chicken breast meat from *S. Enteritidis* over this period (Table 3). In addition, FRBCP are by definition frozen, and can remain in people's freezers long after they are purchased implying cases from FRBCP can occur far past the intervention date.

Looking at the last three months of 2019, only 6.7% of cases were attributed to FRBCP, 5.7 percentage points (p-value 0.07) lower than the first nine months of the year. Though the difference in the percentage attributed to FRBCP is insignificant at the 0.05 cut-off, it is only just insignificant, thus we believe this does provide evidence of a downward trend in the percent attributed to FRBCP.

Preliminary FoodNet Canada data for 2020 showed the percent prevalence of *Salmonella* on chicken breast meat moving upwards in to the 20s, and levels on FRBCP to be within a few percent of 0, as there were some products sampled that are exempt from the government directive (Public Health Agency of Canada, 2021). Given these trends, the contribution of the FRBCP category to salmonellosis post 2019 is expected to fall further to within a few percentage points of 0.

The Alberta and British Columbia sites had similar results. Chicken breast meat was attributed to 70.3% of salmonellosis cases in British Columbia and 65.0% in Alberta (Appendix A, Table A11). FRBCP results were also similar with attribution in British Columbia at 15.6% and in Alberta, 14.1%. The percent of cases unattributed was different in the two sites, with 13.1% of cases in Alberta unattributed and 9.0% in British Columbia.

Comparing these two sites with the Ontario site, fewer cases in the Ontario site were attributed to chicken breast meat (57.4%), but more to FRBCP (25.3%), and water sources (5.0%). This site difference may be a result of vaccination programs implemented in Ontario for the control of specific *Salmonella* serovars in broiler chickens and the fact that chicken breast meat is more regionally distributed within a province versus FRBCP which tends to have national distribution. For chicken breast meat, the 57.4% was composed of 16.5 percentage points from *S. Typhimurium* in Ontario, though only 4.5 of the 70.1% in the British Columbia and Alberta sites combined (Appendix A, Table A12). Similarly, *S. Heidelberg* contributed 11.2 percentage points, whereas it was only 2.9 in the other sites. *S. Enteritidis*, however, makes up 10.9 percentage points of chicken breast's 57.4% in Ontario, though it is higher in the other two sites at 50.3 percentage points.

The percent attributed to ground turkey and turkey parts was higher in the Alberta site at 4.0% versus the other sites, both at 0.5% (Appendix A, Table A11). This was driven by *S. Newport* (14 cases) and *S. Reading* (12 cases), which contributed 1.7% and 1.5%, respectively, to the overall 4.0% attributed to turkey meat in the Alberta site. *S. Reading* in turkey meat was linked to an outbreak of *Salmonella* infections in Canada (Public Health Agency of Canada, 2020b).

**Table 3**

Percentage point contribution of select serovars on the percentage of cases attributed to chicken breast meat, by year, all sites.

Serovar	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Percentage points										
<i>S. Enteritidis</i>	32.0	35.4	19.9	26.0	41.5	42.8	45.2	48.7	41.0	28.3
<i>S. Typhimurium</i>	17.2	10.0	9.3	9.9	7.7	6.5	5.5	4.1	5.3	5.5
<i>S. Heidelberg</i>	8.0	6.0	11.5	12.4	1.5	2.0	2.4	1.9	3.0	3.2
<i>S. Kentucky</i>	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.4	0.0
<i>S. Infantis</i>	0.4	1.2	0.5	0.0	0.0	4.3	3.2	2.2	1.4	0.0
Count										
Salmonellosis case count	128	141	121	100	223	303	306	288	229	191

**Table 4**

Percentage point contribution of select serovars on the percentage of cases attributed to chicken breast meat, as well as the salmonellosis case count, by year, Ontario site.

Serovar	2010	2011	2012	2013 <sup>a</sup>	2014	2015	2016	2017 <sup>b</sup>	2018	2019
Percentage points										
<i>S. Enteritidis</i>	26.0	0.0	0.0	8.0	0.0	12.4	0.0	0.0	24.0	0.0
<i>S. Typhimurium</i>	20.5	16.6	14.5	16.7	15.8	17.0	19.0	9.0	11.1	15.4
<i>S. Heidelberg</i>	13.3	5.7	16.9	22.5	5.3	3.3	9.4	14.2	4.6	5.3
<i>S. Kentucky</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>S. Infantis</i>	0.0	0.0	0.0	0.0	0.0	11.3	0.8	0.0	1.7	0.0
Count										
Salmonellosis case count	72	58	64	68	35	50	63	33	40	35

<sup>a</sup> Year vaccination intervention begins for *S. Typhimurium*, *S. Enteritidis*, *S. Heidelberg* and *S. Kentucky*.

<sup>b</sup> Year vaccination intervention begins for *S. Infantis*.

**Table 5**

Percentage point contribution of select serovars on the percentage of cases attributed to chicken breast meat, as well as the salmonellosis case count, by year, British Columbia site.

Serovar	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Percentage points										
<i>S. Enteritidis</i>	39.9	54.8	35.6	51.5	51.5	48.2	67.2	69.7	44.7	49.2
<i>S. Typhimurium</i>	0.0	0.0	5.2	0.0	4.3	0.0	0.0	0.0	0.0	0.0
<i>S. Heidelberg</i>	3.9	7.1	6.2	0.0	1.2	1.3	0.0	0.0	5.2	0.0
<i>S. Kentucky</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>S. Infantis</i>	1.6	1.1	0.0	0.0	0.0	3.4	1.1	0.9	1.8	0.0
Count										
Salmonellosis case count	56	83	57	32	102	85	91	87	57	53

The Alberta site had a lower amount attributed to water at 0.2%. The 5.0% attributed to water in the Ontario site was composed of many serovars contributing 0.2 percentage points or more. Of interest were three serovars found only in human cases and water: *S. ssp* I 4,[5],12:B: contributing 1.5 percentage points, *S. Bovismorbificans* contributing 0.8 percentage points and *S. Hartford*, 0.6. The Ontario site samples rivers in the region's watershed whereas Alberta samples irrigation water and in the British Columbia site there is a combination of both. This may have an impact on the distribution of serovars in these sources.

### 3.2.3. *Salmonella enteritidis* model

Essentially all of the cases in the *S. Enteritidis* model were attributed to the chicken meat category. Chicken breast garnered 73% of cases, FRBCP 15%, ground chicken 12%, and less than 1% were attributed to water and other sources. The model with all the serovars generated similar results (Table 2), though the *S. Enteritidis* only model attributes a higher percentage of cases to chicken breast meat and less to the water route.

### 3.3. Limitations

The *S. Enteritidis* model had only one subtype on which to attribute cases. Its ubiquity did not provide a reasonable level of source specificity. For the other models, serovar was not an ideal subtype for source attribution. Though many of the less common serovars of *Salmonella* are more source specific, the most common serovars are not particularly so.

It is also difficult to ascertain the strength of the ecological link. Food products that cause disease may be purchased or sourced outside of the sentinel sites and have different levels of contamination or other factors making them unrepresentative. Similarly, contact with food-animal manure or water may occur outside of the site. Food-animal manure samples in the site might not represent the food animals processed in food production facilities that make the products sold in the site's local stores.

Some sources were not sampled every year and thus might not be

representative of the entire time period. In the same manner, a commodity sampled one year used for attribution results for that year might not be representative of the specific situation in a different year. In particular, a significant percentage of salmonellosis is attributed to ground chicken, which was last sampled in 2013. Also, modelling concentration data for the exposure weights on a yearly basis would provide a more accurate picture of temporal effects. In terms of the FRBCP intervention, additional analysis of post 2019 data will provide a more complete picture of the intervention's impact. Also, a better understanding of the roll-out dates of vaccination programs (including type of vaccine and the serotypes covered) in the sentinel sites within each province would help to better pinpoint their impact.

There are other potential sources of salmonellosis that were not collected by the FoodNet Canada program and were not included in the models such as eggs, raw vegetables and fruits (Christidis et al., 2019). FoodNet Canada has done limited sampling of leafy greens, fresh berries, herbs, and fresh cut fruits though *Salmonella* was not detected. Regarding commercial table eggs, though they are a source of outbreaks, they are not considered to be a substantively important source of sporadic salmonellosis given that average exposure was estimated to be six orders of magnitude less than chicken meats and three less than recreational water (Christidis et al., 2019), and thus they are not currently sampled by FoodNet Canada. Household pets, specifically reptiles, are known sources of *Salmonella* infection, and are absent in the models as well; this is a common drawback of source attribution studies (Mughini-Gras, Franz, & van Pelt, 2018). The impact of these missing sources is likely to be low considering the low proportion of unattributed cases and is mitigated by the focus on chicken breast meat and FRBCP.

It is worth noting that specific food types sampled at FoodNet Canada were used to represent broad food categories. For instance, chicken breast meat was a proxy for many types of chicken meat, such as whole carcass, wings, legs, thighs, etc. If these other products had been sampled, the chicken breast results may be lower to accommodate the other sampled products, and the results for ground chicken and FRBCP may be affected as well.

The exposure weights used in the models are for Canada as a whole and are not specific to exposure patterns within the sentinel sites, making the site-specific results less accurate. Also, the Alberta site specific results used irrigation ditch water as the environmental proxy, which may have a different set of biases than the river watersheds sampled in the Ontario site, and the combination of the two sources in British Columbia.

Finally, since sites use their own coding system for outbreaks, the same outbreak may have different codes in different sites, therefore some outbreaks may be over-represented in the analysis. In addition, some cases that are classified as endemic using epidemiologic data may in fact be outbreak cases when further subtyped and analysed.

#### 4. Conclusion

This study estimated the percentage of salmonellosis cases in Canada attributed to key sources with a focus on different retail chicken meat products, as well as the temporal and spatial differentials to evaluate the impact of industry and government interventions in the poultry industry. Overall, the top sources of salmonellosis were chicken breast meat, followed by FRBCP, ground chicken, and water.

The salmonellosis incidence rate in the FoodNet Canada sentinel sites fell by one third with a parallel drop of one third in the percent of cases attributed to chicken breast meat between 2015 and 2019. Decreases in the contribution of many of the top serovars to the percentage of cases attributed to chicken breast meat indicates some emerging success with broiler breeder chicken vaccination programs in the provinces with FoodNet Canada sentinel sites. In addition, some promising prevalence results for FRBCP in late 2019 suggested the CFIA intervention may be having an impact, though more data post intervention is needed to be more conclusive. This analysis provides another method in the source attribution toolkit for other countries to consider who are developing a one-health approach to controlling *Salmonella* infection.

#### CRedit authorship contribution statement

**Matt Hurst:** Conceptualization, Methodology, Software, Validation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Andrea Nesbitt:** Writing – review & editing. **Stefanie Kadykalo:** Data curation, Writing – review & editing. **Brendan Dougherty:** Writing – review & editing. **Juan Carlos Arango-Sabogal:** Investigation, Writing – review & editing. **André Ravel:** Conceptualization, Methodology, Writing – original draft, Visualization, Supervision.

#### Declaration of competing interest

The authors have no declarations of interest regarding the work done in this submission.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2023.109636>.

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