Source attribution of Campylobacter infections in Denmark - Technical Report

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Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

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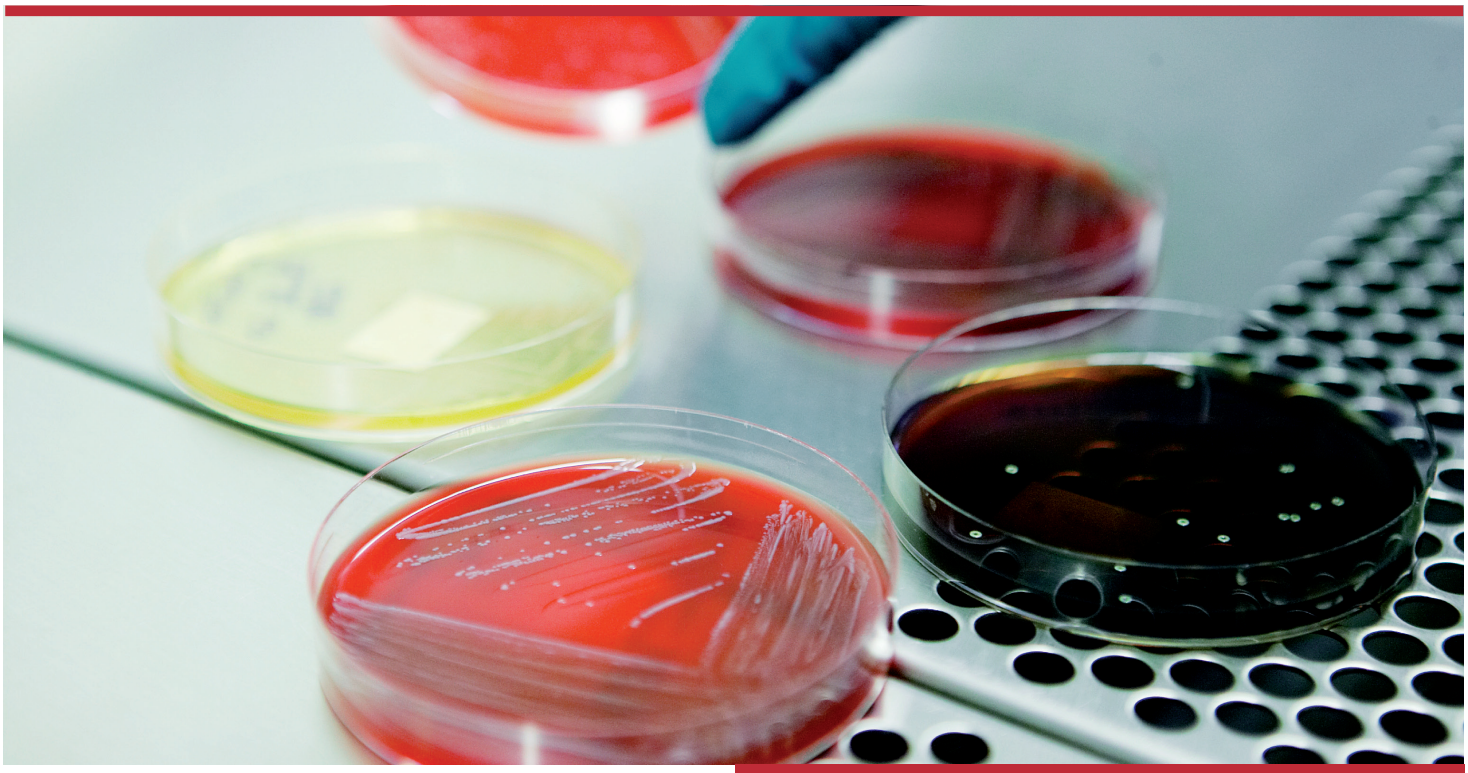
Technical Report



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SOURCE ATTRIBUTION OF CAMPYLOBACTER INFECTIONS IN DENMARK

Technical Report

1. edition, October 2017

Photo: Mikkel Adsbøl
ISBN: 978-87-93565-10-4

This report is available at
www.food.dtu.dk

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List of abbreviations

CEA: Comparative exposure assessment approach

FVST: Danish Veterinary and Food Administration (Fødevarestyrelsen)

MLST: Multi-locus sequence typing

MSA: Microbial subtyping approach

Summary

Campylobacter spp. is the most common foodborne pathogen in Denmark, with 4,677 cases reported and an estimated burden of disease of around 2,000 disability adjusted life years (DALYs) in 2016. *Campylobacter spp.* have been detected in many sources and are considered to be widespread in production animals and in the environment. Initiatives to reduce *Campylobacter* prevalence in broiler production have not had the desired effect in terms of reduction of the public health burden of campylobacteriosis in the population. Identifying the relative contribution of all potential sources of a pathogen is crucial to prioritize food safety intervention strategies. The objectives of this project were to 1) estimate the proportion of human *Campylobacter* cases that can be attributed to main animal and environmental sources, and 2) estimate the relative contribution of different transmission routes to human *Campylobacter* cases. A third objective was to explore the possibility of combining previously developed models for each purpose and evaluate whether the output is relevant and useful for decision-making.

We applied a microbial subtyping approach (reservoir level attribution) and a comparative exposure assessment approach (exposure level attribution) to estimate the relative contribution of sources of campylobacteriosis. Data were collected in the period between January 2015 and March 2017, with all human and part of the animal isolates subtyped by Multi Locus Sequence Typing (MLST). We restricted the data to include only *C. jejuni*, the species most frequently causing disease.

The microbial subtyping approach attributed 731 MLST typed human isolates to eight food, animal and environmental sources for which MLST type distribution was available. Results showed that the most important source of *C. jejuni* infections was domestic chicken (338 cases, 95% Confidence Interval (CI) 263-411), followed by cattle (139 cases, 95% CI 84-200) and imported chicken (69 cases, 95% CI 43-100); these estimates correspond to attribution proportions 46%, 19% and 9%, respectively. Imported duck meat was estimated to contribute to less than 2% of the cases, and no cases were attributed to domestic duck. Around 13% of the cases could not be attributed to any source. We estimated that 30 cases (95% CI 8-62) were attributed to exposure to dogs, and that 27 cases (95% CI 4-46) were attributed to exposure to contaminated seawater. A scenario analysis including a different data source for the reservoir “domestic chicken” (data from cecal samples at the slaughterhouse, instead of the meat samples collected at the slaughterhouse or retail used in the baseline model) suggested a lower proportion of cases attributed to this source and a corresponding increase of the source cattle. The data applied in this scenario were constituted by fewer samples, and thus we considered the results of the baseline model as more robust.

The comparative exposure assessment approach estimated the relative contribution of 10 food, animal and environmental sources and transmission routes. Due to substantial gaps of data and large uncertainties in the exposure model, environmental transmission of *Campylobacter* through sand was excluded from the model. In addition, exposure to *Campylobacter* through direct contact with farm animals (broilers, cattle and pigs) was not considered due to the large bias caused by immunity of people regularly exposed to the same strains of the pathogen. Excluding these two categories reduces the usefulness of the results, as these are considered sources of importance from the environmental reservoirs. Results suggested that consumption of chicken meat is the most important source of exposure to *C. jejuni*, contributing with around 0.8 CFU in a random serving (95% CI 0-5.655) and nearly 70% of overall exposure at the population level. The second and third most important sources were consumption of ducks and unpasteurized milk. Among non-food routes, the transmission route contributing with highest exposure was *contact with dogs*, but the estimated attributed proportion was lower than 1%.

The two models were consistent in identifying chicken as the most important source of campylobacteriosis in Denmark. However, results could only be integrated to explain the contribution of different transmission routes for the cattle reservoir, where the comparative exposure assessment was able to distinguish between exposure via consumption of beef and unpasteurized milk; the results of the models were coherent for these sources. For remaining sources and transmission routes, either due to lack of data or due to large uncertainties (which can derive for example from lack of knowledge on population at risk, of the susceptibility of risk groups or bias introduced by immunity), discrimination between different transmission pathways from main reservoirs was not possible.

One of the purposes of our study design was to integrate the two models to derive more complete source attribution estimate. A careful evaluation of the models' performance and inherent uncertainty makes us conclude that combining the two models did not provide additional information and that the estimates of the microbial subtyping approach already to some degree account for relative exposure to the sources and are more robust than the combined results. Moreover, because we were able to include a wide variety of sources using the microbial subtyping model, including food, animal contact and environmental sources, the microbial subtyping approach is more comprehensive than previously and provided valuable evidence on the most important sources of the pathogen, even if it is not able to point out the exact exposure route.

1. **Introduction**

Campylobacter spp. is the most common foodborne pathogen in Denmark, with 4,677 cases reported in 2016 (Anon., 2017). As other foodborne diseases, campylobacteriosis is largely underreported, and the true incidence of disease in the population is much larger. We estimate that *Campylobacter* led to more than 55,000 cases and the loss of around 2,000 healthy years of life in 2016 (based on Pires, 2012).

Campylobacter spp. have been detected in many sources and are considered to be widespread in production animals and in the environment (Boysen et al. 2013). Broiler chicken meat is recognized as the largest single source of foodborne campylobacteriosis, and Denmark, like several other countries, has implemented a number of initiatives to reduce *Campylobacter* prevalence in broiler production (Rosenquist et al. 2009). However, these interventions have not had the desired effect in terms of reduction of the public health burden of campylobacteriosis in the population. The lack of public health effect may be related to other factors counterbalancing the effect of the implemented interventions, particularly with the role of other sources of exposure. Identifying the relative contribution of all potential sources of a pathogen is crucial to prioritize food safety intervention strategies (Sara M Pires et al. 2009).

The process of partitioning the human disease burden of a foodborne infection to specific sources is known as *source attribution*, where the term source includes reservoirs (e.g. animal reservoirs like pigs, cattle, pets) and vehicles (e.g. food products like pork or beef). A variety of methods to attribute foodborne diseases to sources are available, including approaches based on analysis of data of occurrence of the pathogen in sources and humans, epidemiological studies, intervention studies, and expert elicitations. Each of these methods presents advantages and limitations, and the usefulness of each depends on the public health questions being addressed and on characteristics and distribution of the hazard.

Source attribution methods have been extensively used to investigate the contribution of food and animal sources for various diseases. Measuring the proportion of *Salmonella* infections that is attributable to different sources has proven particularly useful in several countries and regions, with Denmark pioneering the *One Health* efforts to guide food-safety interventions based on scientific evidence. Attribution models provide a tool to guide policy-makers in prioritisation and implementation of control efforts in various sources. The usefulness has been demonstrated in Denmark, where *Salmonella* control programmes in the various animal sectors have resulted in a proportional reduction in human cases from the different reservoirs (Wegener 2010).

Source attribution methods can attribute disease to different points in the transmission pathway: at the reservoir level, i.e. at the very origin of the pathogen (such as the animal or environmental reservoir); at the point of exposure, (i.e. the point of consumption of a contaminated food or direct exposure to a contaminated animal (e.g. pet) or environment); or the point of processing of a food. The point of attribution also determines the usefulness of the different methods to address different risk management questions. As an illustration, the aim can be to identify the most important reservoirs of the pathogen in order to eliminate or reduce the agent at the origin (which requires reservoir-level attribution), or to identify the most important risk factors for disease, for example undercooking, poor handling practices or hygiene, eating unwashed vegetables or fruits (which requires point-of-exposure attribution). On the other hand, public health questions are often more complex and aim at identifying the complete set of interventions that would lead to a reduction of the disease burden. In this case, integration of methods or

results from more than one method will add insight to the contribution of different sources and strengthen confidence in the results.

Several studies have developed or applied methods for source attribution of *Campylobacter* infections (e.g. (Boysen et al. 2013; Domingues et al. 2012; Evers et al. 2008; Mughini Gras et al. 2012; Mullner et al. 2009). These studies have been useful to direct food safety efforts in the different countries, and also to highlight the need for integration of knowledge from other studies to provide more complete evidence for interventions. In particular, they highlight the need for integration of evidence on the most important reservoirs of *Campylobacter* and on the relative contribution of different transmission routes from some of these reservoirs.

1.1. Objectives

The overall aim of this project was to estimate the relative contribution of different sources to human campylobacteriosis in Denmark. The specific objectives were to:

- Estimate the proportion of human *Campylobacter* cases that can be attributed to main animal and environmental sources.
- Estimate the relative contribution of different transmission routes from each source to human *Campylobacter* cases.
- Explore the possibility of combining previously developed models for each purpose and evaluate whether the output is relevant and useful for decision-making.

2. Methods

We applied two source attribution methods to address the objectives of this project: a microbial subtyping approach for reservoir allocation (Model 1), and a comparative exposure assessment approach to add the contribution of transmission routes (Model 2). Both methods rely on data on the occurrence of the pathogen. The microbial subtyping approach attributes disease at the reservoir level and requires data from human and sources' isolates subtyped with the same method. The comparative exposure assessment approach attributes disease at the point of exposure and allows for the estimation of the relative contribution of different transmission routes for human infection, including foodborne, animal contact and environmental. It requires prevalence, concentration and exposure data on all routes. Table 1 presents an overview of the principles and data requirements of the two approaches.

Table 1. Principles and data requirements of the microbial subtyping and the comparative exposure assessment approach.

Source attribution approach	Principle	Data requirements
Subtyping approach	Compare the subtypes of isolates from different sources (e.g., animals, food) with the same subtypes isolated from humans	Characterization of the hazard by subtyping methods (e.g. MLST). Collection of temporally and spatially related isolates from humans and various sources.
Comparative exposure assessment	Determine the relative importance of the known transmission routes by	Occurrence of the hazard (prevalence and

	estimating the human exposure to the hazard via each route.	concentration) in all putative sources. Information on the changes of the level of the hazard in the main steps of the transmission chain. Human consumption/ exposure data.

The results of the two models were integrated in an attempt to complement evidence on the most important reservoirs of *Campylobacter* (Model 1) with information on the relative importance of different transmission routes from those main reservoirs (Model 2). Figure 1 presents a general overview of the integration of the two models. The two models were built separately, each with their own purpose, and combing them in this study is an exploratory and novel approach.

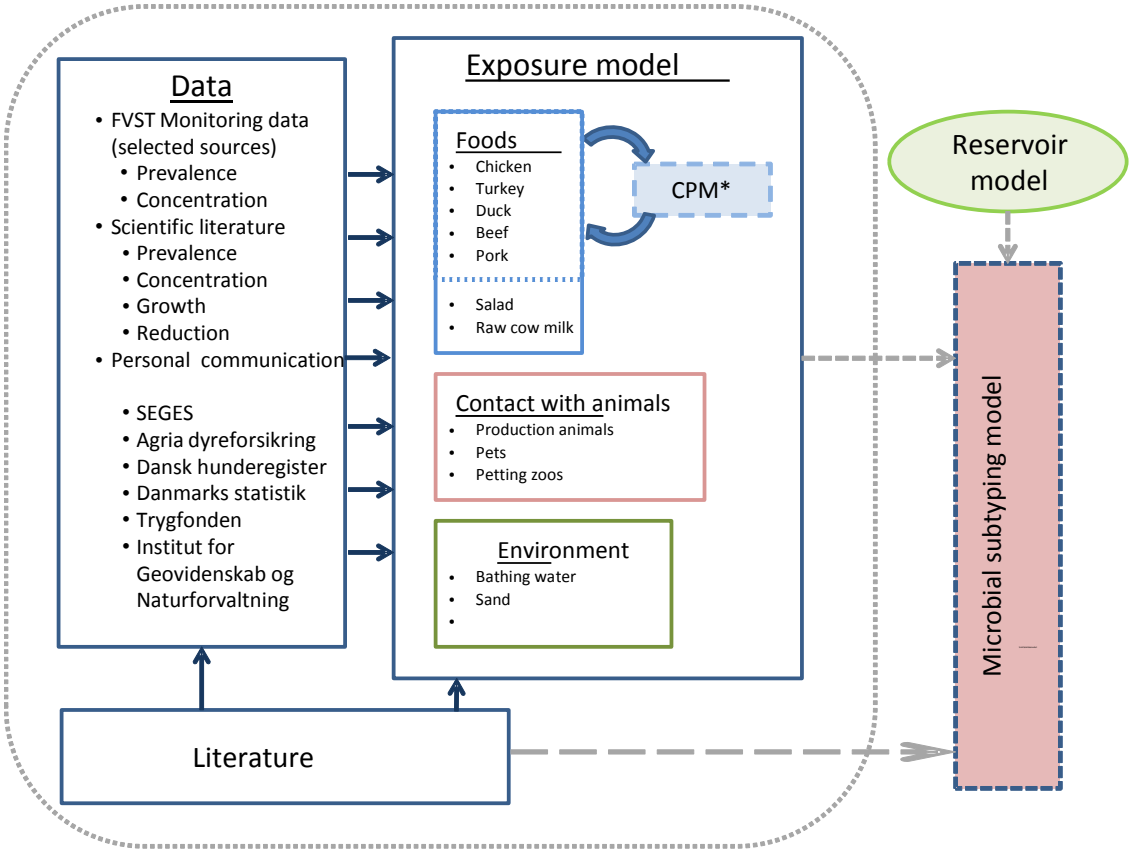

Figure 1. Overview of *Campylobacter* source attribution models applied.

*CPM: consumer phase model. Modes the reduction of Campylobacter throughout the food chain..

2.1. Data overview

Campylobacter occurrence data used in the two models were provided by the Danish Veterinary and Food Administration and the Statens Serum Institute. Data were collected in the period between January 2015 and March 2017, and all human and part of the animal isolates were subtyped by Multi Locus Sequence

Typing (MLST). Both models included data on *C. jejuni* only. Tables 2 and present an overview of the data used for both models.

Table 2. Overview of the data used for the microbial subtyping model (Model 1).

	Source	Number of MLST types in source (Number of isolates)	Number of MLST types matched to human (Number of isolates)
Danish	**Human**	136 (731)	136 (731)
	Broilers*	42 (132)	32 (121)
	Cattle*	38 (208)	21 (191)
	Pig*	6 (22)	4 (16)
	Chicken	46 (176)	35 (161)
	Duck**	1 (1)	0 (0)
	Dogs	19 (25)	16 (22)
	Bathing water	2 (2)	1 (1)
	Vegetables	1 (1)	0 (0)
Imported	**Chicken****	46 (90)	25 (65)
	Duck**	16 (20)	8 (8)
	Turkey**	7 (9)	6 (7)

*Fecal samples. **Meat samples.

Table 3. Overview of contamination data used in the comparative exposure assessment model (Model 2).*

	Source	Type of sample	Total number of samples	Number of positive isolates
Danish	**Chicken meat**	With skin	2511	569
		Without skin	3503	287
	Chicken meat, free range	With skin	15	13
	Chicken meat, organic	With skin	227	190
		Without skin	30	17
	Duck	With skin	13	8
	Turkey	With skin	2	0
		Without skin	4	0
	Vegetables	Salad	327	3
Imported	**Chicken meat**	With skin	1411	605
		Without skin	3505	315
	Chicken meat, free range	With skin	57	46
		Without skin	1	1
	Chicken meat, organic	With skin	5	4
		Without skin	6	4
	Duck	With skin	79	25
		Without skin	10	0
		No information	47	0
	Turkey	With skin	44	4
		Without skin	521	7
	Vegetables	Salad	839	0

*Data for beef not available and were based on an older study (FVST kontrolprojekt 2001-2002).

Food consumption and other exposure data (utilized in Model 2) were retrieved from multiple sources. The amount of each food type consumed per person per meal were collected from the National Danish Survey of Diet and Physical Activity (DANSDA) (Knudsen et al. 2014). This survey is a nationwide and cross-sectional survey in a representative sample of the Danish population. The frequency of exposure to non-food routes was estimated based on literature review and expert elicitations (Table 4). It was not possible to find Danish and recent references for all exposures, which introduces uncertainty about the applicability to the Danish situation today.

Table 4. Overview of exposure data used in the comparative exposure assessment model (Model 2).

Input parameter	Data source	Reference
Amount of food consumer per person per meal event	DANSDA*, Danish statistics	Statistics Denmark. (Evers et al. 2008)
Exposure to pets (dogs <2 years of age)	Literature, Statistics	(Evers et al. 2008) Statistics Denmark. (Evers et al. 2008)
Exposure to petting zoo animals	Literature	
Exposure to farm animals	Literature	Ogden et al., 2005
Exposure to bathing water	Literature.	Jeppesen og Guldbæk, 2006

2.2. Source attribution approaches

2.2.1. Microbial subtyping

The microbial subtyping approach involves characterization of isolates of the pathogen by phenotypic and/or genotypic subtyping methods (e.g MLST). The principle is to compare the subtypes of isolates from different sources (e.g., animals, food) with the same subtypes isolated from humans. The microbial subtyping approach is enabled by the identification of strong associations between some of the dominant subtypes and a specific reservoir or source, providing a heterogeneous distribution of subtypes among the sources. Subtypes exclusively or almost exclusively isolated from one source are regarded as indicators for the human health impact of that particular source, assuming that all human infections with these subtypes originate only from that source. Human infections caused by subtypes found in several reservoirs are then distributed relative to the prevalence of the indicator types. This approach utilizes a collection of temporally and spatially related isolates from various sources.

The applied model was developed by (Hald et al. 2004) and adapted by (Boysen et al. 2013). The model is built in a Bayesian framework and estimates a set of unknown parameters that account for the differences in the ability of different subtypes to cause infection and of different sources to act as a vehicle for infection. The equation used to estimate the expected number of human domestic cases attributed to each source was:

$\lambda_{ij} = p_{ij} * qi * a_j$,

where λ_{ij} is the expected number of cases of type *i* from source *j*, p_{ij} is the number of isolates of type *i* in source *j*, q_i is the ST-dependent factor, and a_j the source-dependent factor. The equation represents a multi-parameter prior, where $_{qi}$ and a_j are parameters of unknown value. These parameters were included as distributions; a hierarchical prior and a uniform prior, respectively. The use of a hierarchical prior was adapted after (Mullner et al. 2009), using a lognormal distribution N(0, τ). The prior distribution for τ was

gamma (0·01, 0·01). The source-dependent factor, a_j, was assumed equal for Danish-produced duck and imported duck meat. A Markov Chain Monte Carlo simulation, specifically the Gibbs sampler, was applied to compute the posterior distributions for a_j and q_i. Five independent Markov chains of 40,000 iterations were run. Convergence was monitored using methods described previously (Hald et al. 2004). The model was run in OpenBugs version 3.2.3.

2.2.1.2. Scenario analysis

Data on the distribution of MLST subtypes in 9 animal or food sources were available: domestic broilers, domestic chicken, domestic duck, domestic turkey, imported chicken, imported duck, imported turkey, dogs and bathing water (see table 2). Among *chicken* samples, we were able to distinguish between conventionally-produced chicken and organic chicken. We assumed that all data represented the closest point possible from the original reservoir of Campylobacter, i.e. domestic meat samples represent domestic production animals, imported meat samples represent foreign production animals, and samples from dogs and bathing water represent Danish pet animal and environmental reservoirs.

Because the sources *broilers* and *chicken* represent the same animal reservoir, we included only one of these in the model. To select which data source to include, we ran two models and selected the one that yielded results with narrower uncertainty intervals (*scenario 1*, including chicken; and *scenario 2*, including broilers).

To investigate if there were differences in distribution of MLST subtypes in conventional and organic chicken that could explain different contribution of these sources for disease, we ran a third model including domestic conventional chicken and domestic organic chicken as two sources (*scenario 3*).

2.2.2. Comparative exposure assessment

The principle of the comparative exposure assessment approach is to determine the relative importance of the known transmission routes both within the same reservoir and between reservoirs by estimating the human exposure to that pathogen via each route. This approach requires, for each known transmission route, information on the prevalence and dose/concentration of the pathogen in the source, of the changes of the prevalence and quantity of the pathogen throughout the transmission chain, and of the frequency at which humans are exposed by that route. With this information, the exposure dose for each transmission route is estimated. These exposures are then compared, and the human disease burden (e.g. the observed laboratory-confirmed infections or estimated total number of infections) caused by the specific pathogen is partitioned to each of the various transmission routes, proportionally to the size of the exposure dose. The estimates of exposure dose for each transmission route can be subsequently combined with a dose-response model to predict the number of infections from each route.

The comparative exposure assessment approach for source attribution makes use of stochastic modelling techniques similar to those used in traditional microbial risk assessments. Nevertheless, the two methods differ in objectives and level of detail. A risk assessment typically aims at describing the complex dynamics of a pathogen in a single food commodity in the farm-to-consumption continuum, and predicting the public-health impact of interventions strategies. In contrast, the comparative exposure assessment approach aims at partitioning the observed (or predicted) human disease burden to all known transmission routes, including various foods, direct contact with live animals, and environmental exposures. For this

purpose, the various transmission routes are modelled in a more simplified fashion that represents only the main steps in the transmission pathway.

We developed a simplified stochastic model for food, animal contact and transmission routes. The food pathway focused on the retail level and forward. Exposure was estimated based on the initial prevalence and concentration of the pathogen in each product, the probability of cross-contamination during preparation and survival of *Campylobacter* after cooking, and the amount of each product consumed in the population per person per day independently of age. Figure 2 represents the generic flow-chart applied for each food transmission route.

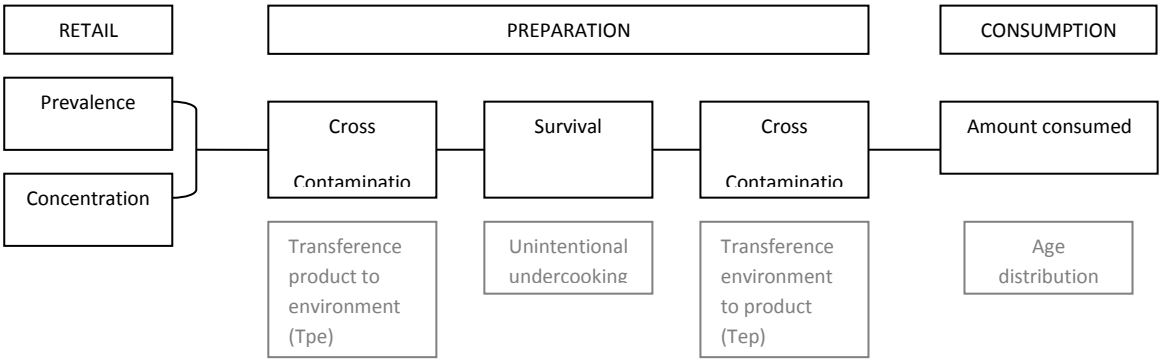

Figure 2. General structure of the foodborne transmission chain of the comparative exposure assessment model.

The preparation step included handling and cooking of the product, cross-contamination to other products, and potential bacterial growth before preparation and before consumption. We applied a consumer phase model (CPM) to estimate cross-contamination in meat products as described by (Nauta et al. 2008). The model estimates the amount of *Campylobacter* that can be transferred and survive after preparation, taking into account levels of hygiene. It was assumed that food products are kept in a cold chain until the preparation stage, but that temperature abuse may occur during transport and storage at the consumer. On the basis of this assumption, potential growth before preparation was considered. The estimation of survival of the pathogen in the food route depends on the process of preparation. If the product is not heat-treated, the probability of survival was considered 1 (one); if heat-treatment is applied, the probability of survival would be a value between 0 and 1. Despite the different effects the wide variety of cooking processes can have (e.g. boiling, baking, frying, grilling etc.), and in order to simplify the model, we considered preparation as a single step, and survival after preparation was expressed as a single probability.

The amount consumed of each food item per person per consumption even (in grams) used in the model was based on the national data as described above. The estimation of the proportion of imported and domestic foods consumed was estimated based on the total amount of imported and domestic food items available for consumption in the country.

For the environmental and direct contact transmission routes, data on the number of people in the population at risk (i.e. number of people in Denmark with direct contact with farm animals, number of pet owners, number of visits of a petting zoo, etc) were retrieved from national statistic sources and combined with data from literature reviews to estimate frequency of exposure. When sufficient data were available, variables were described as probability distributions to describe uncertainty in input data (Table 5). The model was developed in @risk 4.5.1. (Palisade Corporation, 2002). For all transmission pathways, the output of the model was total exposure per exposure event (in CFU).

Table 5. Variables for the calculation of ingestion of *Campylobacter* per food route of the comparative exposure assessment model (CFU per person per event).

Variable	Description	Distribution/ Formula
I	Ingestion of *Campylobacter* pppe[#]	Outcome
P	Prevalence of *Campylobacter* (%)	Input data
Conc	Concentration of *Campylobacter* (CFU/ g)	Input data
a	(CFU/g)	P * Conc
Consumer phase model (CPM)	Transference and survival after preparation (CFU/g)	See Nauta et al. (2008)
C	Consumption of the food product per person per day (g)	Input data

[#]pppe: per person per event

3. Results

3.1. Point of reservoir attribution (microbial subtyping)

We estimated that the most important sources of *C. jejuni* infections in the studied time period in Denmark were domestic chicken, cattle and imported chicken (Tables 5 and 6, Figure 3).The results of scenario 1 (chicken) and scenario 2 (broilers) were consistent in the identification of the top three most important sources, but estimated different ranking of these sources. Scenario 3, which attempted to distinguish between conventional and organic chicken, did not converge and thus did not retrieve valid results.

For comparison and discussion purposes, we present the results of scenarios 1 and 2 in detail below, but thereafter refer to scenario 1 as the main results of this model.

Scenario 1 – model with chicken meat

We estimated that the most important source of *C. jejuni* infections was domestic chicken meat (338 cases, 95% Confidence Interval (CI) 263-411), followed by cattle (139 cases, 95% CI 84-200) and imported chicken (69 cases, 95% CI 43-100) (Table 6); these estimates correspond to attribution proportions 46%, 19% and 9%, respectively. Imported duck meat was estimated to contribute to less than 2% of the cases, and no cases were attributed to domestic duck. Around 13% of the cases could not be attributed to any source. We estimated that 30 cases (95% CI 8-62) were attributed to exposure to dogs, and that 27 cases (95% CI 4-46) were attributed to exposure to contaminated seawater.

Table 6. Number and proportion of *Campylobacter jejuni* cases attributed different sources (mean, median and 95% Confidence Interval (CI)).

	Number of cases				Attribution proportion (%)		
	Mean	**Median**	**95% CI**		**Mean**	**95% CI**	
Chicken DK	338.1	338	[261.3,	411.6]	46.3	[35.7,	56.3]
Cattle DK	139.4	136.9	[83.5,	199.8]	19.1	[11.4,	27.3]
Pig DK	5.1	4.3	[0.7,	14.1]	0.7	[0.1,	1.9]
Duck DK	0				0		
Chicken IMP	69.1	67.8	[43.0,	100.6]	9.5	[5.9,	13.8]
Duck IMP	11.9	11.1	[4.3,	23.6]	1.6	[0.6,	3.2]
Turkey IMP	14.2	12.8	[3.1,	32.9]	1.9	[0.4,	4.5]
Dog	30.1	28.2	[8.5,	62.1]	4.1	[1.2,	8.5]
Bathing seawater	27.6	28.4	[4.0,	46.4]	3.7	[0.6,	6.3]
Unknown	95.7	98.3	[47.8,	146.3]	13.1	[6.5,	20.0]
Total	731						

Scenario 2 – model with broilers

The scenario using broilers isolates as a source of data for the domestic broiler/chicken reservoir estimated a lower proportion of cases attributed to this source (27%, 199 cases (95% CI 122-283), ranking it as the second most important source of campylobacteriosis (Table 7, Figure 3). The estimated attribution proportion for cattle was however very similar (28%, 207 cases (95% CI139-264)), and the sources' confidence limits overlapped. This scenario attributed a higher number of cases to imported chicken (14%) and dogs (6%), and a larger proportion of cases could not be attributed to any source (16%).

Table 7. Number and proportion of *Campylobacter jejuni* cases attributed different sources (mean, median and 95% Confidence Interval (CI)).

	Number of cases				Attribution proportion (%)		
	Mean	**Median**	**95% CI**		**Mean**	**95% CI**	
Broilers DK	198.7	197.5	[121.7,	282.7]	27.2	[16.6,	38.7]
Cattle DK	207.3	201.5	[139.4,	264.4]	28.4	[19.1,	36.2]
Pig DK	4.6	3.8	[0.6,	13.3]	0.6	[0.1,	1.8]
Duck DK	0				0		
Chicken IMP	98.6	97.5	[67.0,	135.2]	13.5	[9.2,	18.5]
Duck IMP	12.7	11.7	[4.3,	27.0]	1.7	[0.6,	3.7]

Turkey IMP	12.9	11.2	[2.4,	32.7]	1.8	[0.3, 4.5]
Dog	45.7	44.2	[12.4,	87.7]	6.3	[1.7, 12.0]
Bathing seawater	30.6	31.6	[7.1,	48.2]	4.2	[1.0, 6.6]
Unknown	119.9	126.3	[76.9,	172.9]	16.4	[10.5, 23.7]
Total	731				100	

Scenario 3 – model splitting domestic chicken into conventional and organic meat samples

The model where human cases of *C. jejuni* were attributed to 10 sources (i.e. splitting domestic chicken into conventional and organic meat samples) did not converge, and thus we will not present results.

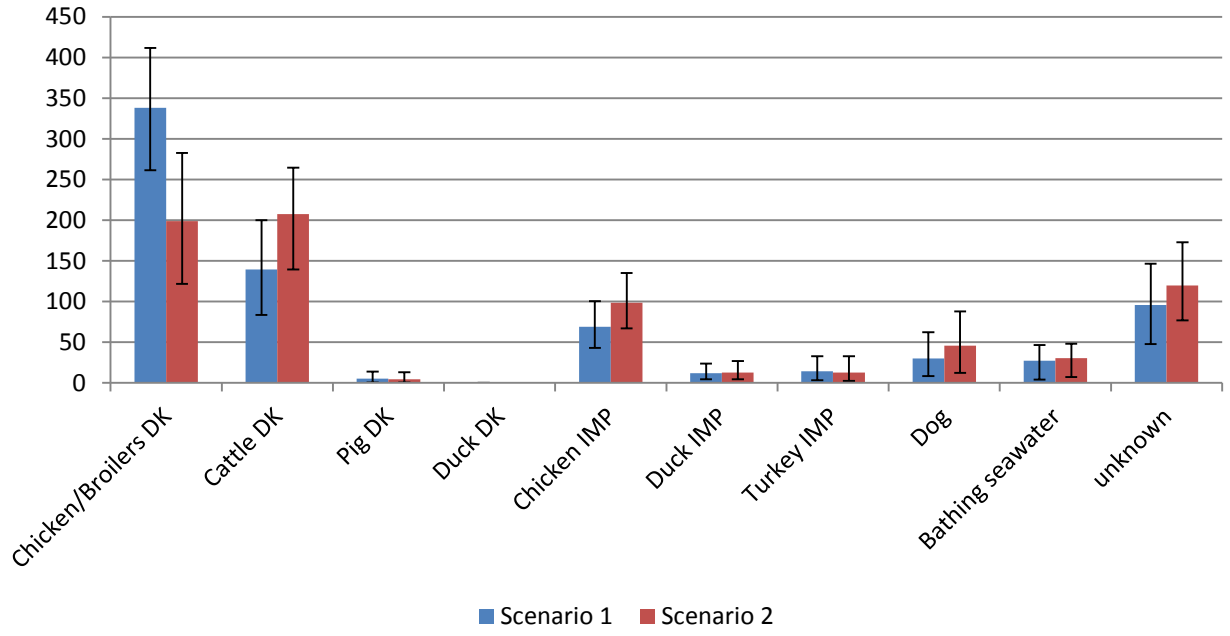

Figure 3. Number of cases of *Campylobacter jejuni* attributed to domestic and imported foods, contact with dogs and bating seawater in Denmark.

3.2. Point of exposure attribution (comparative exposure assessment (CEA))

Due to substantial gaps of data and large uncertainties in the exposure model, environmental transmission of *Campylobacter* through sand, and direct contact with farm animals (broilers, cattle and pigs) were excluded from the model. Excluding these two categories reduces the usefulness of the results, as these are considered sources of importance from the environmental reservoirs.

The results of the CEA show that consumption of chicken meat is the most important source of exposure to *C. jejuni*, contributing with around 0.8 CFU in a random serving (95% CI 0-5.655) and nearly 70% of overall exposure at the population level (Table 8, Figure 4). The second and third most important sources were

consumption of ducks and unpasteurized milk. Among non-food routes, the transmission route contributing with highest exposure was *contact with dogs*, but the estimated attributed proportion was lower than 1%.

Table 8. Mean exposure to *Campylobacter jejuni* per random exposure event (mean CFU and 95% Confidence Interval, CI), and proportion of exposure attributable to food, animal contact and environmental transmission routes.

Transmission route	Mean	95% Confidence Interval	Attribution proportion (%)
FOOD			
Vegetables	0.004	[0.001,0.004]	0.3
Duck	0.222	[0,0.589]	19.1
Chicken	0.783	[0,5.655]	67.4
Raw milk	0.129	[0.005,7.173]	11.1
Turkey	0.022	[0,0.139]	1.9
Beef	0.00001	[0,0.0001]	0.0007
Pork	0.0005	[0,0.01]	0.04
CONTACT WITH ANIMALS			
Petting zoo goats	0.00001	[0.0000001,0.00006]	0.0009
Pets	0.001	[0.0000004,0.006]	0.09
Direct contact with farm animals	Not included		
ENVIRONMENT			
Bathing seawater	0.0000008	[0.0000006,0.000008]	0.00007
Contact with sand	Not included		

*Colony forming units per person per day.

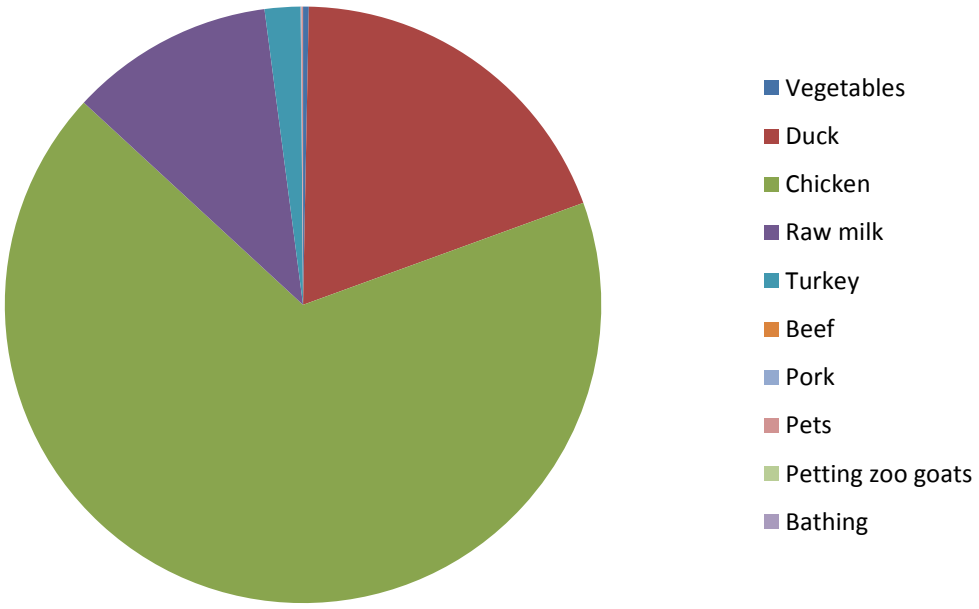

Figure 4. Proportion of *Campylobacter jejuni* cases attributed to food, animal contact and environmental transmission routes (%) using comparative exposure assessment.

3.3. Integrating the MSA and the CEA

The usefulness of integrating the two models was limited. Adding the CEA did not add to the knowledge of relative contribution between reservoirs to campylobacteriosis, mainly due to data gaps and large uncertainties, but also because the microbial subtyping model already accounts for the exposure step in the risk pathway to some degree. The microbial subtyping model is not able to distinguish between different routes within the same reservoir, but since data is lacking on many of the exposure routes, the outputs from the CEA model are limited and in some cases could be misleading.

Model 1(MSA) and model 2(CEA) were consistent in identifying chicken as the most important source of campylobacteriosis in Denmark (Figure 5). Model 1 distinguished between domestic and imported chicken (which model 2 did not), and if we add these two estimates the total proportion of cases attributed to the chicken is relatively similar. On the other hand, model 1 considered “cattle” as a reservoir, not distinguishing between different transmission-pathways from this reservoir until human exposure. If we assume that model 2 was able to do that by estimating exposure via consumption of beef and unpasteurized milk, the results of the models were also coherent. While this is an excellent example of the integration of models and results that was intended in this project, it proved to be possible only for this reservoir. For remaining sources and transmission routes, either due to lack of data or due to large uncertainties (which can derive from lack of knowledge on population at risk, of the susceptibility of risk groups or bias introduced by immunity), discrimination between different transmission pathways from main reservoirs was not possible. Still, we highlight below the main similarities and discrepancies between models 1 and 2.

The two models were also consistent in the proportion of campylobacteriosis attributed to turkey. On the contrary, the two approaches were fundamentally different in the estimated relative contribution of ducks: model 1 did not attribute any cases to domestic duck and attributed below 2% to imported duck, whereas the exposure model (model 2) estimated that 19% of exposure was attributed to this source. The approaches were also in disagreement in the relative importance of dogs (4% in model 1 versus 1% in model 2) and bathing seawater, also higher for model 1 (4%, versus nearly 0%).

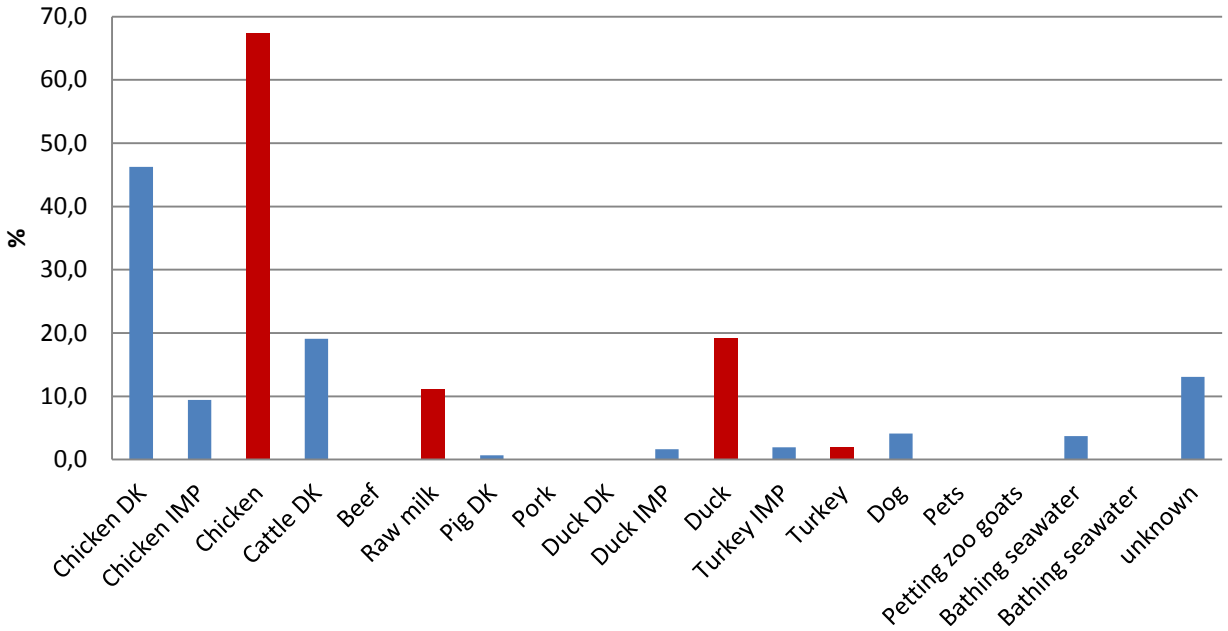

Figure 5. Proportion of *Campylobacter jejuni* cases attributed to sources and transmission routes (%). Bars in blue present the results of the Microbial Subtyping Approach, and bars in red present the results of the Comparative Exposure Assessment.

4. Discussion

We applied two source attribution models to estimate the relative contribution of different food, animal contact and environmental sources of campylobacteriosis in Denmark. While the purpose of our study design was to integrate the two to derive more complete source attribution estimates, a careful evaluation of the models' performance and inherent uncertainty makes us conclude that the estimates of the microbial subtyping approach are more robust, and thus that we should focus on these to make inferences about the ranking of *Campylobacter* sources. Moreover, because we were able to include a wide variety of sources in this model, including food, animal contact and environmental sources, we believe that estimates obtained with the microbial subtyping approach are complete and provide valuable evidence on the most important sources of the pathogen.

Our results show that domestic chicken is the most important source of *Campylobacter* infections in Denmark (with 46% of cases attributed), followed by cattle (19%) and imported chicken (10%). They also show that contact with dogs and bathing in contaminated recreational waters are relevant sources (4% of cases attributed to each of these sources). Imported turkey and duck were responsible for less than 2% of cases, and pigs were estimated to be the least important source, with an attribution proportion below 1%. No cases were estimated to be attributed to domestic duck, and 13% of cases could not be attributed to any source.

These estimates are coherent with the results of a previous study using MLST data and the same approach for source attribution of campylobacteriosis in Denmark (Boysen et al. 2013). That study attributed 406 human cases to six animal food sources and estimated that 38% of cases were attributed to Danish chicken,

14% to imported chicken, and 16% to cattle; no environmental or non-food animal sources were included in that model.

In this study, we were able to include bathing water and dogs as additional sources of *Campylobacter*. The contribution of these sources for campylobacteriosis in the country had not been quantified yet, but several studies from other countries had pointed as these as important sources of *Campylobacter* (Evers et al. 2008; Mughini Gras et al. 2012; Pintar et al. 2017).

The microbial subtyping approach has strong advantages when compared to other source attribution methods (S.M. Pires et al. 2014). It relies on the distribution of isolates (characterized by robust typing methods with appropriate discriminatory power, such as MLST) in humans and animals and identifies the most important reservoirs of the pathogen. Therefore, it is useful to prioritize interventions at production level, reducing uncertainty due to cross-contamination and the risk of attributing to an ''accidental'' source. In addition, because it is of relatively easy application when new data become available, it is able to follow trends over time.

However, this approach does not provide evidence on the route of transmission between some reservoirs and human exposure. Several processes that may change the relative importance of sources and pathways can take place along the transmission chain, e.g. decontamination, preparation/cooking, cross-contamination, growth, and these should be considered when interpreting and comparing attribution estimates. We were able to compensate for this lack of information by using data from a point in the transmission pathway that reflects the type of transmission to humans. Specifically, we used data from slaughterhouse samples to represent domestically produced animals (which are in most cases a representation of meat products), meat samples to represent imported meats, and the animal or environmental reservoir to which humans are exposure through direct contact. Still, we were not able to account for potential cross-contamination of other sources in the other points of the transmission chain (e.g. salads and other food products that will be eaten unheated).

5. Conclusions

- The most important source of *Campylobacter* infections in Denmark remains to be broiler chicken, being responsible for 46% human cases each year.
- Cattle are the second most important reservoir, contributing with nearly 20% of cases.
- Among imported meats, imported chicken is the most important source, with around 10% of all cases attributed to this source; remaining imported meats play a minor role for disease.
- Contact with dogs and bathing in contaminated recreational waters are relevant sources of campylobacteriosis, with 4% of cases attributed to each of these sources.
- The microbial subtyping approach is at this point the most robust method to estimate the relative contribution of different sources for campylobacteriosis and the addition of an exposure pathway mode did not improve accuracy or provide new information.
- Even though we were not successful in improving our knowledge on the contribution of transmission routes by integrating the two applied source attribution models, the results of the microbial subtyping approach were comprehensive and provide valid and robust source of information.

6. Perspectives

To be able to overcome current limitations in terms of knowledge on the most important transmission routes and risk factors, we will integrate the microbial subtyping approach with the case-control study conducted in the same population. This project will be implemented in collaboration with SSI and is to start in September. The output of the study will be evidence on the relative contribution of main reservoirs for human campylobacteriosis in Denmark, and of the relative importance of different risk factors for these cases.

Acknowledgements

We would like to thank Ana Sofia Duarte for her contribution and input in the implementation of model 2, Louise Boysen for the structuring of model 2 and extensive literature review and data collection, and Johanne Ellis-Iversen for the critical review of this report.

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ISBN: 978-87-93565-10-4