European Food Safety Authority

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Overview of methods for source attribution for human illness from food-borne microbiological hazards[1, 2]

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2008-005)

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PANEL MEMBERS

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SUMMARY

For food safety policy, it is important to know not only the fraction of incidence of human illness due to specific pathogens that is attributable to specific foods, but also what is attributable to other sources like environmental exposure, direct animal contact and human to human contact. This is a difficult process that can be based on different information sources that do not necessarily give the same answers. On this basis, and after dialog and interaction between EFSA and the European Commission, EFSA decided to produce a document with the aim of providing an overview of methods for source attribution of human illness from food-borne microbiological hazards. Thus, the present report summarises the methods available for source attribution for human illness, identifies strengths and weaknesses, as well as data requirements for each of the methods.

Attribution to sources of human food-borne illness can be achieved using different methods such as microbial subtyping, outbreak summary data, epidemiological studies, comparative exposure assessment, and structured expert opinion. Each method of source attribution has different strengths and weaknesses and addresses different points in the food chain. The choice of method depends on the specific question that needs answering and the data and resources available.

Source or reservoir attribution using microbial subtyping has mainly been applied to *Salmonella* and, so far, only in a few countries. Serotyping and phage-typing are the preferred

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[2] Original title: "Overview of methods for source attribution for human cases of food borne microbiological hazards"

typing methods for this purpose, but new genotypic-based methods may prove to be valuable in the future, and also for other pathogens such as *Campylobacter* and VTEC. Reservoir attribution of human salmonellosis has provided guidance to risk managers and policy makers on the implementation and evaluation of control strategies for major reservoirs. The philosophy behind the approach is that control of the reservoir will prevent subsequent human exposure, regardless of the transmission route or vehicle. By collating results from surveillance programs that are in place and comparing these to cases of human illness, the method provides added value to data that are already being collected.

Outbreak investigations give public health officials important information about immediate control of individual events. In many countries surveillance of outbreaks is undertaken and summaries are available at an international level. Records over many years provide a relatively detailed dataset, making outbreak data attractive also for use in attribution models. The foods implicated in causing human disease can be assessed using aggregated data from many outbreak investigations and the most common food vehicles involved can be identified, with the caveat that the source of human infection is often not identified in a significant proportion of outbreak episodes. Although source attribution using outbreak data is a promising approach, there are gaps in the datasets available at EU level.

Case-control studies of sporadic infections are a valuable tool to identify relevant risk factors to human food-borne infections, including sources of exposure and predisposing, behavioural or seasonal factors. By calculating the population attributable fractions, the relative importance of the different risk factors can be estimated. A primary limitation of the method is the accuracy of the recall about exposures from interviewed participants, which can lead to either an over- or under-estimation of the contribution of specific sources. In addition, many participants will need to be enrolled in order to have sufficient statistical power to determine the importance of common exposures.

Given the current data limitations, it is concluded that comparative exposure or risk assessment between major categories (food, direct animal contact, environments, person-to-person) needs further development and more data to be ready for decision support purposes. However, within the food category, comparative analysis of different transmission routes and sources is feasible if sufficient data is available.

Expert Opinions have always been used for source attribution, and recently more explicit, quantitative methods have been introduced. Experts are able to combine and weigh data from the different approaches as discussed above for which currently no analytical methods exist. Protocols to reduce bias in expert estimates have been developed in other areas of risk assessment but have not yet been fully applied to source attribution.

Although a variety of approaches have been used to better define the source of foods responsible for human infections, none of these approaches is likely to be sufficient on its own. Comparing and compiling results from more than one method may improve robustness.

For source attribution, there is a need for harmonization and structured categorization of food items taking into account the legal definition of water as food. Ideally, harmonisation and categorisation should be based on both the food commodity and the processing/preservation methods in order to gather data by various countries/organisations/research teams that are comparable and to enable exchange of data. The implicit conclusion, therefore, is that the scientific and accurate attribution of food-borne illnesses to specific foods requires developing a comprehensive program that combines many of the discussed methods and data. Such a system can be achieved with increased resources and cooperation among food safety institutions.

Data gathering for purposes of attribution should be question driven and by representative sampling. Baseline studies, as carried out under the Zoonoses regulations, are an important move in the right direction. Similarly, a common approach to epidemiological studies is recommended. Several recommendations for data requirements related to the different source attribution methods are given.

Key words: source attribution, reservoir attribution, microbial subtyping, outbreak summary data, epidemiological studies, comparative exposure or risk assessment, structured expert opinions.

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BACKGROUND AS PROVIDED BY EFSA

One of the main objectives of epidemiology as a discipline is to implicate sources and uncover reservoirs and vectors of human illness. In recent years, efforts to quantify the (relative) importance of specific food sources and animal reservoirs for human cases of food-borne illness have been gathered under the term "human illness attribution". For food safety policy, it is important to know the fraction of the total incidence of enteric pathogens attributable to foods, and which foods are contributing to that fraction. This is a difficult process that can be based on different information sources that do not necessarily give the same answers.

During discussions with the Commission regarding the most appropriate way in which to answer a request for an opinion on a quantitative microbiological risk assessment on *Salmonella* in meat[3], where EFSA was asked for the relative contribution of different meat categories, such as carcasses, fresh meat and products thereof, minced meat and meat preparations to cases of food-borne *Salmonella* infections in humans, it has become apparent that there is a need for a document reviewing the methodologies available for carrying out source attribution and for identifying the best approaches and the data requirements.

According to Article 23 of EFSA's founding regulation, EFSA shall "promote and coordinate development of uniform risk assessment methodologies in the fields falling within its mission". Therefore, it would be timely if such a review of source attribution was prepared by EFSA. In addition to identifying the methods available, the document should indicate the types of data required for each of the methods, along with its strengths and weaknesses.

TERMS OF REFERENCE AS PROVIDED BY EFSA

ESFA requests the BIOHAZ Panel to:

- Summarise the methods available for source attribution for human food-borne illness
- Identify the strengths and weaknesses of each method
- Identify the data requirements for each of the methods

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[3] Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on a quantitative microbiological risk assessment on *Salmonella* in meat: Source attribution for human salmonellosis from meat. *The EFSA Journal* 625, 1-32.

ASSESSMENT

1. INTRODUCTION

One of the main objectives of epidemiology as a discipline is to implicate sources and uncover reservoirs and vectors of human illness. In recent years, efforts to quantify the (relative) importance of specific food sources and animal reservoirs for human cases of food-borne illness have been gathered under the term "source attribution" or "human illness attribution". For food safety policy, it is important to know the fraction of the total incidence of human illness due to enteric pathogens that is attributable to foods, and which foods are contributing to that fraction. This can be based on different information sources that do not necessarily give the same answers. In addition to the different sources of information, different authors use different categories of exposure sources. A basic structure for data evaluation is proposed in Figure 1. In this opinion, reservoirs are defined as any biological or non-biological system in which the pathogen normally lives and multiplies (Last, 1995). Transmission routes are defined as physical vectors that may transport the pathogen from the reservoir to humans. The outcome of exposure is determined by the dose-response relation, depending on the properties of the micro-organism, the host and the vector. All steps in the pathway are affected by a wide range of biological, technological and social factors (Käferstein, 2003), and control of one or more of these factors is the objective of food safety policy.

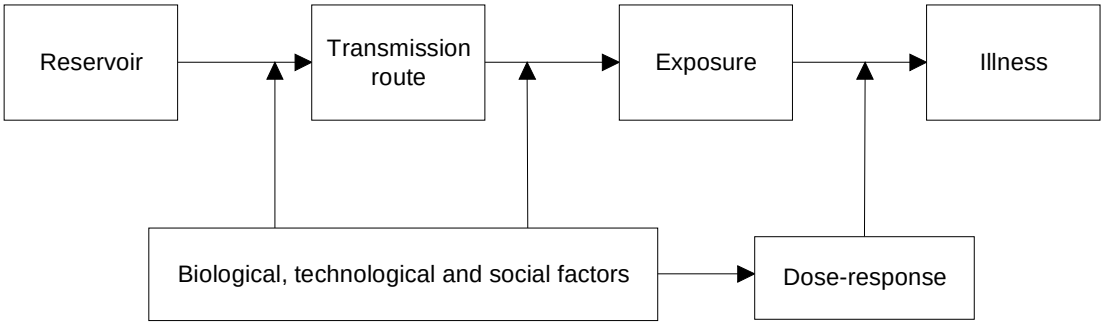

Figure 1: **Causal pathways of (food-borne) infectious diseases.**

The first interest for source attribution is to assess the total food-borne transmission compared with other major routes, such as environmental exposure, direct animal contact and human-human transmission (see Figure 2). Different authors use different definitions to delineate these categories. The Codex Alimentarius Commission definition (CAC, 2007): "Food means any substance, whether processed, semi-processed or raw, which is intended for human consumption, and includes drink, chewing gum and any substance which has been used in the manufacture, preparation or treatment of "food" but does not include cosmetics or tobacco or substances used only as drugs". Hence, this definition includes both drinking water and water used for the production, processing and preparation of foods. According to EU legislation (Regulation (EC) 178/2002), bottled water and drinking water are considered as food. However, in most outbreak investigations and epidemiological studies, drinking water (except if bottled) is included in the environmental transmission route i.e. separate from the food-borne route. Recreational water (e.g. coastal waters or lakes used for bathing) together with soil and air are also included in the environmental category. Human-human transmission may take place by direct physical contact, by pathways that involve the direct living environment, such as

spread by aerosols, by contaminated surfaces etc. Similarly, animal contact may involve direct contact or contamination through the direct living environment. There is no internationally standardised set of definitions for these pathways.

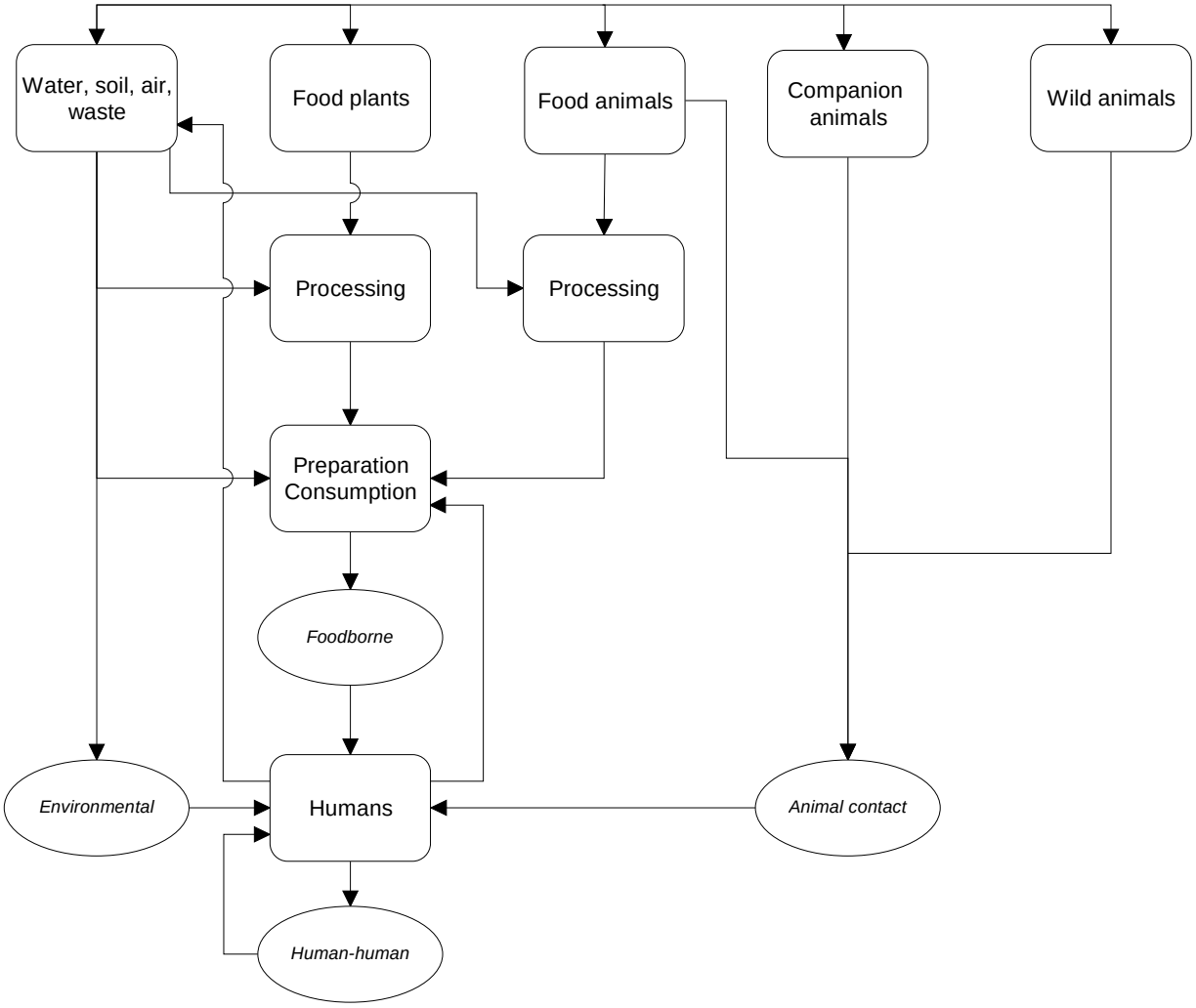

Figure 2: **Major transmission routes for enteric pathogens.** (based on Havelaar et al., in press). See text for definitions.

The task is complicated by exposure taking place in the country of residence or during foreign travel. Although exposure during travel may take place by each of the four major routes, such cases are typically considered by most Member States as a separate category, because national hygiene legislation does not impact on exposure abroad. However, in a European context, the difference between cases acquired within and outside the Community may be less relevant. For example, out of 24,803 Swedish travellers with reported salmonellosis in the period 1997-2003, 17,848 (72%) had travelled within the European region. Hence, the primary differentiation of exposure comprises four categories:

- Food-borne
- Environmental
- Direct animal contact
- Human-human

Figure 2 shows that there may be different transmission routes from a single reservoir. For example, if the reservoir of a pathogen is in food animals, transmission may be food-borne, environmental or by animal contact.

Pathogens that cause food-borne disease may enter or change in the food distribution chain at different points. Therefore, the outcome of source attribution i.e. the proportion of cases allocated to the different sources, depends on the point in the food chain at which the attribution takes place. Attribution at the point of production (farm level) may be considered to represent the reservoir of the food-borne pathogen in question, whereas attribution at the point of exposure represents the direct source of the infection. For example, attribution of Shiga toxin-producing *Escherichia coli* O157 infections at the point of production (reservoir) will partition illness to cattle, whereas attribution at the point of consumption may partition these cases further to beef and dairy products.

Attribution approaches

The successful control of any food-borne pathogen requires knowledge about the most important sources or reservoirs as well as the principal routes of transmission. There exist numerous approaches for tracing the sources of human food-borne infections. Microbiological approaches are focussed on the causative agent and involve sampling, isolation, identification and characterisation of the pathogen. By analysing and comparing data on the occurrence of the pathogen in potential sources and/or comparing pathogen subtypes isolated from humans with subtypes isolated from animals and food, it may be possible to make inferences about the sources of human infections. Application of different subtyping techniques to pathogens like verotoxigenic *E. coli* O157:H7 and *Yersinia enterocolitica* biotype 4, serotype O:3 has, for instance, revealed that the dominating reservoirs of these pathogens are cattle and pigs, respectively. The microbiological approach, consequently, requires that the pathogen is identified and has been isolated from both humans and potential sources.

In contrast, most epidemiological methods can also be applied if the pathogen is unknown and usually include analytical studies focused on the patients. Epidemiological studies, often followed by the implementation of appropriate steps of intervention, have been particularly useful for identifying sources or vehicles of certain diseases, sometimes without the causative agents being known. The most famous example is probably the work done by John Snow in 1854, where he, by epidemiological investigation, including plotting cases of illness on a map, was able to identify a water pump as the source of a major cholera outbreak in Soho. He had the handle of the pump removed, and cases of cholera immediately began to diminish. Before this study, cholera was generally believed to be air-borne. Likewise, epidemiological observations have been used to identify milk as the vehicle of a range of infectious diseases including bovine tuberculosis. Intervening in the milk-production chain by introducing pasteurisation confirmed this link.

In recent times where many of the food-borne pathogens we are dealing with have many potential sources, the task of attributing human illnesses to the responsible sources is more complicated. It requires a structured approach, where not only the most important sources and transmission pathways can be identified, but where also the relative importance of each of the sources can be quantified. Human-illness attribution should therefore include all potential sources and transmission routes and not be limited to only a single or few source-disease combinations.

There exist different approaches for human-illness attribution and many different data sources[4] that can be used, see among others Batz et al. (2005). The most commonly applied include:

1. Microbial subtyping
2. Compilation of outbreak data
3. Epidemiological observations and studies
4. Comparative exposure assessment
5. Expert opinion

To determine the overall burden of a given food-borne disease, it is of little importance to distinguish between cases from outbreaks and sporadic cases. However, when the aim is to evaluate trends and identify sources of human infections, this distinction becomes imperative. It is assumed that patients, who have not been associated with a known outbreak, are sporadic, but as our tools for identifying and tracing outbreaks are constantly improving, this distinction is becoming increasingly difficult. For example the reporting of more diffuse and geographically widespread outbreaks are increasing, which to some extent can be explained by increasing international trade and distribution of food, but also by improved detection of such outbreaks. However, detection is very dependent on the surveillance systems in the affected countries, meaning that the apparent geographical distribution and extent of a multi-state outbreak may be biased and the true number of illnesses virtually unknown (Ammon and Tauxe, 2007). Consequently, point-source outbreaks and continuous outbreaks confined to a well-defined geographical area are generally simpler to track, than to track often multiple sources of diffuse outbreaks and of sporadic infections.

Both microbiological and epidemiological approaches are useful for tracking sources of outbreak-related and sporadic cases, but the way in which they are used differs.

Outbreaks are often detected regionally or nationally due to a rapid increase in the number of reported cases in a certain geographic area and/or because of an increase in the number of isolates of a certain pathogen. The epidemiological investigation aims at identifying the specific source, where microbial subtyping results can be included as an essential part of the hypothesis-generating phase and/or to support the results of the epidemiological investigation. For pathogens that are clonally disseminated through the food-production chain (e.g. *Salmonella*), subtyping is most suitable for generating hypotheses about the original reservoir of the source (e.g. pigs) and less useful for pinpointing the actual food product (e.g. sliced ham). This is because strains originating from a particular reservoir at the end of the food processing may end up in many different food products. In such situations, the subtyping results can only serve as support to the results of an epidemiological investigation, but they are in themselves insufficient. In contrast, the use of subtyping in outbreaks caused by pathogens with a relatively unstable DNA is most suitable for pinpointing the source. This is because the chance of finding the outbreak strain in a food product unrelated to the outbreak will be very small. However, to identify the source in the first place and subsequently to isolate the pathogen often requires an epidemiological investigation (e.g. a case-control or a cohort study).

The use of subtyping for tracing sources of sporadic infections requires an extensive collection of representative isolates from all major sources and humans. Even if this is obtainable, typing may still not be very useful if the subtypes of the pathogen are distributed homogeneously among the potential sources and in humans. Consequently, other approaches including analytical epidemiological studies are necessary.

[4] The different types of studies are defined in the Glossary

Retrospective cohort studies are commonly applied in the investigation of point-source outbreaks, whereas case-control studies may be used to test hypotheses of sources to both sporadic cases as well as to continuous and diffuse outbreaks, where there is an ongoing transmission, but where a study population cannot be readily defined. However, the use of case-control studies, particularly for sporadic cases, may become very extensive and laborious, and the results are often difficult to interpret and extrapolate to the entire population. The real challenge is therefore to trace the sources of sporadic infections (or infections associated with diffuse outbreaks).

Most authors use more than one information source to arrive at (numerical) estimates of the proportion of food-borne cases and the major contributing foods. A frequently cited paper by Mead et al. (1999), estimated the incidence of food-borne illness in the USA in the nineteen-nineties, mainly from outbreak data, but supplemented with epidemiological studies (case series and case-control studies) and unstructured expert opinion. Adak et al. (2002) based their estimates for the UK (data for 1992-2000) on outbreak data only. Van Duynhoven et al. (2002) based their estimates for the Netherlands (data for 1990s) on unstructured expert estimates, published previously by the Health Council of the Netherlands (Gezondheidsraad, 2000), supplemented with more recent data from case-control studies on rotavirus, norovirus, sapovirus (De Wit et al., 2003) and *Giardia lamblia*. Vaillant et al. (2005) for France (data from 1990s), Hall et al. (2005) for Australia (data for 2000) used unstructured expert estimates that were informed by the previously published studies and Cressey et al. (2005) used a modified Delphi procedure with facilitated discussion for expert elicitation.

Complete studies that break down transmission routes for specific pathogens in the above mentioned categories are not available in the literature. Some authors have provided estimates of the fraction of food-borne illness. Table 1 gives an overview of published (international) data.

After estimating the proportion of all cases that is food-borne, the next task is to divide the fraction of food-borne infection into specific food sources.

Existing categorisations for foods are not helpful in this context. Different authors on source attribution have used different categorisations making comparisons difficult. Harmonization of categories is essential when data is collected from different sources and when results from more than one study are to be compared or integrated. A classification that is both general and suitable for the use of the different attribution methods and for a variety of pathogens is needed.

Many of the approaches described in this report, especially microbial subtyping and case-control studies, are efficiently used to identify the source of specific cases or outbreaks in a country on an *ad hoc* basis but this does not necessarily mean that it is used continuously and systematically as a general method to attribute human illness to different sources or reservoirs. In this report we discuss the appropriateness of different methods to be used as methods for attributing human food-borne illness to sources and reservoirs in general and not as methods to, for example, trace the source in a specific outbreak.

Table 1: **Estimated percentages of cases of microbial hazards that are attributed to food.**

Reference[&]	Mead	Adak	Duyn	Valliant	Hall	Cressey	Havelaar
Country	USA	UK	NL	France	Australia	New Zealand	NL
Period	1990's	1992-2000	1990's	1990's	2000	2004	2006
Data sources*	E/O/R	O	E/CC	E	E	E	E
Travel-related cases	Included	Excluded	Included	Included	Excluded	Included	Excluded
Aeromonas spp.		0%			25%		
Brucella spp.	50%			50%			
Campylobacter spp.	80%	80%	30-80%	80%	75%	58%	42%
Escherichia coli							
STEC O157	85%	63%	50-90%	50%	65%	40%	40%
STEC non-O157	85%				65%		42%
Enterotoxigenic	70%						
Other diarrheogenic	30%	8%			50%		
Listeria monocytogenes	99%	99%		99%		85%	69%
Mycobacterium avium							42%
Mycobacterium bovis						28%	
Salmonella spp.	95%	92%	>90%	95%	87%	61%	55%
Salmonella Typhi	80%**	80%[$]		80%			
Shigella *spp.*	20%	8%		10%	10%		
Streptococcus, food-borne	100%						
Vibrio cholerae, toxigenic	90%	90%					
Vibrio vulnificus	50%						
Vibrio parahaemolyticus					71%	89%	
Vibrio spp., other	65%	65%		100%			
Yersinia enterocolitica	90%	90%		90%	75%	56%	
Bacillus cereus toxin	100%	100%	100%	100%	100%	97%	90%
Clostridium botulinum toxin	100%			100%			
Clostridium perfringens toxin	100%	94%	100%	100%	100%		91%
Staphylococcus aureus toxin	100%	96%	100%	100%	100%		87%
Adenovirus 40/41		0%			10%		
Astrovirus	1%	11%			10%		
Enterovirus							6%
Hepatitis A virus	50%			5%			11%
Hepatitis E virus							14%
Norovirus	40%	11%	10-20%	14%	25%	40%	17%
Rotavirus	1%	3%	0-10%		2%		13%
Sapovirus		0%					
Cryptosporidium parvum	10%	6%	??		10%		12%
Cyclospora cayatenensis	90%	90%					
Giardia lamblia	10%	10%	<30%		5%		13%
Toxoplasma gondii	50%			50%		32%	56%
Anisakis simplex				100%			
Diphyllobotrium latum				100%			
Fasciola hepatica				100%			
Taenia saginata				100%			
Trichinella spiralis	100%			100%			

[&] Mead: Mead, et al. (1999); Adak: Adak et al. (2002); Duyn: Van Duynhoven et al. (2002); Valliant: Valliant et al. 2005; Hall: Hall et al. (2005), Cressey: Cressey and Lake (2005), Havelaar: Havelaar et al. in press

[*] E: expert estimates, O: outbreaks, R: reported cases, CC: case-control studies

** >70% of cases acquired abroad (i.e. outside USA)

[$] Similar estimate for S. paratyphi

2. Attribution of human illness through microbial subtyping

The critical linkage between public health surveillance data and animal and food monitoring data is possible through the extensive use of subtyping of isolated pathogens. Microbial subtyping is an umbrella term for numerous methods used to distinguish bacterial and viral isolates from one another. Microbial fingerprinting and microbial source tracking (MST) are two other terms that essentially describe the same thing.

2.1. Methodology for attributing human illness from sporadic cases

The microbial subtyping approach involves characterisation of isolates of a specific pathogen by different phenotypic or genotypic subtyping methods. One of the most important and commonly used phenotypic methods is serotyping, where bacteria within the same species are characterised by their antigenic profile. For some pathogens, phage typing may be applied. Phage typing reflects differences between two organisms with the same serotype but different susceptibilities to infection by a defined panel of lytic bacteriophages. Finally, antimicrobial susceptibility patterns may in some situations be useful, particularly if the resistance is located on genes that are clonally disseminated. Otherwise, the epidemiological importance of this typing method is secondary to the implications for therapy and control.

Genotypic typing methods include various gel electrophoresis and sequence-based techniques. An efficient technique for separation of the full bacterial genome is pulsed-field gel electrophoresis (PFGE), which makes use of enzymes that only cut the genome at a few sites ("rare cutters"). This produces large restriction fragments that are easy to compare. Generally, if the number and sizes of fragments are identical the strains are defined as belonging to the same PFGE type. However, interpretation of PFGE data (and other sequence-based typing methods) is not straight forward as the reproducibility of the method with a particular pathogen and the stability of the organism being subtyped influence the variability in the PFGE patterns. The epidemiological context in which the PFGE is applied is therefore crucial to consider. For instance for point-source outbreaks, only isolates displaying indistinguishable patterns should be regarded as outbreak associated, whereas more variability (i.e. patterns differing from each other in two to three band positions) may be quite acceptable for continuous outbreaks and for sporadic cases (Barett et al., 2006). The usefulness of the genotyping methods for human-illness attribution still needs to be demonstrated. So far only a very few research groups have attempted to apply genotypic methods for attribution purposes (see e.g. French et al., 2007). These methods are expected to be applied increasingly for this purpose in the future.

Generally, the results from serotyping and phage typing are readily comparable between laboratories, regions and countries, on the condition that the same typing schemes have been adopted. In contrast, the gel-electrophoresis techniques normally require that strains are analysed on the same gel in order to be fully comparable. However, in some countries methods to electronically transform PFGE patterns into graphics have been or are in the process of being developed (Swaminathan et al., 2006). The results are stored in central databases, which make it possible to compare PFGE results analysed on different gels and in different laboratories, regions and countries. An example of this is the pioneering PulseNet in USA (Gerner-Smidt et al., 2006), and the recently initiated PulseNet International, where several independent networks work together (Swaminathan et al., 2006).

The principle behind the microbial subtyping approach for attribution is to compare the subtypes of isolates from different sources (e.g. animals, food) with those isolated from humans. Microbial subtyping is enabled by the identification of strong associations between some of the dominant subtypes and a specific reservoir or source (i.e. animal species) providing

a heterogeneous distribution of subtypes among the sources. Such subtypes are regarded as indicators for the human health impact of that particular source, assuming that all human infections with these "indicator" 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 (Hald and Wegener, 1999). Microbial subtyping requires a collection of temporally and spatially related isolates from all appropriate sources, and is consequently facilitated by an integrated food-borne surveillance programme focused on the major food animal reservoirs of food-borne diseases.

2.2. Examples and results

Microbial subtyping has become the major tool for reservoir attribution for non-typhoidal *Salmonella* spp. and has been applied for several decades in the Netherlands (Van Pelt et al., 1999) at the National Institute for Public Health and the Environment (RIVM) and in Denmark (Hald et al., 2004) at the Danish Zoonosis Centre (DZC). Although the basic idea behind the two methods is similar, the approaches differ with regard to the statistical methods applied and the number of parameters in the model, e.g. the stochastic Danish model recognizes more food subcategories than the Dutch model, and takes into account the animal/food prevalence and the amount of food source available for consumption each year.

The RIVM is the Dutch National Reference Centre for *Salmonella* and supervises the laboratory-based surveillance of human salmonellosis. Isolates of human cases and non-human sources are submitted to the RIVM for sero- and phage typing. The Dutch approach compares the number of reported human cases caused by a particular *Salmonella* type with the relative occurrence of that type in the animal-food sources. Results of attribution modelling for the Netherlands are shown in Table 2.

Table 2. **Estimated contribution (%) of different reservoirs to laboratory confirmed salmonellosis in the Netherlands** (Van Pelt et al., 1999, Valkenburgh et al., 2007).

Reservoir	1994-98	2001-2	2003	2004	2005
Incidence of salmonellosis (per 100,000 inhabitants)*	27.4	17.7	20.7	15.8	13.2
Pig	25	22	22	23	28
Cattle	11	14	8	13	10
Chicken	21	17	12	16	16
Layers	39	34	46	31	30
Travel/other	4	13	12	17	16

* Van Pelt et al. (2003; 2006).

Throughout 1994-2005, eggs and pork were the two most important sources of human salmonellosis in The Netherlands, accounting for up to two-third of all cases in 2003. The attribution of the various sources was relatively stable over this period. Note that there is an overall decreasing trend of the incidence of salmonellosis in the Netherlands, with the exception of 2003. In this year, the proportion of cases attributed to eggs was also exceptionally high (46%). These findings could not be explained by an unusually hot summer and was explained by an increased import of eggs contaminated with *Salmonella*, as a side effect of an avian influenza outbreak in the spring of 2003 (Van Pelt et al., 2004).

The Danish Zoonosis Centre has for the past decades produced annual estimates of the number of human *Salmonella* infections attributable to the various food animal sources based on a model using microbial subtyping results (Figures 3 and 4). During this period, the validity of

the estimates produced by the attribution model has been improved considerably. The *Salmonella* surveillance programmes have been gradually extended, resulting in more abundant data and the application of computer-intensive methods has made it possible to move from a deterministic to a stochastic approach. The output of the model in 2005 (Figure 3) shows that pork and imported chicken, along with table-eggs, are the major sources of human salmonellosis in Denmark. Imported and domestically produced beef, as well as domestically produced broiler meat, each account for a minor proportion of the human cases.

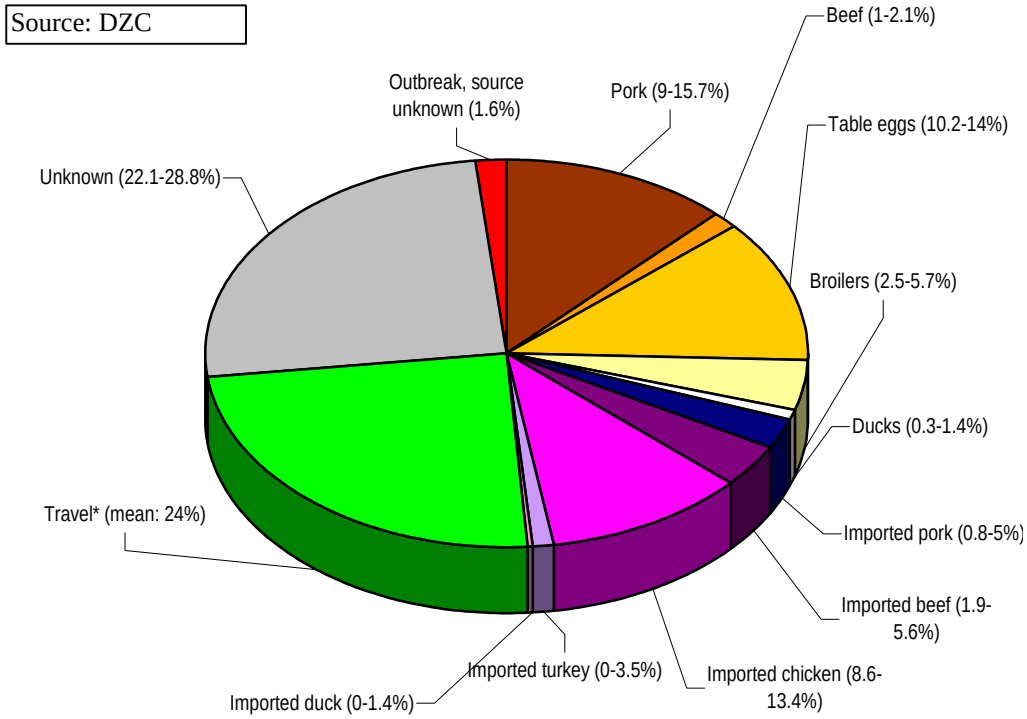

Figure 3. **Estimated major sources of human salmonellosis in Denmark in 2005** (Anonymous, 2006).

Source attribution using microbial subtyping has proved to be a valuable tool to inform risk managers in Denmark, providing evidence for the need for initiating food safety initiatives as well as monitoring the effect of control programmes in place (Figure 3). Figure 4 shows that Denmark has experienced three waves of human salmonellosis each associated with a different major source: broiler meat in the end of the 80's, pigs/pork in the mid-90's and table-eggs in the end of the 90's. At each peak a new or revised control programme was implemented resulting in a decline in the number of human cases that could be associated with that source.

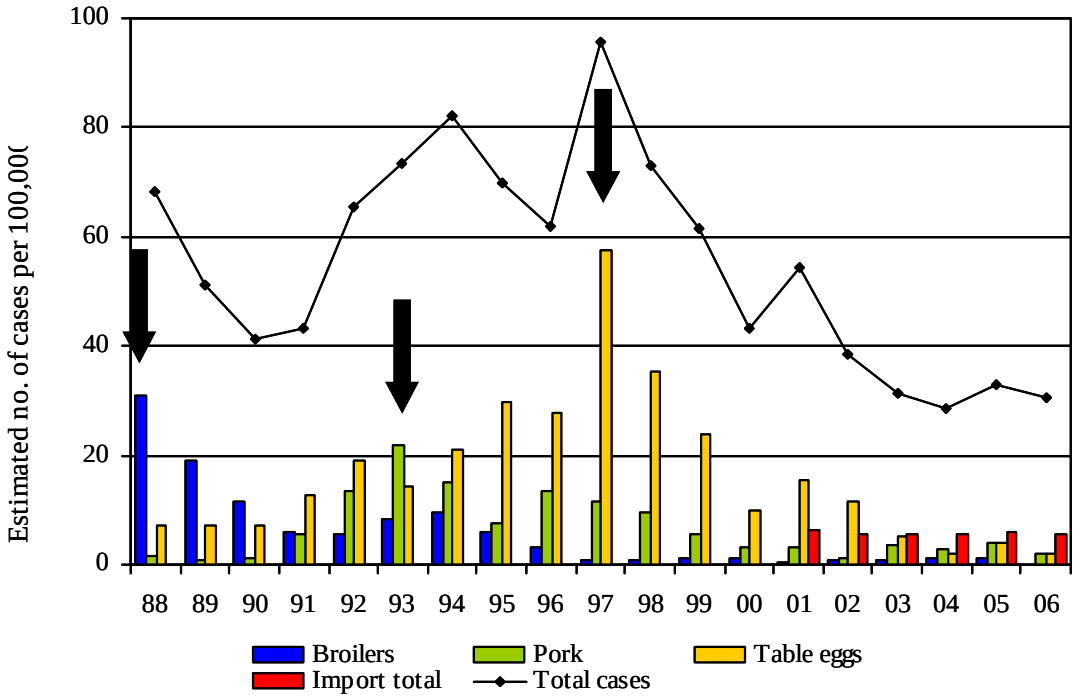

Figure 4. **Trends in the attribution of major sources of human salmonellosis in Denmark 1988-2006** (Imports only included from 2001 onwards) (Anonymous, 2007).

The application of different subtyping techniques has also revealed important reservoirs of other food-borne pathogens including verotoxigenic *E. coli* O157:H7 and *Yersinia enterocolitica* biotype 4, serotype O:3, which have been traced to the ruminant and porcine reservoirs, respectively. For *Campylobacter*, a suitable subtyping method has been difficult to identify, as *Campylobacter* appears to consist of innumerable clones that are only vaguely associated with specific reservoirs/sources. However, the application of multilocus sequence typing (MLST) of *Campylobacter* isolates from foods and humans may be the way forward as indicated by the preliminary results from New Zealand, where this approach is currently being developed (French, 2007). For pathogens such as *Listeria monocytogenes*, it is well recognised that contamination primarily arises in the production process, post harvest. This means that even if strains of *L. monocytogenes* can be isolated from the reservoir level (i.e. farm level), attribution at the reservoir level makes little sense.

2.3. Data requirements

Ideal data set

The application of source attribution through subtyping requires an integrated surveillance of most major sources (e.g. food animals and food) and humans providing a collection of representative isolates from animal/food sources and humans, followed by the use of appropriate discriminatory typing methods. Furthermore, it is important that large outbreaks are detected. The reason is that unrecognised outbreaks caused by types occurring in only one or few sources will tend to overestimate the total number of infections originating from the reservoir harbouring this type, whereas unrecognised outbreaks caused by homogeneously distributed types will tend to underestimate the total number of infections from the reservoir in

question. Inclusion of information regarding the amount of animal-food product available for consumption improves the quality of the results and is particularly useful for interpreting the results e.g. whether a certain reduction in the number of cases associated with a particular source is a result of risk management actions or due to reduced consumption. Finally, it may also be most relevant to obtain information about the number of travel-related cases, as these obviously can not be attributable to a domestically produced or imported food source.

It is emphasized that it is important to have an in-depth understanding of the data that are used for this approach i.e. the data that serve as input to the model. Knowledge of the epidemiology of the pathogen in the study population (animals, food and humans) and the surveillance systems (e.g. epidemiological units, representativeness of the samples, etc.) in which the relevant data are collected is equally important. The results of a single model, for instance using only a single year's data, should be looked at with a critical eye and the model should be applied repeatedly to increase the confidence in the model parameters.

As the approach attributes sources at the reservoir level, data collected as close as possible to the point of production (e.g. farm or slaughterhouse) should be given preference. Still, the data should, to the widest extent possible, reflect what the human population is exposed to. In that perspective, it is recommended that representative data should be chosen over amount of data. Moreover, clustered data should be avoided, meaning that a precise and sensible epidemiological unit should be defined (flock, batch or sample, whichever is most relevant). Active surveillance data is preferable to passively acquired data, and the use of results from veterinary diagnostic submissions should be avoided, as those data are not representative of the human population exposure.

Existing data set

The availability and representativeness of subtyping data from animal and/or food and human sources differ greatly between Member States (MS) of the European Union (EU). This is reflected by the varying levels of detail of the official prevalence data that are being reported to EFSA and ECDC. However, this does not mean that more detailed data are not available for some MS that were involved in national and international research projects. Currently, EFSA is carrying out baseline studies where MS collect data on the occurrence of specific food-borne pathogens in specific food animal production types according to a standardised protocol (e.g. *Salmonella* in layers, broilers and pigs). Results from these studies provide not only comparable prevalence estimates, but also a means for adjusting the official data reported by the MS. Unfortunately, the subtyping of isolates i.e. phage tying and antimicrobial resistance testing, is not obligatory and performed on a voluntary basis. ECDC is receiving data of human food-borne illness from various existing networks such as the Basic Surveillance Network (BSN) and two dedicated surveillance networks (i.e. Enter-Net and Euro-TB). Unfortunately, subtyping data is not abundantly available at the discriminatory level that is required for human illness attribution modelling.

Recommendations for improving the available data

- Collection of representative *Salmonella* isolates from the major reservoirs/sources obtained through monitoring of animals and food (e.g. as part of the Zoonosis Directive) and humans.
- Application of discriminatory and definitive epidemiological marker methods providing knowledge of the distribution of the different subtypes in the major reservoirs/sources including:

  - Serotyping for all *Salmonella* isolates
  - Phage typing of *S.* Enteritidis and *S.* Typhimurium isolates and/or application of other methods that can distinguish between strains within these serovars e.g. antimicrobial susceptibility testing.

- Application of new subtyping methods for *Salmonella* attribution e.g. multiple locus variable number tandem repeats analysis (MLVA). MLVA is increasingly used for surveillance of human *S.* Typhimurium infections and tracing of outbreaks, but its usefulness for source attribution needs to be explored.
- Collection of data from additional sources for pathogens like *Campylobacter* and VTEC, where focus mainly has been on broiler chickens and cattle, respectively.
- Development and or application of other typing techniques for source attribution of other pathogens e.g. use of multi-locus sequence typing (MLST) for *Campylobacter* spp. Definition of meaningful subtypes of *Campylobacter* and VTEC based on these subtyping techniques.

2.4. Strengths and weaknesses

When the microbial subtyping approach is applied at the point of production, it allows the identification of the most important reservoirs of the specific pathogen. Since pathogens can be transmitted through a variety of sources, interventions that control the pathogen at the reservoir level, before the dissemination of the pathogen to numerous transmission pathways, will result in important declines in human infections. Therefore, attribution at the reservoir level is particularly useful in prioritisation of food safety interventions.

A limitation is that the method is constricted to clonally disseminated pathogens that are heterogeneously distributed among the reservoirs. The fact that the attribution is being made at the reservoir level also means that the different pathways through which the pathogen can be transmitted to humans are not investigated. However, attributing human infections to the primary source reduces uncertainty due to cross-contamination at any point on the transmission pathway and, therefore, the risk of attribution to the wrong source. Moreover, the method could be complemented with the exposure assessment approach (see 6.4), as the blending of the two methods and of their strengths is thought to be useful for risk management and for the prioritisation of control strategies.

So far, the approach has been applied to the attribution of reported cases only. If one should consider making attribution for the total number of (estimated) cases (e.g. obtained from risk assessments or disease burden estimates), bias may be introduced due to higher reporting rates for more severe cases of human illness, which may be related to more virulent serotypes.

The Danish model, as described above, attributes the number of human *Salmonella* infections caused by different *Salmonella* subtypes (serotypes and phage-types) as a function of the prevalence of these subtypes in animal and food sources and the amount of each food source consumed. The differences in abilities of the *Salmonella* subtypes to cause human disease (q) and of the food sources to act as a vehicle for infection (a) are accounted for in a multi-parameter prior. Posterior distributions for these factors are estimated by fitting the model to the reported number of cases per *Salmonella* subtype per year using a Bayesian framework with Markov Chain Monte Carlo (MCMC) simulation. Bayesian inference and MCMC requires good statistical skills for application and monitoring of convergence. Otherwise misinterpretation and wrong results may result. However, by using good modelling practice MCMC is an extremely powerful tool and its use will continue to increase in the future.

2.5. Conclusions and future prospects

Attribution through microbial subtyping requires integrated surveillance of the pathogen in most major (food) animals, food and humans, providing a collection of isolates representing what the human population is exposed to through food, direct contact or the environment (including water). This is then followed by the use of appropriate discriminatory typing methods (Hald et al., 2004). For several pathogens other reservoirs than the food-related, such as pets or wild animals, may be important. In that case, data on the distribution of the pathogen types in the different reservoirs is required.

The method has provided guidance to risk managers and policy makers on the implementation and evaluation of control strategies for major reservoirs. The philosophy behind the approach is that control of the reservoir will prevent subsequent human exposure, regardless of the transmission route or vehicle. By collating results from surveillance programs that are in place and comparing these to cases of human illness, the method provides added value to data that already are being collected.

3. Using summary outbreak data for source attribution

Outbreaks occur when food safety measures fail at any point (or points) along the chain from primary production to the consumer. Standard textbook definitions of outbreaks tend to be either:-

- two or more related (i.e. epidemiologically linked) cases of a similar disease. A typical scenario is a group of people suddenly becoming unwell with diarrhoea and vomiting after e.g. a wedding reception or large party. Cases are linked in place and time, by clinical features and, after an outbreak investigation, by food exposure.
- an increase in the observed incidence of disease over the expected incidence. This implies that there is ongoing public health surveillance through which the expected level of disease can be determined and that analysis of new data in real-time shows an increase above baseline levels. Outbreaks might be localised or dispersed geographically. Outbreaks due to nationally or internationally distributed contaminated foods are typically identified in this way.

In practice, outbreaks are detected in a number of ways e.g. by complaints from members of the public, through surges in admission to hospital or, typically, through laboratory-based surveillance of food-borne pathogens. Undertaking outbreak investigations gives public health officials important information about immediate control of individual events. In many countries surveillance of outbreaks is also undertaken and summaries are available at an international level. Records over many years provide a relatively detailed dataset, making outbreak data attractive for use in attribution models. The foods implicated in causing human disease can be assessed using aggregated data from lots of outbreak investigations. Epidemiologists can evaluate trends and identify the most common food vehicles involved. Furthermore using data from outbreaks, as well as peer-reviewed journals, means that frequently occurring food vehicles can be identified – the greatest health gains are made by tackling common problems.

3.1. Methodology

The first step in a food attribution model using outbreak data is to calculate the number of cases of infectious intestinal disease by pathogen. This is simply the number of laboratory-confirmed cases by pathogen multiplied by the ascertainment ratio (multiplier) for each pathogen (where available). In some countries there is sufficient information held on foreign travel to allow the

proportion of disease acquired aboard to be subtracted from the total. Using the outbreak dataset the next step involves calculating the percentage of food-borne transmission for each pathogen. This percentage is applied to the number of cases of infectious intestinal disease in order to give the number of cases of food-borne infectious intestinal disease by pathogen. It is then possible to partition the pathogen-specific number of cases of food-borne infectious intestinal disease by examining the proportion of cases in the outbreak dataset that are attributable to different food types. The level of resolution about individual foods will depend on the amount of detail captured about food types in the outbreak surveillance system e.g. "beef" as opposed to "ground beef"; "quiche" as opposed to "ham, onion and mushroom quiche". Adding up the pathogen-specific estimates of food-borne disease by food type yields the food attribution estimates. To express these in risk terms, for example, the risk per million servings means dividing the number of cases of food-borne disease by food type, by the number of servings (if known). The levels of uncertainty in the data and in the processes of estimation and extrapolation may be incorporated into the final model, using appropriate statistical techniques.

An example of the output from a study by Adak et al. (2005) is shown below.

Table 3: **Outbreak data by food vehicle: General outbreaks of infectious intestinal disease involving one food vehicle, England and Wales.** (Source: Adak et al., 2005)

Food group	**All salmonellae**	***Campylobacter***	**Other bacteria**	**Viruses**	**Protozoa**
	Number of outbreaks (**%** to nearest whole number)				
Poultry	108 (23)	15 (54)	49 (25)	4 (6)	0 (0)
Red meat	51 (11)	0 (0)	83 (42)	0 (0)	0 (0)
Eggs	69 (14)	0 (0)	0 (0)	0 (0)	0 (0)
Seafood	19 (4)	1 (4)	2 (1)	23 (36)	0 (0)
Milk	8 (2)	6 (21)	9 (5)	0 (0)	1 (100)
Other dairy products	4 (1)	0 (0)	2 (1)	0 (0)	0 (0)
Vegetables/fruit	10 (2)	1 (4)	5 (3)	6 (9)	0 (0)
Rice	4 (2)	0 (0)	12 (6)	0 (0)	0 (0)
Complex foods	202 (42)	4 (14)	32 (16)	11 (17)	0 (0)
Infected food handler	3 (1)	1 (4)	1 (1)	20 (31)	0 (0)
Total	478	28	195	64	1

3.2. Examples and results

Two major international studies have primarily relied on outbreak data for attribution to food categories. Adak et al. (2005) extracted from the UK national surveillance dataset outbreaks reported as food-borne that involved a single vehicle of infection, identified by epidemiological and/or microbiological investigations. Outbreaks in which investigators implicated either no food vehicle or more than one food, and those within which no pathogen was confirmed by laboratory testing, were excluded. Foods were classified into broad categories, such as poultry, and more specific food types, e.g. chicken. The percentage of outbreaks due to each food type for each pathogen, the pathogen-specific totals by food-type, the food-specific totals for all disease and the risk expressed as cases per million servings were calculated. In these analyses

contaminated chicken was consistently responsible for a considerable proportion of food-borne disease.

The Food-borne Illness Risk Ranking Model (FIRRM) (Food Safety Research Consortium, 2004) is largely based on US outbreak data collected by CDC and collated by the Center for Science in the Public Interest (www.cspinet.org/foodsafety/index.html). The FIRMM attribution data are more detailed than those used by Adak et al. (2005), who distinguish only 5 groups of micro-organisms (*Salmonella*, *Campylobacter*, other bacteria, viruses and protozoa) and a smaller number of food categories. In the US a large proportion of outbreaks is attributed to multi-ingredient and to multi-source outbreaks. As a consequence, attributable fractions for specific food groups are typically lower in the US than in the UK dataset. For many pathogens, however, the proportion of cases attributed to produce (vegetables, including salad, and fruit) is higher in the US than in the UK, as is the proportion of cases of STEC O157 attributed to beef.

How to handle complex foods is a key decision in food attribution using outbreak data. The problem arises because contaminated food vehicles often comprise multiple ingredients, any one of which might have been the original source of contamination. The classic example is an outbreak of *S*. Typhimurium in which a ham quiche is implicated. Quiches tend to contain egg, milk or cream, cheese and ham in a pastry case. Any one of these might have been a plausible source of contamination. Unless there is microbiological corroboration of the organism in a source ingredient, to which food group would you ascribe the organism – pork meat, dairy products or eggs? The danger is of being biased by biological plausibility. To overcome this, Adak et al. (2005) created a "complex foods" group to accommodate dishes consisting of ingredients of various food types in which the precise source of infection was not verified. It remains to be seen how other groups will cope with this.

3.3. Data requirements

Ideal data set

In an ideal world, MS would be able to describe the true burden of food-borne disease i.e. they would be able to extrapolate from their laboratory-based surveillance data to the total burden of illness because they would have calibrated their national surveillance systems. This would give a good picture of the burden of food-borne illness across the EU. Outbreaks would be investigated consistently and comprehensively using analytical epidemiology combined with clinical and food microbiology that pinpointed not only the contaminated food vehicle(s) but also the contaminated source ingredients. Food vehicles would be described in specific, not generic terms e.g. "minced/ground raw beef" as opposed to "beef". Outbreak surveillance datasets would contain sufficient denominator data (on the population at risk) to enable attack rates to be calculated. All outbreaks of infectious intestinal disease would be captured, whatever the route of transmission, to enable better estimates of the proportion of food-borne disease transmission by pathogen, as well as the implicated foods. There would be consistent data on the settings in which outbreaks occurred and the results of environmental health investigations would have been recorded so that the food handling faults that had contributed to an outbreak were transparent.

Existing data set

Although source attribution using outbreak data is a promising approach, there are large gaps in the datasets available at EU level. Despite substantial data on food-borne outbreaks, not all information contributes to gaining insight into the importance of various food-borne pathogens, outbreak settings and contributing factors at a Community level because almost three quarters

of all food-borne outbreaks are reported on a yearly basis in summary form rather than in the form of raw data. These data still provide information on the total number of people involved, hospitalisations and deaths, but may not inform on the number of human cases that can be assigned to individual sources and locations in these reported outbreaks.

The reporting of outbreaks is facilitated by the availability of structured harmonized networks for reporting. For example the establishment of the food-borne viruses in Europe (FBVE) network database in 1999 to monitor trends in outbreaks of gastroenteritis due to noroviruses (NoV) increased the rates of reporting of NoV outbreaks. The completeness of the dataset on NoV outbreaks has also increased. However, further harmonization of the surveillance systems on Norovirus is needed and countries that do not participate at present need to be encouraged to join (Kroneman et al., 2008). The availability of detection methods, preferably internationally standardized methods, is a prerequisite for surveillance of food-borne outbreaks. This is still often a challenge with emerging pathogens such as protozoa or non-O157 EHEC for which available outbreak data will rely heavily on the availability of an expert laboratory to detect the pathogen.

Recommendations for improving the available data

A major step forward would be to work towards a minimum dataset to be collected in each MS and to encourage the reporting of disaggregated data to EFSA. To make this possible, it is essential that surveillance experts in each MS be closely involved with the analysis and subsequent interpretation of the dataset, since they are best placed to understand the biases in their own, and hence the aggregated data.

Although the level of detail of the data currently gathered at the European level is not sufficient to allow this approach to answer the ToR, recent developments at EFSA and ECDC should improve the data gathered in the future (EFSA, 2007a, b; ECDC, 2008).

3.4. Strengths and weaknesses

Using outbreak data for disease attribution has certain advantages. It is possible to take into account foreign travel, as well as avoiding assumptions inherent in the use of expert elicitation and publication bias. A major advantage is that outbreak data are observed at the public health endpoint and can, therefore, be considered a direct measure of attribution. However, outbreak data on certain pathogens are often limited, and it is difficult to deal with complex foods (Adak et al., 2005).

Extrapolating information from outbreak datasets in an attempt to describe food-borne disease burden is not straightforward. A major limitation is investigation bias. Large outbreaks, outbreaks associated with the food service and institutions, and outbreaks that have a longer duration or cause serious disease are more likely to be investigated and reported (O'Brien et al., 2002). Thus, the data may not reflect what occurs in sporadic cases.

A second major limitation is that the method assumes that the relative pathogen-specific contribution of each food type to both sporadic and outbreak associated disease is similar and, therefore, that the outbreak experience can be generalized to sporadic disease. However, certain vehicles may be more likely to be implicated in outbreaks than others, especially if investigators preferentially collect data on the types of food perceived as high risk, or when laboratory methods vary in sensitivity according to food type. A systematic vehicle detection bias might underestimate the contribution and risks attributable to foods less commonly implicated in outbreak investigations, e.g., salad items, fruit, or background ingredients such as herbs and spices.

A third limitation is that in many outbreaks it is not possible to find an etiological agent and/or identify a source of infection. D'Aoust (2000) published a detailed overview of *Salmonella* outbreaks, but published outbreaks are a biased fraction of all outbreaks. In a review of 1,763 outbreaks of food-borne disease in England and Wales, in which food vehicles reported to a systematic surveillance system were compared with those published in the peer-reviewed literature, publications in the peer-reviewed literature favoured the unusual food vehicle or novel event (O'Brien et al., 2006). This is not entirely surprising given the mission of peer-reviewed journals but it might also influence expert judgments, and hence expert reviews.

3.5. Conclusions and future prospects

Although source attribution using outbreak data is a promising approach, there are large gaps in the datasets available at EU level.

4. Epidemiological studies for source attribution of sporadic cases

4.1. Methodology

Identifying sources of sporadic infections can be performed using analytical epidemiological studies (e.g. case-control or cohort studies), which involve interviewing persons with and without the infection, and case-series studies, which require only interviewing persons with the infection. Cohort studies are used less often for attribution of sporadic food-borne infections, as they usually require interviewing more persons than practical, most of whom would likely not be infected.

Case-control studies are a valuable investigative tool, providing rapid results and being less expensive than cohort studies, but caution should be exercised unless results are confirmed by other evidence. In some studies, case-series instead of case-control studies are used, particularly to estimate the fraction of travel-related cases.

Case-control study is defined as an analytical epidemiologic study design in which individuals who have the disease under study, also called cases, are compared to asymptomatic, and therefore assumed to be free of disease, individuals (controls) regarding past exposures. The relative role of exposures is estimated by comparing the frequency of exposures among cases and controls. When infections are associated with an exposure, the proportion of cases attributed to that exposure can be calculated and is defined epidemiologically as the "population attributable fraction" (PAF) (Clayton and Hills, 1993).

A case-control study can be expanded to attribute the human disease burden of food-borne infections to specific sources; an example is the recently published study for sporadic *Campylobacter* infections in Australia (Stafford et al., 2008). In addition to individual case-control studies, a systematic review of published case-control studies of sporadic infections of a given food-borne disease can provide a summary of the estimated population attributable fractions for each exposure, and this can be combined for overall estimates of the burden of illness caused by that pathogen attributed to each exposure.

Case-series studies of sporadic infections are commonly conducted, particularly for uncommon diseases that are frequently associated with a given exposure, which, by its turn, is uncommon among the general population.

Another epidemiological approach is the intervention study. Intervention studies are the "gold standard" in epidemiological research, particularly if a randomised, double-blind design is applied. Intervention studies can be designed as small-scale (e.g. at farm level) or larger-scale

(e.g. interventions at a national level) studies to control a certain food-borne disease. For example, the 1999 dioxin crisis in Belgium could be considered as an intervention (Vellinga and Van Loock, 2002); the use of a disaster as an epidemiologic tool offers a unique opportunity to observe exceptional changes in the occurrence of infections or other diseases. The causes or consequences of the crisis can serve as treatment in an uncontrolled natural experiment.

4.2. Examples and results

Case control and other epidemiological studies of sporadic laboratory-confirmed cases have been conducted to attribute the proportion of food-borne diseases that are caused by specific foods of food preparation and handling practices. Table 4 summarises the results of case-control studies of sporadic cases of illnesses caused by different microorganisms, published in the peer-reviewed literature since 1995, in which particular food groups or food items were identified as risk factors for illness (Medline search terms = "*Microorganism specie*" and "sporadic" and "case-control-study").

4.3. Strengths and weaknesses

Case–control studies have several advantages and limitations as an analytical epidemiology tool. Those strengths and weaknesses are summarised as follows:

Strengths of case-control studies:

1. *Good for rare diseases, illnesses with long latency or with a poorly defined source population*
2. *Requires relatively little time to conduct and are relatively inexpensive*
3. *Retrospective, uses existing data*
4. *Possibility of exploring multiple exposures.*
5. *Captures risk factors not commonly included in surveillance data e.g. contact with pets or behavioural factors.*

Weaknesses:

1. *Reliance on recall and/or historical data on exposure, which can lead to misclassification of exposure (recall bias).*
2. *Temporality can be difficult to establish:* The chronologic order of the exposure and disease, which is easy to elucidate in prospective cohort studies, may be uncertain from the results of a case–control study because it may not be possible to know if the exposure occurred before the disease.
3. *Difficult to select or find controls that are representative of the study population (selection bias)*
4. Confounding and bias
5. *Problem with common exposures, particularly if the allowed exposure window is long.*
6. *Misclassification of the outcome i.e. case detection (e.g. immunity in population can impede associating exposure with illness.*

Table 4: **Examples of case-control studies of food-borne human illness considering different foodstuffs as independent risk factors.**

Year(s) of study	Country	Biological agent investigated	Cases: Controls	Food implicated as an independent risk factor	Population Attributable Fraction	Reference
2002-2004	US (8 FoodNet Sites)	Non-typhoidal salmonellae	442: 928 infants <1 year of age	Meat - type not specified	8% overall	Jones et al. (2006)
2002-3	US (8 FoodNet Sites)	Highly resistant *Salmonella* Newport-MDRAmpC	215: 1154	Uncooked ground beef	4.6%	Varma et al. (2006)
2002-2003	US (8 FoodNet Sites)	*S.* Enteritidis	218:742	Chicken prepared outside the home		Marcus et al. (2007)
2002-3	Netherlands	*S.* Typhimurium	232: 3409	Undercooked meat - type not specified	7%	Doorduyn et al. (2006)
2001-2002	Australia	*S.* Birkenhead	111: 234	Food from a fast food chicken chain	Not calculated	Beard et al. (2004)
2000	Spain	Non-typhoidal salmonellae	21: 84 hospitalised children <3 years old	Meat products – type not specified	Not calculated	Bellido Blasco et al. (2007)
1996-1997	US (5 FoodNet Sites)	*S.* Enteritidis	182: 345	Eating chicken outside of the home	35% (all cases); 28% (domestically- acquired cases)	Kimura et al. (2004)
1996	France	*S.* Typhimurium	101: 101 children < 14 years of age	Undercooked ground beef	35%	Delarocque-Astagneau et al. (2000)
1994-1995	Spain	Non-typhoidal salmonellae	44: 69. Children aged 1 to 7 years (Note: controls were laboratory-confirmed cases with enteric viruses or *Campylobacter*)	Minced meat –type not specified	Not calculated	Bellido Blasco et al. (1998)
1993-1994	Norway	Non-typhoidal salmonellae	94: 226	Poultry purchased abroad		Kapperud et al. (1998)
2004	Kasai (Japan)	Hepatitis E Virus	45 volunteers with experience in eating deer meat. 45 volunteer without experience as controls	Uncooked deer meat	Eating uncooked deer meat is an epidemiological risk factor for HEV infection	Tei et al. (2004)
2000	Six European cities	*Toxoplasma* infection	Pregnant women positive for anti-*Toxoplasma gondii* IgM. Pregnant women seronegative as controls	Uncooked or cured meats products	Between 30 and 63 % was attributed to consumption of undercooked or cured meats	Cook et al. (2000)

Year(s) of study	Country	Biological agent investigated	Cases: Controls	Food implicated as an independent risk factor	Population Attributable Fraction	Reference
2003	San Francisco (USA)	*Crystospridium*	In inmunocompetent population. Cases (n=26), Controls (n=62)	Drinking water	The major risk factor was travel to another country(matched odds ratio [95% confidence interval]:24.1 [2.6, 220]	Khalakdina et al. (2003)
2006	Northern Peruvian altiplano	*Fasciola hepatica*	Children. Cases (n=61). Controls (n=61)	Consumption habits	An association between fascioliasis and four variables (40 analysed) were identified: the habit of drinking alfalfa juice (OR=4.5; 95% CI 1.8-11.1; P<0.001); familiarity with aquatic plants (OR=4.3; 95% CI 1.8-10.6; P<0.001); dog ownership (OR=5; 95% CI 1.7-15.1; P=0.002); and raising more than five sheep (OR=0.3; 95% CI 0.1-0.8; P=0.01).	Marcos et al. (2006)
2000-2003	United States	*Listeria monocytogenes*	249 case patients	Foods prepared in a commercial establishment	*L. monocytogenes* infection was associated with eating melons at a commercial establishment (odds ratio, 2.6; 95% confidence interval, 1.4-5.0) and eating hummus prepared in a commercial establishment (odds ratio, 5.7; 95% confidence interval, 1.7-19.1)	Varma et al. (2007)
2001-2002	Five Australian States	*Campylobacter*	881 *Campylobacter* cases and 833 controls aged 5 years	Meat (chicken)	The population attributable risk proportions indicate that eating chicken meat, either cooked or undercooked may account for approximately 30% of *Campylobacter* cases that occur each year in Australia	Stafford et al. (2007)
12 month study	United Estates	*Campylobacter*	1316 patients, 1 matched control subject for each case patient	Chicken and other meats	The largest population attributable fraction (PAF) of 24% was related to consumption of chicken prepared at a restaurant. The PAF for consumption of non-poultry meat was 21%	Friedman et al. (2004)
2007	Spain	Rotavirus, *Campylobacter* and *Salmonella*	117 patients and 84 controls	Exposure to meat products (eating or environmental exposure in the kitchen), some kinds of pets, and attendance at day care		Bellido et al. (2007)
1999	Belgium	VTEC	Cases 27 and controls 69	Exposure to different foods	Consumption of fish appeared to be a risk factor for infection (adjusted odds ratio (OR) 3·25, *P*=0·04). Contact with dogs (OR 0·27, *P*=0·04) and consumption of shellfish (OR 0·19, *P*=0·05) showed a negative association, corresponding to a decrease in risk	Piérard et al. (1999)

Case-control studies are observational and are potentially subject to the effect of extraneous factors which may distort the findings. The term confounding or confounding factor used in this context, refers to an extraneous variable that satisfies both of two conditions: it is a risk factor for the disease being studied, and it is associated with the exposure being studied but is not a consequence of exposure (Schlesselman, 1982). An example of confounding factor is age. Adjusting for the effects of confounding factors is evidently important in observational epidemiological studies, and can be dealt with in the study design by matching or stratifying sampling of study subjects, or in the data analysis by stratified or multivariate analyses (Kleinbaum et al., 1982: Rothman, 1986; Schlesselman, 1982).

Another type of bias frequently referred to in epidemiological research is "recall bias", namely the propensity of diseased subjects (cases) when interviewed, to scrutinize their memory and report more accurately on past exposure and possible causes of their disease than non-diseased subjects (controls) would do. Such recall bias has been documented (Hogue, 1975, Klemetti and Saxen, 1967, Lindefors-Harris et al., 1991).

Acquired immunity is rarely considered in case-control studies (Piérard et al., 1999). Unwitting inclusion of immune controls in a case-control study will tend to reduce statistical associations towards the null hypothesis (i.e. under-estimate the impact of an exposure on illness) if immunity is present and protective. Immune controls may be consumers of food contaminated with *Salmonella* spp. but are no longer susceptible to it. Given recent data from Denmark that suggests that most *Salmonella* infections are asymptomatic (Simonsen et al., 2007), control for acquired immunity among the control group should be considered in future studies. Other factors such as breast feeding or prior antibiotic use (Bellido-Basco et al., 2007) and the use of a gastric-acid reducing medication (Varma et al., 2004) are other factors that may interact with the outcome and consequently should be considered in case-control studies.

4.4. Data requirements

Ideal data set

To conduct a case–control study, we must start by identifying a group of people who have the disease in question, typically called **cases.** Those data can be obtained through hospital registries or clinic records listing all patients having a certain disease. We can also locate cases through local health department disease registries. Cases can also be found in a predefined group that includes medical records, such as high schools and some industrial plants, or in a prepaid health insurance group.

Once individuals with the disease under study (cases) are identified, individuals without the disease (**controls**) should be identified. Selection of controls for case–control studies is one of the most difficult design issues in epidemiology because the apparent difference in prior exposure of cases and controls may be thought to be the result of the factors that cause the disease but may actually be the result of the process used to select the controls (an error called selection bias). Controls should be representative of the source population from which cases were derived. Although challenging and often expensive, the surest approach is to draw a random sample of controls from the source population from which the cases came. Other sources of control groups include special groups such as friends, neighbours or relatives of the cases. Hospital- or clinic-based controls are frequently used but often are not representative of the source population. This happens when the reasons for attending the hospital or clinic may be different for cases and controls.

Once the person planning a case–control study has identified the outcome of interest (disease or health condition) and the factors to be studied, a method for collecting information (e.g., a self-administered questionnaire, an interview form or a medical examination form) is developed. Data should include information about the outcome of interest. Data analysis involves calculating the odds ratio as a measure of association between the disease and each of the factors of interest. The odds ratio can be used to determine if there is an association and to quantify the magnitude of such an association. To enable source attribution from analytical epidemiology requires not only knowledge of risk factors but also population-attributable fractions i.e. the proportion of cases in any study that can be explained by exposure to a particular risk factor. From well conducted case-control studies, it is possible to assess the relative role of several different exposures by estimating the population attributable fraction (PAF) (Havelaar et al., 2007). PAF can be calculated by a multivariate risk factor analysis, Friedman et al. (2004) used this multivariate analysis to calculate the population attributable fraction (PAF´s) for sporadic *Campylobacter* infection for all risk factors in the final model by using the logistic model case-control method described by Bruzzi et al. (1985).

According to Batz et al. (2005) there is no consensus about the ideal data set for food attribution, however, there is nearly universal agreement that none of the current data sources are sufficient on their own because of methodological limitations or gaps in available data. Furthermore in general, data are spread over a wide range of agencies and researchers, resulting in myriad studies covering different aspects of the food attribution problem. These issues make it difficult to accurately and dependably attribute illnesses to the foods responsible as pathogen vehicles.

Existing data set

There is a wide range of existing data which could provide helpful information for case-control studies. This includes systems which could help to identify human cases as well as information on foods related to single cases or outbreaks which could be used to generate hypotheses and to design questionnaires for case-control studies.

Several countries produce annual reports on zoonoses, which combine surveillance data with data on food and animal monitoring. In addition, etiologic analyses of food-borne outbreaks, detailing illnesses by pathogen, food source, and additional risk factors, are performed. ECDC collects information on human cases of infectious diseases within EU and every year EFSA publish a community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks in the European Community.

Recommendations for improving the available data

Conducting multinational (multi-MS) studies of sporadic food-borne diseases, where cases and controls in several MSs are interviewed during the same time period and applying the same questionnaire could provide very useful information regarding sources in EU and in the individual MSs. Comparing such results with monitoring data from food and animals may give indications of the effect of MS-specific interventions.

4.5. Conclusions and future prospects

Epidemiological methods are particularly suitable for evaluating the current burden of food-borne illness, to identify major risk factors and to monitor trends over time. Most countries collect information based on cases reported by medical microbiological laboratories, which is only a small proportion of all cases. Moreover, this proportion differs between countries and

may vary within one country over time. More research is needed to provide a more complete picture of the burden of food-borne illness and its development over time (Havelaar et al., 2007).

5. Source attribution by comparative exposure or risk assessment

5.1. Methodology

The most recent development in attribution methods is the application of risk assessment methodologies, to quantify exposure to pathogens from a multitude of sources. Current methods estimate exposure per person per day, averaged over a specified population (e.g. all inhabitants of one country). Exposure is estimated separately for all relevant specific sources that can be part of categories such as food, animal contact and environment

For food sources, the average exposure is estimated by multiplication of (averages of) the daily intake per person of the food product, the fraction of contaminated products at retail, the concentration of pathogens in contaminated products at retail and the fraction of pathogens that is eventually ingested by consumers. For foods that are ready to eat or consumed raw, this fraction is 1; for foods that are cooked before consumption the fraction is between 0 and 1 and can result from undercooking and/or cross-contamination. For environmental (water) exposure, visiting frequency, water intake and pathogen concentration are taken into account. For animal contact, calculations involve the frequency of human-animal contact, the (probability of) ingestion of faeces per contact, the fraction of contaminated animals and the pathogen concentration in contaminated faeces.

Results of all exposures can be cumulated to calculate the total exposure or can be ranked to identify the most significant sources of exposure. There are currently many data gaps, and uncertainty analysis is an essential component of the calculations.

5.2. Examples and results

An example of the approach is a Dutch exposure assessment for *Campylobacter* spp. (Evers et al., 2008). These authors estimated the mean dose of *Campylobacter* ingested per person per day averaged over the entire Dutch population by different routes including consumption of food (animal or vegetable origin; raw or prepared), direct contact with animals (pets, farm animals and petting zoo animals); and water (swimming in or drinking water). Thirty-one routes related to these categories were investigated. Approximately 2/3 of the average exposure was related to direct contact with animals, whereas 1/3 was related to food. (Surface) water contributed only 1% to the total exposure. Within the food routes, raw or partly cooked foods (chicken liver, milk, herring, and vegetables) were the major sources of exposure, with chicken meat being the most important source of exposure from cooked meats.

Relative exposure is not synonymous with relative risk. An estimation of relative risk requires combining the exposure estimates with a dose-response model. The difference between relative exposure and relative risk is due to the fact that the dose-response relationship is non-linear. Whether this difference is significant, depends on the sizes of the doses occurring. If the size range includes small doses only, relative risk and relative exposure will give similar results as the dose-response relationship is almost linear in the small dose range.

An example of estimating relative risk is the comparative risk assessment of *Listeria monocytogenes* carried out by the US Food and Drug Administration[5]. This study identified deli meats as the major source of listeriosis in the US population.

Comparative exposure or risk assessment has not yet been applied apart from the above mentioned studies on *Campylobacter* and *Listeria monocytogenes*.

5.3. Data requirements

Ideal data set

Ideally, if comparative exposure or risk assessment of a specific pathogen from different food sources is to be performed then data on prevalence and numbers of the pathogen in the different food products at retail are necessary. Data on the effect of undercooking and cross-contamination, as well as data on frequency of consumption, portion size and fractions consumed raw and prepared, is also necessary. If exposures from foods are to be compared with exposures from other sources like animal contact and the environment, then data on prevalence and numbers from these sources are needed. Thus, calculations on exposures from animal contact require data on prevalence and numbers in animal faeces, data on (probability of) ingestion of faeces per exposure event and data on contact frequency for the exposed population. Similarly if the environmental exposure should be estimated then data on numbers in water and other environmental sources, ingested volumes of the sources as well as data on contact frequency for the exposed population are required.

In practice, data availability will force the risk assessor to use expert opinion or assume identical values for parameter values between transmission routes for a smaller or larger part of the parameters.

Existing data set

A general overview of available data on the occurrence of pathogens in different foods at EU-level appears from the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Food-borne Outbreaks in the European Union (EFSA, 2006). From this report it can be seen that for most pathogens, data on numbers at retail are lacking or not easily accessible.

Recommendations for improving the available data

In general, collection of data should be question-driven and focussed. Thus, if it is decided, i.e. by risk managers and/or risk assessors that comparative exposure or risk assessment of a specific pathogen from different food sources is needed, then data on the prevalence and numbers of that pathogen in retail products must be made available for all products for which a preliminary analysis has indicated that they may contribute significantly to the risk and also for which there is substantial uncertainty. In addition, data on food storage, handling, preparation and consumption that reflect the diversity of consumer habits in the EU should be available.

[5] Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods; FDA/Center for Food Safety and Applied Nutrition; USDA/Food Safety and Inspection Service; Centers for Disease Control and Prevention. September 2003: www.foodsafety.gov/~dms/lmr2-toc.html.

5.4. Strengths and weaknesses

The method requires many parallel exposure assessments to be made and is therefore resource intensive, even though the models used are relatively simple. It needs reliable data for the occurrence of pathogens in all putative transmission routes and on preparation and consumption. In case of comparison with animal contact and environmental transmission routes, additional information on contact frequencies and (probability of) ingestion of faeces given contact are necessary. As discussed above, such data are currently only available to a limited extent, or the variables may be very difficult to measure in practice (latent variables), resulting in broad uncertainty intervals. Comparative risk assessment is the only approach that in principle allows the high level of detail needed for e.g. estimating the proportion of cases attributable to minced meat or other meat categories.

Combining the Dutch exposure estimates for *Campylobacter* with a dose-response relation resulted in a predicted incidence of infection and illness much higher than observed in epidemiological surveys (Evers et al., 2008). Recent serosurveillance studies offer an explanation for this as these suggest that there is a high incidence of asymptomatic *Campylobacter* infections in the Dutch population (Ang et al., 2007). Clustering of exposure, asymptomatic infections and/or immunity may need to be taken into account in order to reach agreement between epidemiological and risk assessment estimates.

5.5. Conclusions and future prospects

Given the current data limitations, it is concluded that comparative exposure assessment of transmission routes from different categories (food, animal contact, environment) needs further development and more data to be ready for decision support purposes. However, within the food category, comparative analysis of different sources is feasible, if sufficient data is available. The method can potentially integrate food chain information produced by national surveillance programmes or by special studies, and complements approaches that use identified cases of human illness as a starting point.

6. Source attribution by expert opinion

6.1. Methodology for attributing human illness by expert opinion

Most papers on food-borne illness attribution have used expert opinion to fill data gaps, or to combine data from information from different studies and scientific approaches into one single estimate. Experts use a broad set of information from pathology, epidemiology, microbiology, ecology, technology, and consumer behaviour to arrive at an estimate of the proportion of cases transmitted by a particular pathway. Published approaches were usually unstructured in the sense that they were organised on an *ad hoc* basis, guided by specific data gaps and no standardised protocols were used for these expert elicitations. Structured expert elicitation has been developed since the 1950's to support forecasting studies and is also widely applied in different domains of risk analysis (Cooke, 1995). Some recent studies have applied structured expert elicitation to food attribution problems.

In general, several steps are taken in a structured expert judgement study (Cooke and Goossens, 2001). Preparatory steps include a definition of the decision problem, identification and description of target variables, identification of variables to evaluate the performance of the experts, identification and selection of experts, development of documentation, try-outs and expert training. After these preparatory steps, the elicitation study proper is performed, either as

individual interviews or in group sessions. Data analysis includes aggregation of individual expert's assessment, possibly applying performance based weights, robustness and discrepancy analysis, and reporting.

For food attribution, specific details of the study domain need to be addressed such as decisions on the point of attribution, on how group transmission pathways in a limited number of categories, how to deal with cross-contamination etc. This requires careful preparation and pre-testing of protocols.

6.2. Examples and results

A recent study in the USA has been published on the internet and in the peer-reviewed literature (Hoffman et al., 2006; 2007). In this study were included 44 experts from different backgrounds (government, industry, academia) and different scientific disciplines (medicine, food science, public health, microbiology, and veterinary medicine. Expert estimates were compared with estimates based on outbreaks, as published earlier on the basis of CDC data. As expected, the degree of agreement between the two data sources varied with the pathogen. There was close agreement for pathogens with dominant transmission routes (*Vibrio* spp. *Cyclospora cayatenensis*) but substantial disagreement for pathogens with multiple routes (*Campylobacter* spp., *Toxoplasma gondii* and *Cryptosporidium* spp.). Data for *Salmonella* spp. are presented in EFSA (2008). The experts considered poultry to be the main source of salmonellosis, whereas outbreak data suggest eggs to be the dominant source. Pork appears to be a relatively small source of salmonellosis in the USA, based on outbreak data and in particular on expert estimates. These data cannot be directly transferred to the EU.

In the Netherlands (Havelaar et al., in press), 16 experts (from research and industry with backgrounds in microbiology, epidemiology and food science) provided their estimates on sources for 17 pathogens. There were two steps in the attribution. First, experts were asked to quantify the contribution of five major pathways (food, travel, environment, direct human-human transmission and direct animal-human transmission). Second, experts were asked to split the food pathway into 11 categories (eggs, chicken and other poultry, pork, beef and lamb, dairy products, fruit and vegetables, other foods incl. composite foods, infected humans and animals, fish and shellfish, bread, grains, pastas and bakery products, beverages). Results for salmonellosis are presented in EFSA (2008).

6.3. Data requirements / experimental design

Ideal data set

There are several ways to perform an expert elicitation. A classical approach is the Delphi method, aimed at reaching consensus. Typically, point estimates are presented. Alternative methods involve paired comparisons (ranking all alternatives on some criterion), discrete event probabilities ("rain tomorrow"). A full quantitative approach involves asking the experts for distributions for continuous quantities (typically, the 5-, 50-, 95-percentiles are asked).

Performance measures are an important component of expert elicitations. They allow the experts to be weighed, based on their answers to seed variables. These are variables from the experts' field whose values are known to the analyst but become known to the experts only *post hoc*. Two performance measures are used: calibration (accuracy) and information (precision). More information is given in Cooke and Goossens (2001).

A formal procedure for expert elicitation was developed at the Delft University of Technology, and primarily applied in the domain of external safety (Cooke and Goossens, 2001). Subsequent studies have applied this procedure to problems in the life sciences (Havelaar et al., in press; Horst et al., 1997; Van der Fels-Klerx et al., 2002, 2005). The protocol involves the following steps:

- Preparation
- Definition of case structure
- Identification of target variables
- Identification of query variables
- Identification of seed variables
- Identification of experts
- Selection of experts
- Preparation of elicitation document
- Dry run exercise
- Expert training session
- Elicitation
- Expert elicitation session
- Post elicitation
- Combination of expert assessments
- Discrepancy and robustness analysis
- Feedback
- Post-processing analysis
- Documentation

Existing data set

Expert opinion has been a key component of national studies on source attribution of enteric pathogens. These include the following examples:

Mead et al. (1999), present estimates of total and reported cases, hospitalizations and deaths by 28 enteric pathogens in the USA. These data are then combined with % food-borne transmission to estimate the total incidence of food-borne illness and deaths. Different approaches were used for estimates of food-borne transmission, and extensive documentation was provided in an annex to the paper. Expert opinion was used to complement data gaps. The procedure used varied between pathogens. Some examples: *C. botulinum*: 100% by definition, Brucella: extrapolation from outbreak data. STEC non O157: assumed comparable with STEC O157. No uncertainty was reported.

Hall et al. (2005), present estimates of total cases by 16 enteric pathogens in Australia and combine these data with % food-borne transmission to estimate total incidence of food-borne illness. Attribution was based on a combination of outbreak data, literature review and a Delphi process. The Delphi process included 10 experts, to supplement literature data. After written preparation, a meeting was organized to arrive at consensus. Uncertainty in the estimates was included by Monte Carlo simulation.

Cressey and Lake (2005), present estimates of disease burden (DALYs) by 6 enteric pathogens in New Zealand. The data were combined with % food-borne transmission to estimate total burden of food-borne illness. The elicitation process included 14 experts, using a two pass modified Delphi procedure with facilitated discussion. The study also included attribution to some food groups. Minimum, most likely and maximum values per expert were collected and

the mean values of each were used to define Pert distribution for Monte Carlo simulation. Experts were equally weighed.

Hoffmann et al. (2007) used a US FoodNet update of estimates on total cases of food-borne illness, hospitalizations and deaths by 11 enteric pathogens and present attribution to 11 food types. The data were based on the opinion of 42 experts, using a qualitative and quantitative approach. In the qualitative approach, experts were asked if they considered the food group likely to be a source. If not, then the group was excluded from the quantitative elicitation. In this step, best estimate and a 90% confidence interval were asked. Descriptive statistics and results from Tobit regression (best estimates are bounded by sum = 100%) are presented.

Havelaar et al. (in press) present the fraction of total cases in the Netherlands of 17 enteric pathogens transmitted by five major pathways and eleven food groups within the food pathway. A structured elicitation involving 16 experts was organized. Experts were asked to present their 90% confidence interval for each variable. Joint probability distributions were then calculated by probabilistic inversion, including uncertainty. Experts were not weighed for their performance.

Recommendations for improving the available data

Expert estimates have always played an important role in food attribution studies. Structured protocols and advance mathematical analysis are beginning to be applied and are potentially powerful tools to obtain consistent and complete estimates. Such studies, when carefully planned and executed, may provide policy-relevant information.

6.4. Strengths and weaknesses

Expert judgements are subjective by nature and may be biased by the specific background and scientific expertise of the respondents. Methods exist to evaluate the expert's performance by evaluation of their answers to seed variables, i.e. variables to which the answer is known to the analyst but not generally available (Cooke, 1995). Experts are evaluated on their information and on their calibration. An expert is considered to provide informative results if the relative uncertainty in his estimates are small. An expert is well calibrated if the mean value of his estimate is close to the observed value for the seed variable. A structured procedure also helps to avoid many other pitfalls that may arise when asking experts for their subjective estimates. These structured approaches require more resources and technical expertise than conventional, unstructured evaluation and need a multidisciplinary approach. This may hamper their acceptance in practice.

Expert estimates typically combine information from different sources, which can be considered both a strength and a weakness. There are currently no analytical approaches for combining data from e.g. outbreak studies and epidemiological studies of sporadic cases, hence expert judgement is the only feasible way. The actual evaluation of the data and the weight put on any single data source is intractable, however.

6.5. Conclusions and future prospects

Expert opinion is frequently used in many domains of risk assessment and has always been a component of food safety risk assessment. Recent developments have been to introduce more explicit and more structured approaches in several domains including source attribution. Expert

elicitation is a relatively economical way of obtaining quantitative estimates, and methods have been developed to reduce bias in such studies, e.g. by performance-based weighting. The full potential of such approaches has not yet been applied in source attribution studies and should be further implemented. Currently, different mathematical methods are being used to aggregate individual expert estimates to an overall estimates (including it's uncertainty) and the performance of these methods needs to be compared. These developments should lead to an improved protocol for source attribution by expert elicitation, that could be used at a European as well as on a global scale.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

It is important to know the fraction of incidence of human illness due to specific pathogens that is attributable to foods and which foods are contributing to that fraction in addition to the contribution of other sources like environmental, direct animal contact and human to human contact.

Attribution to sources of human food-borne illness can be achieved using different methods such as microbial subtyping, outbreak summary data, epidemiological studies, comparative exposure assessment, and structured expert opinion.

Each method of source attribution has different strengths and weaknesses and addresses different points in the food chain. The choice of method depends on the specific question that needs answering and the data and resources available.

Source or reservoir attribution using microbial subtyping has mainly been applied for *Salmonella* and so far only in a few countries. Serotyping and phage typing are the preferred typing methods for this purpose, but new genotypic-based methods may prove to be valuable in the future also for other pathogens like *Campylobacter* and VTEC.

Reservoir attribution of human salmonellosis has provided guidance to risk managers and policy makers on the implementation and evaluation of control strategies for major reservoirs. The philosophy behind the approach is that control of the reservoir will prevent subsequent human exposure, regardless of the transmission route or vehicle. By collating results from surveillance programs that are in place and comparing these to cases of human illness, the method provides added value to data that are already being collected.

Outbreak investigations give public health officials important information to be used for immediate control of individual events. In many countries surveillance of outbreaks is undertaken and summaries are available at an international level. Records over many years provide a relatively detailed dataset, making outbreak data attractive also for use in attribution models. The foods implicated in causing human disease can be assessed using aggregated data from many outbreak investigations and the most common food vehicles involved can be identified, with the caveat that the source of human infection is often not identified in a significant proportion of outbreak episodes. Although source attribution using outbreak data is a promising approach, there are gaps in the datasets available at EU level.

Case-control studies of sporadic infections are a valuable tool to identify relevant risk factors to human food-borne infections, including sources of exposure and predisposing, behavioural or seasonal factors. By calculating the population attributable fractions, the relative importance of the different risk factors can be estimated. A primary limitation of the method is the accuracy of the recall about exposures from interviewed participants, which can lead to either an over- or

underestimation of the contribution of specific sources. In addition, statistical power to determine the importance of common exposures will require enrolment of many participants.

Given the current data limitations, it is concluded that comparative exposure or risk assessment between major categories (food, direct animal contact, environments, person-to-person) needs further development and more data to be ready for decision support purposes. However, within the food category, comparative analysis of different transmission routes and sources is feasible if sufficient data is available.

Expert Opinions have always been used for source attribution, and recently more explicit, quantitative methods have been introduced. Experts are able to combine and weigh data from the different approaches as discussed above for which currently no analytical methods exist. Protocols to reduce bias in expert estimates have been developed in other areas of risk assessment but have not yet been fully applied for source attribution.

As described in this report, a variety of approaches have been used to better define the source of foods responsible for human infections. However, none of these approaches is likely to be sufficient on its own. Comparing and compiling results from more than one method may improve robustness.

For source attribution, there is a need for harmonization and structured categorization of food items, taking into account the legal definition of water as food. Ideally, harmonization and categorisation should be based on both the food commodity and the processing/preservation methods in order to gather data by various countries/organisations/research teams that are comparable and to enable exchange of data.

The implicit conclusion, therefore, is that the scientific and accurate attribution of food-borne illnesses to specific foods means developing a comprehensive program that combines many of the discussed methods and data. Such a system can be achieved with increased resources and cooperation among food safety institutions.

RECOMMENDATIONS

Data gathering for purposes of attribution should be question driven and by representative sampling. Baseline studies, as carried out under the Zoonoses regulations, are an important move in the right direction. Similarly, a common approach to epidemiological studies is recommended.

Methodology and data requirements for use of subtyping data for source attribution

General recommendations for food-borne pathogens like *Salmonella*, *Campylobacter* and VTEC:

It is recommended to perform intensive monitoring of all major reservoirs (i.e. food animals) and/or food including imported food and humans providing;

- A collection of representative isolates from the major reservoirs/sources and humans. It is better to choose representative data instead of amount of data.
- Avoid clustered data, i.e. precise and meaningful definition of epidemiological unit e.g. flock and NOT isolate.
- Avoid using animal isolates from diagnostic submissions (not representative).If available include information on the number of travel-related cases and the number of outbreak-related cases; particularly important for large outbreaks.

Application of discriminatory and definitive epidemiological marker methods providing knowledge of the distribution of the different subtypes in the major reservoirs/sources is recommended. For *Salmonella* this includes:

- Serotyping for all *Salmonella* isolates
- Phage typing of *S*. Enteritidis and *S*. Typhimurium isolates and/or application of other methods that can distinguish between strains within these serovars.

It is recommended to apply and evaluate new subtyping methods for *Salmonella* attribution e.g. multiple locus variable number tandem repeats analysis (MLVA). MLVA is increasingly used for surveillance of human *S*. Typhimurium infections and tracing of outbreaks, but its usefulness for source attribution needs to be explored.

It is recommended to apply and evaluate other typing techniques for source attribution of other pathogens e.g. use of. multi-locus sequence typing (MLST) for *Campylobacter* spp. and VTEC based.

Methodology and data requirements for use of outbreak data for source attribution;

It is recommended to develop a minimum dataset including standardized food categories and reporting of contributing factors linked to outbreaks, to be collected in each MS. Disaggregated data should be reported to EFSA.

Methodology and data requirements for use of epidemiological studies for source attribution of sporadic cases;

It is recommended to conduct multinational (multi-Member State) studies of sporadic food-borne diseases, where cases and controls in several MSs are interviewed during the same time period and applying the same questionnaire.

Methodology and data requirements for comparative exposure or risk assessments;

Comparative exposure assessment should be developed as the most appropriate tool to identify food categories within the same reservoir (i.e. pig, cattle, poultry) that pose a greater risk to individual consumers or the population as a whole.

Risk assessors and risk managers should agree on specific scenarios with respect to storage, handling, preparation and consumption that reflect the diversity of consumer habits in the EU.

Data on the prevalence and number of the investigated organisms in retail products together with data on frequency of consumption and portion size must be available for those products to be compared. In addition, depending on products, data on the effect of undercooking and cross contamination, and fraction consumed raw and prepared may also be necessary.

Methodology and data requirements for source attribution by structured expert opinions

A structured expert survey could provide the EC with a set of estimates for attribution of several pathogens to food. Such a study would need to take into account different exposures and consumer habits within different regions of EU.

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GLOSSARY

Case control study	The observational epidemiologic study of persons with the disease of interest and a suitable control group of persons without the disease. The relationship of an attribute to the disease is examined by comparing the diseased and non-diseased with regard to how frequently the attribute is present [(1)].
Case series study	A group or series of case reports involving patients who were given similar treatment (*For the purposes of this document; "those patients who have the same clinical symptoms"*). Reports of case series usually contain detailed information about the individual patients. This includes demographic information (for example, age, gender, ethnic origin) and information on diagnosis, treatment, response to treatment, and follow-up after treatment [(2)].
Cohort study (syn. longitudinal study)	The analytic method of epidemiologic study in which subsets of a defined population can be defined who are, or have been, or in the future may be exposed or not exposed, or exposed to different degrees, to a factor or factors hypothesized to influence the probability of occurrence of a given disease or other outcome [(1)]. *In this document, the following interpretation is used:* *"A research study that compares a particular outcome (such as lung cancer) in groups of individuals who are alike in many ways but differ by a certain characteristic (for example, female nurses who smoke compared with those who do not smoke)."*
Intervention study	A study involving intentional change in some aspect of the status of subjects, e.g. introduction of a preventive or therapeutic regimen, or designed to test a hypothesized relationship [(1)].

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