Attribuer les cas humains d'infections d'origine alimentaire à des sources spécifiques

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Review

Attributing the Human Disease Burden of Foodborne Infections to Specific Sources

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Abstract

Foodborne diseases are an important cause of human illness worldwide. Humans acquire these infections from a variety of sources and routes of transmission. Many efforts have been made in the last decades to prevent and control foodborne diseases, particularly foodborne zoonoses. However, information on the impact of these interventions is limited. To identify and prioritize successful food safety interventions, it is important to attribute the burden of human illness to the specific sources. Defining scientific concepts and harmonizing terminology for "source attribution" is essential for understanding and improving attribution methodologies and for sharing knowledge within the scientific community. We propose harmonized nomenclature, and describe the various approaches for human illness source attribution and their usefulness to address specific public health questions.

Introduction

Pathogens commonly transmitted to humans through foods are responsible for a high burden of human illness and death worldwide. The World Health Organization (WHO) estimates that 1.8 million children die each year from diarrhea, and much of the childhood diarrhea is caused by pathogens that are commonly acquired from contaminated food or water. Furthermore, even in developed countries up to one third of the population each year has an infection from a pathogen commonly transmitted through foods (WHO, 2005). Humans acquire these infections through a number of routes, including eating contaminated food, contact with live animals, and contact with a contaminated environment. Foodborne transmission is recognized as being responsible for a major proportion of these infections, and foodborne diseases may involve many different food sources and commodities. Several countries have implemented intervention programs during the last decades to prevent and control foodborne diseases, particularly foodborne zoonoses (Wegener *et al.*, 2003; EFSA, 2006). However, precise measurement of the public health impact of such interventions has been difficult, in part because information on the attribution of the burden of foodborne diseases to specific sources is often insufficient. To prioritize appropriate food safety interventions, it is crucial to attribute the human disease burden of each foodborne infection to specific sources (FAO/WHO, 2006).

A variety of general methods to attribute one or more foodborne diseases to specific sources has been developed, including microbiological approaches, epidemiological

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approaches, intervention studies, and expert elicitation approaches. Each of these general methods presents advantages and limitations, and the usefulness of each depends on the public health questions being addressed (Batz *et al*., 2005). Several groups are using attribution methods, but these often have different nomenclature and food categorization schemes. Defining scientific concepts and harmonizing terminology are essential for understanding and improving attribution methodologies and sharing knowledge across the scientific community. In this paper, we propose harmonized nomenclature; describe different approaches to human illness attribution; and discuss the advantages, limitations, and applicability of each approach in answering different questions along the farm-to-consumption continuum, while emphasizing that the choice of the attribution method will depend on the pathogen and the public health question being addressed.

Definitions

Human illness "source attribution" may be defined as the partitioning of the human disease burden of one or more foodborne infections to specific sources, where the term *source* includes animal reservoirs and vehicles (e.g., foods). The human disease burden can be measured by the number of laboratory-confirmed (reported) infections or by the estimated total number of infections. To enable comparisons and to account for morbidity and mortality, the human disease burden can be expressed as disability adjusted life years (DALYs).

Attribution of human illness to specific sources requires categorization of the sources. Harmonization of the categorization scheme is needed for comparisons and integration of results from various models and approaches. Such a system should be hierarchical, while accommodating different levels of detail required for different purposes. The categorization scheme should fit with food consumption databases and be internationally standardized.

General Approaches for Source Attribution

Microbiological approaches

One general method for attribution of the human disease burden of foodborne infections to specific sources is "microbiological approaches." Microbiological approaches for source attribution include the microbial subtyping approach and the comparative exposure assessment approach. Both approaches involve isolation of the pathogen from the various sources and from ill humans. The microbial subtyping approach requires a representative distribution of the subtypes of the pathogen in the different sources and humans, but does not depend on estimates of the prevalence of the subtypes in each source. The comparative exposure assessment approach requires estimates of the prevalence and concentration of the pathogen in each of the sources of exposure.

Microbial subtyping approach. The microbial subtyping approach involves characterization of isolates of a specific pathogen by phenotypic and/or genotypic subtyping methods (e.g., serotyping, phage typing, antimicrobial susceptibility testing, pulsed-field gel electrophoresis, sequence-based subtyping). The principle is to compare the subtypes of isolates from different sources (e.g., animals, food) with those isolated from humans. The microbial subtyping approach is enabled by the identification of strong associations between some of the dominant subtypes and a specific reservoir or source, providing a heterogeneous distribution of subtypes among the sources. As a first step, subtypes isolated exclusively or almost exclusively from one source are regarded as "indicator subtypes," and the human infections caused by each indicator subtype are assigned (attributed) to that specific source. The relationship between the relative occurrence (i.e., proportion of positive samples or positive isolates) of each indicator subtype in the source and the incidence of human infections caused by that indicator subtype is then determined. Finally, human infections caused by subtypes found in several sources are assigned to specific sources proportional to the occurrence of the indicator subtypes. The application of this approach assumes that the distribution of subtypes in the collection of isolates in each source used in the attribution exercise is similar to the true distribution of subtypes in each source. Because the microbial subtyping approach utilizes a collection of temporally and spatially related isolates from various sources, it is facilitated by an integrated foodborne disease surveillance program that is focused on the collection of isolates from the major food animal reservoirs of foodborne diseases.

There have been several applications of the microbial subtyping approach for *Salmonella* source attribution (e.g., Van Pelt *et al*., 1999; Sarwari *et al*., 2001). The most advanced application of the microbial subtyping approach for *Salmonella* was developed in Denmark (Hald *et al*., 2004). Using data from the integrated Danish *Salmonella* surveillance program, a mathematical model was developed to quantify the contribution of each of the major food animal sources to human *Salmonella* infections. The "Danish *Salmonella* source account" model attributes domestically acquired laboratory-confirmed 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, using a Bayesian framework with Markov chain Monte Carlo simulation (Gilks *et al*., 1996). This microbial subtyping approach has proved to be a valuable tool in focusing food safety interventions to the appropriate animal reservoir in Denmark and provides an example of potential synergy between quantitative risk assessment and public health surveillance (Hald *et al*., 2004).

Another example of the microbial subtyping approach is the use of multilocus sequence typing (MLST) of *Campylobacter jejuni* isolates from foods and humans, being applied in the United Kingdom (Dingle *et al*., 2002) and New Zealand (French, 2007). In this microbial subtyping approach, MLST is used to identify lineages in bacterial populations by indexing the variation present in seven housekeeping genes located in various parts of the chromosome (Dingle *et al*., 2001). With the development of novel analysis tools such as the ClonalFrame algorithm (Didelot and Falush, 2007), MLST has been used to identify clonal complexes associated with different isolation sources that, in some instances, correspond to different host species. As an increasing number of isolates, including isolates from various sources, are added to the MLST database (accessible on the internet), there will be increased precision in the attribution of human infections to host sources (McCarthy *et al*., 2007). Recently, MLST data were utilized to attribute the sources of human *C. jejuni* infections in New Zealand using two microbial subtyping models, the ClonalFrame algorithm

and the Danish *Salmonella* source account; both models gave similar results (French, 2007).

When the microbial subtyping approach is applied at the point of production, it quantifies the contribution of the most important reservoirs of the specific pathogen, assuming isolates are available from those reservoirs. Because pathogens can be transmitted through a variety of sources, interventions that control the pathogen at the reservoir level, before the dissemination of the pathogen through numerous transmission pathways, should result in important declines in human infections.

A limitation is that the microbial subtyping approach is restricted to pathogens that are heterogeneously distributed among the sources. Furthermore, the microbial subtyping approach will only attribute illness to those sources from which isolates are available. The microbial subtyping approach is also data intensive and requires a sufficiently large and representative sample from each source. Independent of the typing method used, the application of strain typing to make inferences on the sources of human infections must be done with appreciation of the strengths and limitations of typing methods (Olsen *et al*., 1993; Tenover *et al*., 1997). Clonality is defined as the high probability that two isolates that are identical by typing are related to each other, and the confidence in this probability becomes greater when more than one typing method is applied (Olsen *et al*., 1993).

Comparative exposure assessment approach. The principle of the comparative exposure assessment approach is to determine the relative importance of the known transmission routes by estimating the human exposure to that pathogen via each route. The comparative exposure assessment approach requires, for each known transmission route, information on the prevalence and dose of the pathogen in the source, the changes of the prevalence and quantity of the pathogen throughout the transmission chain, and the frequency at which humans are exposed by that route. These data provide an estimate of the exposure dose for each transmission route. The exposure doses are compared and the human disease burden (e.g., the observed laboratory-confirmed infections or estimated total number of infections) caused by the specific pathogen is partitioned to each of the various transmission routes, proportionally to the size of the exposure dose. The estimates of exposure dose for each transmission route can be combined with a dose–response model to predict the number of infections from each route, similar to what is done in traditional microbial risk assessments. The comparative exposure assessment approach for source attribution differs from traditional risk assessment in its objective and level of detail. A risk assessment typically aims at describing the complex dynamics of a pathogen in a single food commodity during food processing, and predicting the relative public-health effect of different interventions strategies—alone and in combination. In contrast, the comparative exposure assessment aims at partitioning the observed (or predicted) human disease burden to all known transmission routes, including various foods, direct contact with live animals, and environmental exposures. For this purpose, the various transmission routes are modeled in a more simplified and less detailed way that represents only the main steps in the transmission pathway.

A comparative exposure assessment approach was used to attribute human *Campylobacter* infections to various transmission routes in the Netherlands (Evers *et al*., 2008). Human exposure across the major transmission routes was estimated in a stochastic model as the mean dose of *Campylobacter* ingested per person per day, averaged over the entire Dutch population. Thirty-one transmission routes related to ingestion of food and direct contact with animals and water were investigated. Another example of this approach was developed to attribute human *Campylobacter* infections in New Zealand, where a model was used to explore the relative importance of four of the most commonly identified routes of exposure: food (poultry and red meat), drinking water, freshwater swimming, and occupational contact (livestock). The model estimates the mean daily dose to which an average individual is exposed to from each route, and, by comparing the relative exposure doses, human *Campylobacter* infections were attributed to the sources (McBride *et al*., 2005).

The strength of the comparative exposure assessment approach is that it attributes human illness to sources of exposure, taking into account the different transmission routes from the same reservoir, e.g., estimating the role of dairy products and beef from the cattle reservoir. However, this approach is often limited by a lack of sufficient data (e.g., comparable prevalence data or data on exposure through different routes), which results in large uncertainties around the estimates.

Epidemiological approaches

A second general method for attribution of the human disease burden of foodborne diseases to specific sources is "epidemiological approaches." Epidemiological approaches for source attribution usually involve interviews of patients to elicit the patient's recall of foods consumed or other exposures before illness began. Some patient interviews are undertaken as a routine activity under the existing public health surveillance infrastructure. Additional studies may be performed to determine factors associated with apparently sporadic infections or in association with investigations of outbreaks of human infections. An outbreak is defined as the occurrence of two or more cases of a similar illness resulting from the exposure to a common source (Olsen *et al*., 2000), while sporadic cases represent cases that have not been associated with known outbreaks (Engberg, 2006). In some circumstances, cases classified as sporadic may belong to undetected outbreaks. Identification of possible sources of apparently sporadic infections and outbreaks may be undertaken using analytical epidemiological studies, which involve interviewing persons who are (or will become) ill and persons who are not (or will not become) ill, or case-series studies, which involve interviewing only individuals who are ill.

Studies of sporadic infections. Several types of studies have been performed to identify possible sources of apparently sporadic human infections. Case–control studies are the most commonly used analytical epidemiological studies for identifying possible exposures associated with sporadic infections. To allow sufficient enrollment of patients, case–control studies of sporadic infections are often conducted over an extended period of time and commonly use public health surveillance to ascertain culture-confirmed cases. Typically, selected case-patients and a corresponding group of asymptomatic, and therefore assumed to be uninfected, individuals

(controls) are interviewed, and 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 the exposure can be calculated and is defined epidemiologically as the "population attributable fraction" (PAF) (Clayton and Hills, 1993). The PAFs can be used to partition the human disease burden to specific sources (Stafford *et al*., 2008).

Numerous case–control studies of sporadic infections of diseases commonly transmitted through food, including zoonotic diseases, have been published. Some of these studies calculate only measures of association, e.g., *Salmonella* Enteritidis infections in Denmark (Mølbak and Neimann, 2002) and *Campylobacter* infections in Denmark (Wingstrand *et al*., 2006), while others estimate the PAF, e.g., Shiga toxin–producing *Escherichia coli* O157 infections in the United States (Voetsch *et al*., 2007). Case–control studies, when combined with data on the human health burden of the disease under study, can also be utilized to attribute that burden to specific sources. A good example is the recently published study for sporadic *Campylobacter* infections in Australia (Stafford *et al*., 2008), where the authors determine the PAF for various exposures, and combine the PAF estimates with estimates of the burden of *Campylobacter* to partition the burden to specific sources.

Case–control studies are a valuable tool to identify potential risk factors for human infections, including sources and predisposing, behavioral or seasonal factors (Engberg, 2006). Moreover, in addition to individual case–control studies, a systematic review of published case–control studies of sporadic infections of a given pathogen can provide an overview of the relevant exposures and risk factors for that infection, and a summary of the estimated population attributable fractions for each exposure. An overall population attributable fraction derived from a meta-analysis or weighted summary of several case–control studies of a certain pathogen can be combined with estimates of the burden of illness caused by that pathogen to estimate the burden of illness attributed to each exposure.

A limitation of epidemiological approaches, particularly case–control studies, is that cases that are not associated with a recognized outbreak reflect a mixture of possible sources of exposure, and it may be difficult to distinguish between these exposures. Another limitation is that the statistical power to determine the importance of common exposures often requires enrollment of many participants. Furthermore, a limitation of case–control studies is the lack of accuracy in the recall of exposures by participants (ill and well participants). This misclassification of exposures commonly leads to an underestimation of the burden of illness attributed to specific exposures (Stafford *et al*., 2008).

Cohort studies, another type of analytical epidemiological study, are used less often for sporadic infections, since they usually require interviewing more persons than is practical, most of whom are not infected. Examples of cohort studies performed to determine the overall disease burden attributable to specific pathogens include the study of sporadic infections by de Wit *et al*. (2002) and the Infectious Intestinal Disease study in the United Kingdom (Wheeler *et al*., 1999).

Case-series studies of sporadic infections are commonly conducted, particularly for uncommon diseases that have a well-recognized source of infection, to which persons without the infection are infrequently exposed. Examples of case series studies of sporadic infections and the common source for that disease include botulism associated with home-canned foods (Sobel *et al*., 2004), *Vibrio vulnificus* and oysters (Shapiro *et al*., 1998), and *Salmonella* Typhi infections and foreign travel in the United States (Ackers *et al*., 2000). When an exposure is uncommon in the general population, case series studies of a sporadic infection can be used to determine the frequency of that exposure among the cases, and that frequency can be considered against the proportion of the burden of illness caused by that disease that is attributed to that specific exposure.

Analysis of data from outbreak investigations. Another epidemiological approach for source attribution involves conducting an analysis of the information available from outbreak investigations. An investigation of an individual outbreak may involve both microbiological and epidemiological data; the epidemiological data may be derived from interviewing only infected persons (i.e., a case-series) or from interviewing both infected and noninfected persons. Typically, the microbiological data in an individual outbreak investigation are used to generate hypotheses about the source of the outbreak or to support the results of an epidemiological investigation. Many outbreak investigations are successful in identifying the specific source for the human infections. By conducting an analysis of data from outbreak investigations, the most common food vehicles involved in outbreaks can be identified. A simple analysis or summary of outbreak investigations is useful for attributing illnesses to foods, but often the implicated food item in an individual outbreak is a "complex" food, containing several food items, many of which could be the specific source of the infection. Several methods have been used to include the information of complex foods involved in outbreaks when conducting an analysis of data from outbreak investigations to attribute human illness to sources.

An example of an analysis of data from outbreak investigations for source attribution was developed in the United Kingdom. This analysis used data from individual outbreak investigations reported through national surveillance and population-based studies to estimate the number of human foodborne infections in England and Wales associated with specific food sources (Adak *et al*., 2005). In this method, individual outbreak investigations that implicated complex foods were not included in the analysis. An alternative method for conducting an analysis of data from outbreak investigations is being developed in the United States. In this method, food items implicated in foodborne outbreaks reported to the Centers for Disease Control and Prevention via the electronic Foodborne Outbreak Reporting System are used to attribute illnesses to specific foods. Food items are categorized into a hierarchical scheme, according to their ingredients. Foods that contain ingredients that are members of a single commodity are considered "simple foods," while foods that contain ingredients that are members of multiple commodities are considered "complex foods." As an example, ground beef is a simple food, whereas meat pie is a complex food. Each implicated food is assigned to one or more mutually exclusive food commodities, according to its ingredients. For outbreaks that have implicated a simple food item, all illnesses are attributed to that single commodity. For outbreaks that have implicated a complex food item, illnesses are partitioned to each commodity in the complex food according to the proportion of illnesses attributed to each of those commodities in outbreaks caused by simple foods. As a result, illnesses in an outbreak due to a

complex food item are attributed to a commodity in the implicated complex food, only if that commodity has been implicated in at least one outbreak due to a simple food. The number of illnesses attributed to each commodity are then summed and used to determine the percentage of disease attributed to each commodity. To estimate the attribution of all foodborne illness in the United States (and not just illnesses involved in reported outbreaks), the percentages of illness for each disease attributed to each commodity were weighted by the estimated annual burden of illness for each pathogen.

A limitation of an analysis of data from individual outbreak investigations is that many outbreaks may not be detected, investigated, or reported. In many cases, a pathogen is not identified, the source is not elucidated, or both. Also, the quality of evidence varies and classification schemes for the data are not consistently used. Large outbreaks, outbreaks associated with point sources, outbreaks that have short incubation periods, and outbreaks that cause serious illness are more likely to be detected, investigated, and reported. As a consequence, the burden of illness in the population attributed to some food sources may be underestimated, which will likely vary by pathogen (Batz *et al*., 2005). Another limitation is that categorization of foods may differ from one analysis to another. A third limitation of an analysis of data from individual outbreak investigations is that foodborne illnesses included in data from outbreak investigations may not be representative of all foodborne illnesses. Finally, certain food vehicles are more likely to be associated with reported outbreaks than others, which can lead to an overestimation of the proportion of human illnesses attributed to a specific food.

An advantage of data from outbreak investigations is that clear documentation that a specific pathogen was transmitted to humans via a specific food item can be available. Furthermore, an analysis of data from outbreak investigations to attribute foodborne illness to specific sources may sometimes include information on the point of contamination at the farm to consumption chain, particularly when individual outbreak investigations have complete trace back information available. Another advantage is that a wide variety of food vehicles are represented, including less frequently identified food items (e.g., almonds or sprouts). Finally, an analysis of data from outbreak investigations may in some countries or regions be the most readily available source of information for human illness attribution purposes.

Intervention studies

A third general method to attribute the human disease burden of one or more foodborne infections to specific sources is the use of intervention studies. Intervention studies can provide compelling evidence of the burden of illness attributed to a specific source, particularly if the intervention is conducted in a randomized, double-blinded design. Intervention studies have proven useful, for example in developed countries in determining the population disease burden from a particular exposure (e.g., drinking water), and in developing countries in determining the burden of illness from a variety of hygiene-related factors. 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 foodborne disease. Examples of intervention studies include the experiences of reducing the burden of human campylobacteriosis from poultry meat in Iceland (Stern *et al*., 2003) and human salmonellosis in Denmark (Wegener *et al*., 2003).

Accidental interventions, where a decrease or increase in human disease incidence can be observed due to a change in exposure or behavior of the population at risk (e.g., sudden changes in consumption of certain food items) may occur. Examples include the sudden decline in beef consumption due to bovine spongiform encephalopathy in the United Kingdom, and declines in chicken consumption due to outbreaks of avian influenza in Europe. By analyzing changes in consumption and numbers of reported cases of human illness, it is possible to estimate the number of human cases attributable to specific sources. For example, in June 1999, the use of dioxin-contaminated chicken feed in Belgium resulted in the withdrawal of chicken meat and eggs from the market (Vellinga and Van Loock, 2002). A decrease in reported human *Campylobacter* infections also occurred in Belgium, beginning in June 1999. Public health surveillance data from preceding years (1994 to 1998) were used to predict the number of human *Campylobacter* infections expected in 1999. The number of reported cases in 1999 was substantially lower (40%) than the expected, suggesting that 40% of reported human *Campylobacter* infections in Belgium could be attributed to chicken (Velling and Van Lock, 2002).

Expert elicitation approaches

A fourth general method to attribute the human disease burden of foodborne diseases to specific sources is "expert elicitation approaches." Expert elicitations are commonly used to address data gaps. Several studies have used expert elicitations to estimate the proportion of human infections that are foodborne, which can be viewed as a first step in source attribution (e.g., Mead *et al*., 1999; Van Duynhoven *et al*., 2002; Hoffmann *et al*., 2007).

One of the approaches used for expert elicitation is the generalized framework for expert judgment studies from heterogeneous expert panels based on the Group Delphi method (Henson, 1997), which has been adapted for source attribution of foodborne illness in New Zealand (Lake *et al*., 2006). The process involved selection and invitation of experts, distribution of summary sheets for each food–hazard combination, a group meeting and completion of a questionnaire (without discussion). The results of the questionnaires were then aggregated by Monte Carlo simulation of pert distributions with equal weighting of each participant and reported back to the group. A period of discussion followed and the estimates for the questionnaire were then repeated by each expert and re-aggregated. A final report was prepared and sent to each participant with the possibility of further comment. The discussion period was considered valuable in generating consensus and exchanging information (Lake *et al*., 2006). Another example of the use of a systematic expert elicitation approach for source attribution is the study performed to estimate the fraction of human cases of enterically transmitted illness by five major pathways (food, environment, direct animal contact, human–human transmission, and travel) and by 11 groups within the food pathway. In this study, 16 food safety experts were asked to provide their estimates of the most likely range for each of the parameters, and joint probability distributions were created by probabilistic inversion (Havelaar *et al*., 2008).

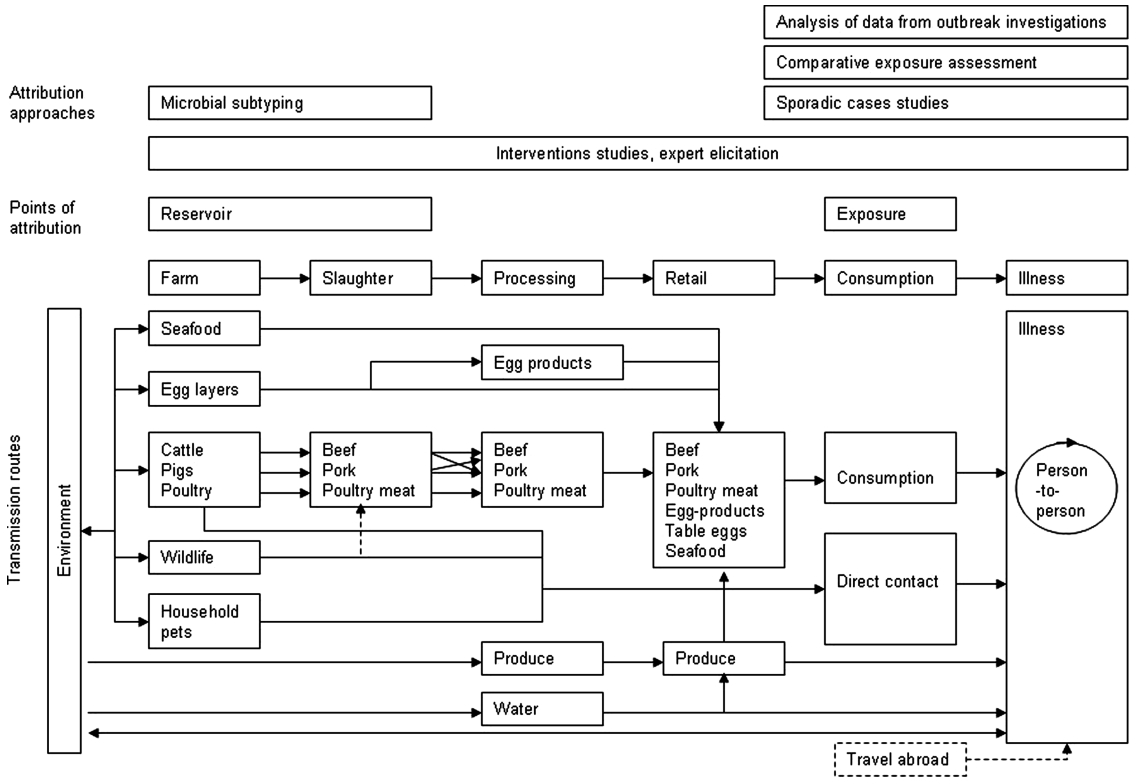

FIG. 1. Routes of transmission of zoonotic pathogens and points of human illness attribution. Travelling abroad is not considered to constitute a route of exposure by itself, as the main routes described will also apply for travelers. Still, it is common to include traveling abroad as a source on its own, which is the reason for including it in this diagram.

Although expert elicitation is useful when data are lacking, a limitation is that the conclusions are based on individual judgment, which may be misinformed or biased (Batz *et al*., 2004). Nevertheless, for some pathogens or diseases, expert elicitations may be the only available method for source attribution.

Points of Source Attribution

Human illness source attribution can take place at different points along the food chain, including at production, distribution and consumption. Source attribution at the point of production most closely represents attribution of the foodborne pathogen at the reservoir level. A reservoir is defined as an animal species or a nonanimal substance upon which the pathogen depends for its survival (Martin *et al*., 1987).

Because pathogens that cause foodborne disease may enter the food distribution chain at different points, the burden of illness caused by one disease attributed to specific sources may vary, depending on the point along the food chain the approach focuses on. For example, because cattle are the most important reservoir of *Escherichia coli* O157:H7, attribution of

Table 1. Harmonized Terminology and Methodology for Attribution of Human Illness to Specific Sources

Concept	*Definitions*	
Human illness source attribution	Partitioning of the human disease burden of one or more foodborne infections to specific sources	
Source	Origin of the pathogen causing infection, including animal reservoirs and vehicles, e.g., foods and water.	
Points of attribution	Points in the food chain where human illness attribution can take place, including production, distribution and consumption	
Approaches	*Methods*	
Microbiological approaches	Microbial subtyping	
	Comparative exposure assessment	
Epidemiological approaches	Studies of sporadic cases	Case–control studies
		Cohort studies
		Case-series studies
	Analysis of data from outbreak investigations	
Expert elicitation approaches		
Intervention studies		

E. coli O157:H7 infections will partition more illness to cattle at the point of production (reservoir) than it will partition to beef at the point of consumption, since other foods, besides beef, may be contaminated with the pathogen.

Some of the general methods to attribute foodborne diseases to specific sources work primarily at one point in the food chain (e.g., epidemiological approaches work primarily at the point of consumption), while other general methods (e.g., expert elicitation approaches) can be more generally applied. The general method for source attribution chosen, and consequently the point of attribution, will depend on the availability of data and on the risk management question being addressed. Figure 1 presents the major transmission routes for foodborne infections, including zoonotic infections, and indicates at which point in the transmission chain the different approaches attribute human illness.

Conclusion

To identify and prioritize appropriate food safety interventions and to precisely measure the impact of interventions aimed at controlling foodborne diseases, it is crucial to attribute the human disease burden of foodborne infections to specific sources. Several general methods for human illness source attribution have been developed and are being utilized, and the usefulness of each depends on the pathogen, the specific public health questions being addressed, the data availability, and the strengths and limitations of the different methods.

The limitations of the different general methods for source attribution may be overcome by the integration of different methods. The described approaches often focus on only one point of the transmission chain (e.g., point of reservoir or point of exposure), which means that choosing only one approach for source attribution may be inadequate to answer specific risk management questions. The integration of source attribution approaches aims at improving the estimates at the same point or at different points of attribution. Examples include blending data from studies of sporadic infections and analysis of data from outbreak investigations (integration at the same point of attribution), blending of case–control studies and the microbial subtyping approach, and integration of the microbial subtyping approach and the comparative exposure assessment approach (integration of different points of attribution).

Like epidemiological approaches in general, source attribution approaches describe an association between the outcome (the burden of a given disease) and the specific sources or exposures. Source attribution methods attempt to attribute the burden of disease at the population level, and do not describe causation of disease at the individual level.

Human illness source attribution is increasingly used to partition human illness to the most important sources and as such support risk management strategies. In this paper, we discussed the available source attribution approaches, and introduced nomenclature that should contribute to the harmonization of concepts, definitions, and methods. Table 1 summarizes our proposal for such harmonization. We conclude by encouraging other scientists that apply human illness source attribution to clearly address and define as a minimum: 1) the sources considered, 2) the point(s) of attribution addressed and 3) the attribution approach(es) chosen, because this is crucial for the sharing of knowledge between research groups and the comparison of results among the scientific community.

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Med-Vet-Net Work Package 28 was established to focus on the development and comparison of human illness attribution methods. Med-Vet-Net is a European Union "Network of Excellence" of 16 institutions in 10 countries, established to facilitate research for prevention and control of zoonoses and foodborne diseases. The kick-off meeting of the work-package joined members of the partner institutes and participants from the Centers for Disease Control and Prevention and the United States Department of Agriculture Food Safety and Inspection Service, and focused on the harmonization of terminology on attribution. A thorough discussion achieved agreement on important concepts, which have been presented here. More information about Med-Vet-Net and the work package can be found at http://www.medvetnet.org/cms/.

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Depuis la rédaction de cet article, d'autres travaux ont été publiés sur le sujet. Deux publications concernent la méthode d'attribution par typage microbiologique (Wilson, Gabriel et al. 2008; Mullner, Jones et al. 2009) et un autre, l'analyse de données issues d'investigation de foyers (Greig and Ravel 2009). Les travaux de Wilson *et al* (2008), portent sur *Campylobacter* et utilisent une méthode de typage génotypique basée sur la variabilité de la séquence de certains gènes (multilocus sequence typing). Alors que le modèle développé par Hald et al (2004) repose sur la prédiction du nombre de cas par source, celui-ci repose sur la distribution des cas entre les sources, mais sans tenir compte des niveaux de consommation, ni des différences entre source et entre types. Les travaux de Mullner *et al* (2009) proposent des variations au modèle de Hald *et al*, afin de traiter le problème de sa surparamétrisation structurelle et de l'adapter à des données de moindre qualité que celles utilisées par les Danois. Le troisième article est un exemple d'attribution à grande échelle. En effet, les données analysées concernent plusieurs pathogènes et une grande variété d'aliments. Elles sont issues de rapports et de publications internationales. Cette étude souligne les différences dans l'importance des sources pouvant exister pour certains pathogènes, dont *Salmonella*, entre différentes zones géographiques (Etats-Unis, Europe, Australie et Nouvelle Zélande,…), la limitation qu'est le biais de publication et la difficulté d'établir un lien spécifique entre certains pathogènes et un aliment, difficulté qui peut être liée notamment aux notions de contamination croisée et de contamination environnementale. Ce dernier point montre l'intérêt des méthodes d'attribution s'intéressant au réservoir animal en amont, telle que l'attribution par typage microbiologique.

5 L'attribution par typage microbiologique

5.1 Pourquoi choisir cette méthode

Comme l'indique la figure 0.7, la méthode d'attribution par typage microbiologique est particulièrement adaptée pour identifier les principaux réservoirs d'un pathogène en amont, au point élevage.

En France, le choix a été fait, pour lutter contre les salmonelles, d'intervenir dès l'élevage. A partir des années 80, un plan d'intervention a ciblé les volailles (poule pondeuse puis poulet de chair) et les mesures prises ont permis de diminuer le nombre de cas humains

(Poirier, Watier et al. 2008). Cependant, les travaux d'évaluation de l'impact du plan *Gallus gallus*, basés sur des analyses de séries chronologiques, soulignent l'impossibilité d'attribuer la baisse des cas liés à Typhimurium spécifiquement à ce plan, étant donné que des mesures étaient également appliquées dans la filière bovin lait et que Typhimurium est un sérotype très présent dans cette filière. Il apparaît donc ici essentiel de disposer d'une méthode permettant de mesurer de façon simultanée le nombre de cas lié à chaque filière, comme l'attribution par typage microbiologique. Ainsi, à partir du nombre de cas lié à chaque filière et de son évolution dans le temps, l'impact des changements de niveau de prévalence dans les filières, induits par les interventions, pourra être évalué de façon spécifique. De plus, les méthodes d'intervention permettent d'identifier des points d'intervention potentiels et de scénariser l'efficience des mesures envisagées (Miller, Liu et al. 2005). Cependant, cette méthode nécessite de disposer d'un grand nombre de données représentatives concernant les cas humains et la contamination des sources potentielles et suppose l'utilisation à grande échelle d'outils de typage discriminants.

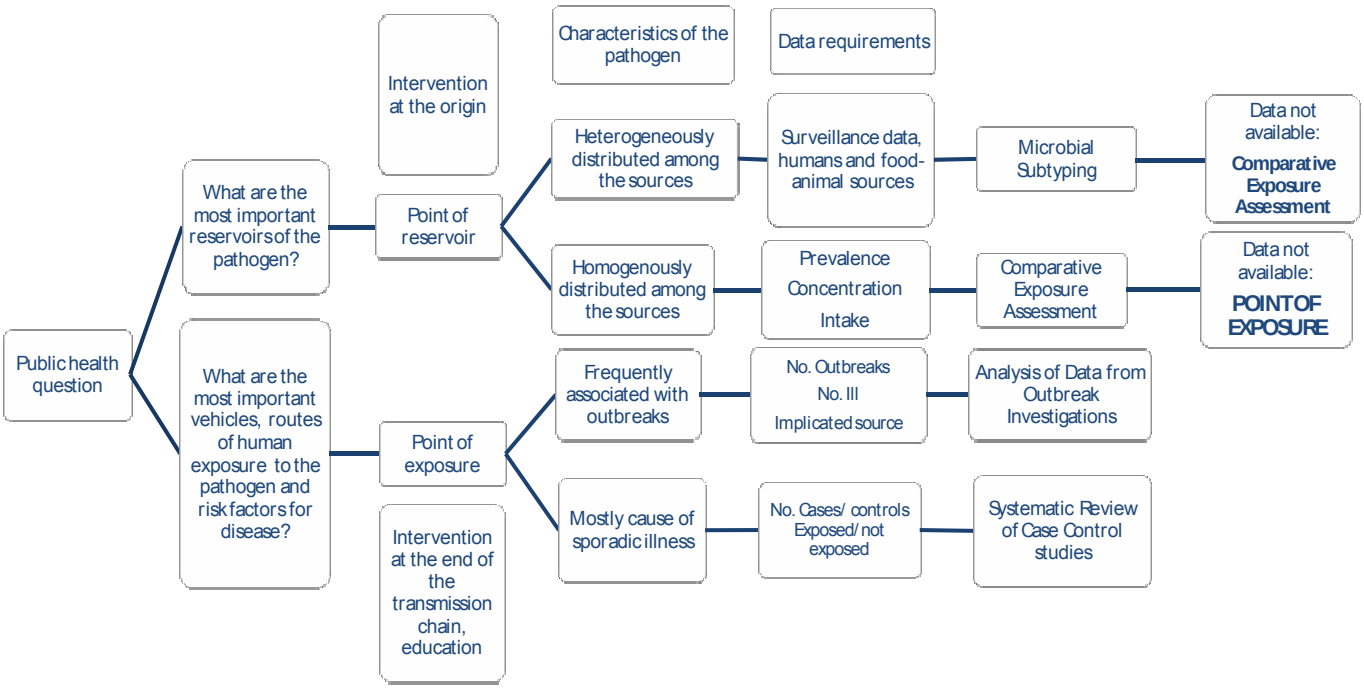

Figure 0.7 : Arbre de décision pour le choix de la méthode utilisée (Rapport final du groupe de travail européen sur l'attribution)

5.2 Principe de la démarche et utilisation du bayésien et de la modélisation bayésienne

La méthode, telle que décrite par Hald et al (2004, Cf § 4.1), repose sur l'estimation du nombre de cas d'un type (de salmonelle) dans une source (λ_{ij}) comme étant proportionnel :

- à la prévalence du type dans la source, ce qui correspond au niveau de contamination (p_{ij});
- à la quantité de source consommée dans la population (M_j) ;
- à la capacité de la source à véhiculer les salmonelles (paramètre source dépendant a_j) ;
- à la capacité du type à causer une infection (paramètre type dépendant q_i).

$$\lambda_{ij} = p_{ij} * M_j * a_j * q_i$$

avec i=(1,… I)) et j=(1,…, J)

Ainsi, l'ensemble des paramètres permettant de cheminer de la contamination des sources observée aux cas humains, notamment en terme de répartition de types microbiologiques est ici pris en compte. Ce nombre de cas attendu est confronté au nombre de cas observé par type, en se basant sur l'hypothèse d'une exposition poissonienne des cas humains :

$$o_i \sim \text{Poisson} (\Sigma_j \lambda_{ij})$$

De par son écriture, le modèle est surparamétré (Mullner, Jones et al. 2009). En effet, le nombre d'équations utilisées correspond au nombre de types (I), alors que le nombre de paramètres à estimer (a_j et q_i) est égal au nombre de types plus le nombre de sources (I+J). C'est pourquoi il est fait appel au bayésien[1] dans ce cas.

Le problème d'identifiabilité du modèle lié à la surparamétrisation a été traité jusqu'ici de deux façons différentes. Dans l'article originel (Hald, Vose et al. 2004), de l'information « informative » est utilisée en prior pour certains paramètres spécifiques au type. Ainsi pour les sous-types au sein d'un sérotype, les paramètres type spécifiques correspondants sont supposés égaux entre eux. De plus, le paramètre type spécifique du sérotype le plus fréquent parmi les cas humains est utilisé comme référence et fixé à une valeur arbitraire. Aucune hypothèse ni aucune information « informative » ne sont utilisées pour les autres paramètres à estimer à qui sont attribués des distributions *a priori* non informatives (lois uniformes). La solution de Mullner *et al* (2009) est toute autre, une structure hiérarchique

[1] Le principe de la démarche bayésienne est explicité dans l'annexe 1.

est introduite sur le paramètre type dépendant. Les q_i sont alors définis comme des observations aléatoires d'une distribution hypothétique des caractéristiques bactériennes. Cette distribution est définie comme étant une loi lognormale dont la moyenne est constante et fixée et dont le paramètre de précision est à estimer. Le nombre total de paramètres à estimer est donc réduit aux paramètres source-dépendants plus l'hyper-paramètre de la distribution lognormale commune des q_i, soit J+1 paramètres.

5.3 Le sous-typage

La méthode d'attribution par typage microbiologique repose sur la caractérisation microbiologique des souches bactériennes. Pour *Salmonella*, la méthode de sérotypage (Cf §1.1.2) est utilisée en première intention. Cependant, deux sérotypes, Enteritidis et Typhimurium, sont toujours isolés très fréquemment parmi les cas humains et sont de plus largement présents dans la plupart des sources. Il est donc nécessaire d'introduire une catégorisation des souches d'Enteritidis et de Typhimurium (Galanis, Lo Fo Wong et al. 2006), permettant si possible d'obtenir des sous-types inégalement répartis parmi les sources. La méthode utilisée jusqu'ici dans les travaux utilisant ce modèle d'attribution est la lysotypie (Hald, Vose et al. 2004; Hald, Lo Fo Wong et al. 2007; Pires, Nichols et al. 2008; Mullner, Jones et al. 2009). D'autres méthodes peuvent cependant être utilisées, elles sont soit d'ordre phénotypique (basées sur les caractères biochimiques des souches), soit d'ordre génotypique (Brisabois 2001; Foxman, Zhang et al. 2005; Deplano, Struelens et al. 2007; Hyytia?-Trees, Cooper et al. 2007; Weill 2008; Foley, Lynne et al. 2009).

5.3.1 Méthodes phénotypiques

Les méthodes phénotypiques portent sur l'expression de caractères génétiques parfois complexes. Le phénotype est donc un indicateur imparfait de la variabilité génétique sous-jacente. Dans le cadre d'études visant à déterminer des corrélations écologiques entre des populations de salmonelles issues de différents réservoirs (humains et animaux en l'occurrence), corrélations qui résultent de la parenté de ces populations, ces méthodes seront à utiliser avec précaution.

La lysotypie consiste à étudier la sensibilité des souches à une série de bactériophages sélectionnés. Cette technique est réservée à quelques centres de référence car sa mise en

œuvre, la lecture et l'interprétation sont délicates. Elle est à l'heure actuelle très peu utilisée en France.

L'antibiotypie est basée sur l'étude de la sensibilité des souches à un panel d'antibiotiques. Elle est fréquemment réalisée dans un but thérapeutique, et est également utilisée dans le cadre d'études épidémiologiques (Kariuki, Gilks et al. 1999; Harbottle, White et al. 2006). La résistance aux antibiotiques chez *Salmonella* est déterminée par des gènes situés pour certains sur le chromosome, auquel cas la résistance sera stable pour une lignée, mais également dans une grande partie des cas sur des éléments génétiques mobiles tels les plasmides. Dans ce cas, le profil de résistance sera moins stable et cette méthode sera à considérer avec circonspection quant aux résultats.

5.3.2 Méthodes génotypiques

Les méthodes génétiques visent à détecter le degré de parenté génétique entre différentes souches. Elles sont à ce titre particulièrement adaptées à une utilisation en attribution.
Trois types de méthodes peuvent être mentionnés ici, les méthodes basées sur la restriction de l'ADN, les méthodes basées sur l'amplification de gène et les méthodes basées sur le séquençage. Seules les méthodes les plus fréquemment utilisées sont présentées ici.

Les méthodes de restriction (RFLP, Restriction fragment length polymorphism) reposent sur l'utilisation d'enzymes de restriction, ou endonucléases, qui reconnaissent des sites de coupure dans l'ADN étudié. Leur action mène à la formation de fragments d'autant plus petits que les sites de coupures seront fréquents, fragments qui sont séparés par électrophorèse selon leur poids moléculaire (et donc leur taille).
L'électrophorèse en champ pulsé (PFGE), actuellement considérée comme la méthode de sous-typage moléculaire de référence pour *Salmonella* (des protocoles standardisés ainsi que des bases de données publiques sont développés pour permettre une comparaison de profils rapide et à grande échelle (Swaminathan, Barrett et al. 2001)), fait partie de ces méthodes. Les enzymes utilisées sont des enzymes reconnaissant des sites rares et générant donc un petit nombre de fragments de grande taille. Ces fragments sont séparés par une technique d'électrophorèse utilisant un champ électrique pulsé. La PFGE est très discriminante ; néanmoins, le polymorphisme obtenu après macro-restriction par l'enzyme XbaI (protocole PulseNet) est variable selon le sérotype. Ainsi le pouvoir discriminant, bon pour Typhimurium est relatif pour Enteritidis.

La ribotypie est une méthode RFLP combinant l'utilisation d'enzymes de restriction avec une révélation des fragments d'ADN migrés à l'aide d'une sonde spécifique, sonde ciblant ici les gènes codant pour les ARN ribosomaux. Le pouvoir discriminant de cette méthode est très variable selon les sérotypes. Néanmoins, elle fait l'objet d'une automatisation permettant standardisation et réduction du temps de manipulation.

Les méthodes d'amplification par PCR (Polymerase chain reaction) sont basées sur l'utilisation de l'amplification de séquences particulières du génome, à l'aide d'une ADN polymérase. Cette méthode permet de détecter la présence ou l'absence de facteurs génétiques particuliers dans le génome de la souche étudiée. Ces méthodes nécessitent de disposer d'amorce correspondant aux facteurs recherchés.
La technique RAPD (Random Amplification Polymorphic DNA[1]), basée sur l'utilisation d'une amorce de séquence aléatoire s'hybridant à plusieurs endroits du génome. A la différence de la PCR classique, l'utilisation d'amorces de séquences arbitraires présente l'avantage de ne nécessiter aucune information préalable sur la séquence de l'ADN étudié. Cette technique fréquemment utilisée permet une bonne discrimination au sein d'un sérotype donné. Néanmoins, de par son manque de reproductibilité et de répétabilité, elle n'est pas adaptée à un suivi de souches à long terme.
La MLVA (Multilocus VNTR[2] Analysis) est basée sur l'examen du polymorphisme de loci comprenant chacun un nombre variable de souches répétées en tandem (VNTR). La méthode consiste à amplifier par PCR ces régions répétées et à analyser la taille des fragments avec un système haute résolution. Il s'agit d'une méthode reproductible et, de plus, particulièrement discriminante pour le sérotype Typhimurium.

Enfin, les méthodes de séquençage reposent sur la détection de variations dans la séquence de certains gènes codant pour les enzymes métaboliques afin de mesurer la distance génétique multi-locus (MLST, Multilocus sequence typing). La grande discrimination du MLST combinée au procédé relativement lent d'accumulation de changements dans la séquence des gènes métaboliques au cours du temps rend cette méthode idéale pour des études épidémiologiques globales.
Une variante de cette méthode est basée sur la détection de polymorphisme pour des nucléotides isolés (SNP, Single nucleotide polymorphims). Cette dernière option est plus simple mais moins discriminante que la méthode MLST.

[1] Desoxyribonucleic acid
[2] VNTR : Variable number of tandem repeat

Les méthodes MLST et SNP sont particulièrement adaptées aux études de génotypage à grande échelle. Le principal avantage des méthodes de séquençage pour le typage des souches est l'interprétation non ambigüe des résultats permettant la création de bases de données internationales.

5.3.3 Utilisation en attribution

La méthode de sous-typage idéale pour l'attribution sera une méthode combinant la facilité de mise en œuvre en routine, un bon pouvoir discriminant, une standardisation permettant l'obtention de résultats harmonisés entre laboratoires, et une bonne répétabilité. La méthode doit présenter également des coûts raisonnables afin d'être utilisable à une large échelle. Les caractéristiques des méthodes présentées selon ces critères sont exposées dans la table 0.7.

	Pouvoir discriminant	Reproductibilité	Stabilité	Lisibilité	Coût de mise en œuvre	Exemples d'application à Typhimurium et Enteritidis
Lysotypie	Passable	Passable	Faible	Faible	Elevé	(Boxrud, Pederson-Gulrud et al. 2007) (Guerra, Schrors et al. 2000; Tsen 2002b; Botteldoorn, Herman et al. 2004; Lukinmaa, Nakari et al. 2006)
Antibiotypie	Variable	Bonne	Passable	Excellente	Moyen	(Kariuki, Gilks et al. 1999; Tsen 2002b; Botteldoorn, Herman et al. 2004)
PFGE	Excellent (Selon le nombre d'enzymes utilisé)	Bonne (protocole standardisé Pulse net)	Passable	Bonne	Elevé	(Kariuki, Gilks et al. 1999; Botteldoorn, Herman et al. 2004) (Guerra, Schrors et al. 2000; Bender, Hedberg et al. 2001; Gudmundsdottir, Hardardottir et al. 2003; Fakhr, Nolan et al. 2005; Gebreyes, Altier et al. 2006; Harbottle, White et al. 2006; Lukinmaa, Nakari et al. 2006; Woo and Lee 2006; Boxrud, Pederson-Gulrud et al. 2007)
Ribotypie	Passable	Bonne	Bonne	Bonne	Elevé	(Guerra, Schrors et al. 2000; Jeoffreys, James et al. 2001) (Millemann, Gaubert et al. 2000; Liebana, Garcia-Migura et al. 2002a)
RAPD	Bon	Passable	Passable	Passable	Moyen	(Malorny, Schroeter et al. 2001; Tsen 2002b; Delgado Ronda, Mun?oz Bellido et al. 2006; Woo and Lee 2006)
MLVA	Bon à excellent	Excellente	Bonne	Excellente	Moyen	(Boxrud, Pederson-Gulrud et al. 2007)
MLST	Bon à excellent	Excellente	Bonne	Excellente	Elevé	(Fakhr, Nolan et al. 2005)
SNP	Excellent	Excellente	Bonne	Excellente	Moyen	

Table 0.7 : Comparaison des performances de systèmes de typage épidémiologique d'après (Foxman, Zhang et al. 2005; Deplano, Struelens et al. 2007)

Problématique

Les salmonelles font partie des agents zoonotiques majeurs dont la maîtrise répond à des objectifs de santé publique. Il s'agit à ce titre d'une zoonose emblématique puisque c'est la première zoonose d'origine alimentaire faisant l'objet d'une approche communautaire intégrée quant à sa surveillance harmonisée et à sa maîtrise dans les filières avicoles et porcines. De plus, alors que production et approvisionnement se globalisent, la France, grand pays producteur de denrées animales, doit faire face à un environnement fortement concurrentiel. Dans ce contexte, les questions de santé publique peuvent constituer des arguments scientifiques forts pour certains pays, afin de limiter les importations ou imposer des contraintes sanitaires sur les denrées importées. Les salmonelles sont au cœur de ce type de préoccupations. Le Danemark, fort d'une démarche volontariste de lutte en filière porcine, a mis en œuvre une démarche d'attribution des salmonelloses humaines aux denrées animales afin de déterminer les contributions relatives de celles-ci et l'allocation des efforts de maîtrise. La France a quant à elle initié très tôt une lutte volontariste contre les salmonelles en filière avicole qui a permis de diminuer le nombre de cas humains. Cependant, les salmonelles demeurent un enjeu de santé publique majeur et dans un contexte de productions animales diversifiées et de rationalisation des efforts des pouvoirs publics, il apparaît crucial pour la France de se positionner sur la démarche d'attribution. Dans le but d'identifier de potentielles interventions au niveau de l'élevage, le choix a été fait de s'approprier l'outil d'attribution par typage microbiologique et d'avoir un regard critique sur cette démarche.

L'objectif de cette thèse a donc été d'évaluer la pertinence de l'outil d'attribution par typage microbiologique en répondant aux deux questions suivantes :

- les données de surveillance produites en France sont-elles suffisantes et adaptées à une telle démarche ?
- le modèle développé par Hald *et al*, est-il robuste et adapté à une utilisation avec des données de surveillance différentes de celles produites au Danemark ?

Les travaux seront ainsi présentés en deux temps. Une première partie est consacrée aux données, sous la forme d'une revue du système de surveillance français puis de l'exposé des

étapes de sélection et d'adaptation des données nécessaires à la démarche d'attribution par typage microbiologique. La deuxième partie concerne plus particulièrement l'outil de modélisation. Dans un premier temps, les essais d'adaptation du modèle en Europe sont présentés au travers d'un projet de publication commun aux participants du projet européen sur l'attribution de sources dans lequel s'inscrit cette thèse. Cet article souligne les difficultés de certains pays dans cet exercice. Nous avons donc cherché à déterminer la raison méthodologique de ces problèmes par une analyse de la sensibilité du modèle à l'information *a priori,* ce qui nous a amenés à proposer une alternative permettant d'obtenir des résultats plus robustes. Enfin, nous nous sommes intéressés à l'impact de la qualité des données sur les résultats d'attribution produits par le modèle à partir des données françaises, dont la particularité est de disposer à la fois de données de surveillance passive et de surveillance active concernant la contamination des sources. Enfin, cela nous permet de dégager les potentialités et évolutions nécessaires du système de surveillance français pour une utilisation des données produites dans une démarche d'attribution, de discuter de l'impact respectif de la méthodologie et de la qualité des données sur les résultats d'attribution et, plus largement, des limites et potentialités de cette démarche.