Investigating Outbreaks of *Salmonella* Typhimurium Using Case-Control Studies, with a Reference to the One Health Approach

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Abstract

An outbreak is defined as the occurrence of disease cases in excess of normal expectancy within a particular area and a given time. Foodborne outbreaks caused by gastrointestinal bacteria such as *Salmonella* Typhimurium are among the most commonly reported and most extensively investigated. The classic outbreak investigation follows a series of well-defined steps which lead to a faster confirmation of the source and hopefully preventing of further cases. These steps are ideally undertaken using a One Health cross-sectorial collaboration approach involving partners from public health, food safety, and the veterinary and environmental sectors. In order to firmly identify the source of the outbreak, descriptive epidemiology is often combined with more robust evidence from analytical epidemiology such as a case-control study. A case-control study assesses whether a specific exposure is associated with illness, firstly by identifying cases (persons known to have been ill) and controls (persons who have not been ill, used as a reference group), and then retrospectively through interviews determining specific exposures for all persons. This information ultimately leads to the calculation of an odds ratio (see Note 3) which indicates the strength of the association between specific exposures and the outcome (illness or no illness). A well-conducted case-control study may substantiate or form core evidence as to the vehicle of a foodborne outbreak and is often a very important investigation tool, particularly in situations where microbiological proof cannot be obtained.

**Key words**  Outbreaks, Clusters, Foodborne, Epidemiology, *Salmonella*, One health, Outbreak investigation, Case-control, Odds ratio, *Salmonella* Typhimurium, Gastrointestinal bacteria

1 Infectious Disease Outbreaks

“Outbreak” is a word which not only generates a wealth of public interest but also often sets in motion a long line of well-defined processes for the infectious disease epidemiologists charged with detecting and investigating the outbreak. The World Health Organization (WHO) defines a disease outbreak as the occurrence of disease cases in excess of normal expectancy [1]. An outbreak is declared based on the sheer number of cases with particular...
reference to the usual or expected frequency of the given disease in
the affected area, in the same population and at the same time of
the year. The size and severity of the outbreak reflect not only the
pathogen involved but also a range of factors relating to the
affected population such as vehicle, exposure, and immunity. Dis-
ease outbreaks are also referred to as “clusters,” “epidemics,” or
“pandemics.” A cluster follows more or less the same definition as
an outbreak and is often considered to be the first signal of an
outbreak, although cases may cluster in space or time. An epidemic
is a severe outbreak with a fast increase in size and geographical
spread, while a pandemic is defined by the WHO as the worldwide
spread of a (new) disease. The recent Ebola virus outbreak in West
Africa is a good example of an epidemic [2] while the Spanish Flu
during World War I and the 2003 outbreak of severe acute respira-
tory syndrome (SARS) have both been categorized as pandemics
[3, 4].

All infectious diseases can give rise to outbreaks. Respiratory
viruses transmitted from person to person such as the seasonal
influenza are responsible for large outbreaks in temperate regions
across the world every year [5] and hemorrhagic viruses which
originally emerge in animals have given rise to serious outbreaks
of Ebola and Lassa fever in Africa [6, 7]. Food represents an
important vehicle for infectious pathogens of substantial public
health significance; in 2010 it was estimated that 600 million peo-
ple became ill and 420,000 died from a foodborne illness [8]. In
high-income countries, foodborne disease outbreaks are among the
most commonly reported and most extensively investigated. This is
partly because of the potential to affect large numbers of people,
considering the extensive distribution of food items, and because of
the strong public health and economic burden [9], making prompt
detection and solving of outbreaks beneficial not only to the indi-
vidual but also to society as a whole. Investigation and control of
foodborne disease outbreaks are facilitated by a number of practical
factors: the ability to detect cases (confirmed infections are often
mandatorily notifiable to health authorities), the potential of
tracing back infections to a single food/contaminated source
(rather than tracing person contact), and being able to prevent
further cases by withdrawing the contaminated food source.

Salmonella is one of the most commonly reported foodborne
infections across the world. The primary symptom of an infection
with Salmonella is acute gastroenteritis (diarrhea, abdominal
cramps, sometimes fever, and occasionally vomiting) which on
average begins 24–72 h after exposure. The disease is usually self-
limiting and in most cases does not require treatment unless symp-
toms are severe or other complications arise. The Salmonella genus
consists of more than 2500 different serotypes. The majority of the
serotypes that are responsible for disease in humans belong to a
single species, Salmonella enterica. Non-typhoidal Salmonellae are
zoonotic bacteria which live in the gut of different animals but can also be transmitted to humans. *Salmonella* Typhimurium is an important non-typhoidal serotype often associated with outbreaks. Transmission of *Salmonella* Typhimurium to humans is by ingestion of contaminated or undercooked food (e.g., pork, beef, and eggs) and by direct contact to the infected animal. Although incidences of *S*. Typhimurium infection have decreased during the past decade in many high-income countries [10, 11], salmonellosis has a higher case fatality than other comparable gastrointestinal infections and is still the leading cause of bacterial foodborne disease outbreaks in Europe, the USA, and Australia [11–13]. It is believed that the majority of *Salmonella* outbreaks remain unrecognized as involved patients do not seek medical attention or clustering of cases in space and time is not detected by responsible authorities. For those outbreaks which are recognized, many are either not investigated or investigated in a poor manner because the necessary resources and skills are not available. However, many published outbreak reports point to successful detection, investigation, and solving of salmonellosis outbreaks. Such *Salmonella* outbreaks are in general associated with food; in Europe it is mainly pork, poultry meat, and eggs [14, 15]; in the USA and Australia eggs are the most frequently implicated vehicle [16, 17] while in Latin America different types of meats are the most common cause of outbreaks [18]. In the USA, several multistate *Salmonella* outbreaks have also been linked to direct animal contact such as pet turtles, hedgehogs, and chickens [19–21]. Outbreaks of *S*. Typhimurium in particular are often linked to meat, especially pork [22] but also chicken and eggs [23, 24], and nonmeat produce such as lettuce and fruit juice [25–27].

Because the pathogen is zoonotic and infection is acquired through animal contact or ingestion of food, detection and investigation of foodborne disease outbreaks is a multidisciplinary task. To fully understand the outbreak epidemiology, prevent further cases, and reduce the risk of a similar outbreak happening again, we need to consider evidence from public health, veterinary, food safety, and environmental sectors in a so-called One Health approach. One Health recognizes that human health is strongly connected to the health of animals and the environment and that successful disease control and interventions require collaboration between the different communities [28]. Although One Health is a relatively new term, the idea as a whole has been operating for decades, and One Health cross-sectorial collaborations are set up in many countries, particularly for detecting and investigating foodborne disease outbreaks. For instance, One Health approaches were crucial in investigating the 2016–2017 European salmonellosis outbreak caused by eggs [29] and for the serious multinational outbreak of *Escherichia coli* linked to bean sprouts, causing more than 30 lives across Europe in 2011 [30].
Detection of a foodborne disease outbreak can occur in several ways. Most commonly, health authorities are notified by local medical or microbiological professionals who observe an unusual increase in the number of a specific illness/complaint (e.g., diarrhea) or in the amount of samples positive for a certain pathogen. In some countries, it is also possible for members of the public to directly contact health authorities and report clusters of disease among their own family or friends after eating the same food at a party or in a restaurant. This contact is by either telephone or email or using special online links. Finally, outbreaks are also detected through disease surveillance systems where algorithms may be applied to national or local surveillance data to identify when the number of cases (or frequency of symptoms) exceeds a specified threshold. Microbiological surveillance, involving subtyping of patient isolates, is often necessary to detect outbreaks this way. The latter method relies on patients seeking medical attention, supplying a sample, analysis of the sample (often subtyping by a reference laboratory), a confirmed diagnosis (see Note 4), and the case notified to the surveillance system. It is therefore the slowest method of detection but also the one that allows for large dispersed outbreaks to be detected.

Once a cluster of disease or symptoms is detected, a formal outbreak investigation can be set in motion. This involves initiating a globally accepted line of processes which consists of the ten outbreak investigation steps, created to harmonize, speed up, and optimize the investigation (Fig. 1). The steps are presented in a conceptual order and several steps may, and often should, be done at the same time. The framework presented is a general version, and many outbreak investigation groups follow slightly amended versions according to the methods and practices which best suit their resources.

The authorities investigating the outbreak will initially consist of representatives from the public health sector. However, when the involved pathogen is confirmed to be foodborne/zoonotic, all relevant actors from the One Health chain should be included. Ideally, the One Health setup should already be established before the outbreak occurs to ensure smooth and efficient communication and coordination. The Danish Central Outbreak Management Group [31] consists of public health representatives (epidemiologists/medical doctors and microbiologists), veterinarians, and food safety experts. This group meets weekly, even if there are no ongoing outbreaks, a deliberate strategy to uphold familiarity between the group members, optimize communication flow, and strengthen overall collaboration. In the outbreak investigation process, steps
### 1. Confirm that an outbreak exists
- Determine if cases cluster and review past incidence of the pathogen in the affected area(s)
- Assess if the cluster reflects changes in diagnostics, surveillance or population

### 2. Verify the diagnosis
- Describe clinical symptoms
- Collect and analyse biological specimens
- Review laboratory results

### 3. Set up the outbreak investigation group
- Include representatives from public health, food safety and veterinary sectors
- Ensure regular meetings and strong communication flow

### 4. Define and identify cases
- Define ‘person’ (type of illness/diagnosis), ‘place’ (location of cases or suspected exposure) and ‘time’ (onset of symptoms, time of diagnosis or time of exposure)
- Create a linelist of cases including clinical, demographic and exposure information

### 5. (Active) case finding
- Search for cases in surveillance records
- Notify GPs and diagnostic laboratories to observe for new cases

### 6. Perform descriptive epidemiology
- Describe the outbreak in ‘person’, ‘place’ and ‘time’

### 7. Generate hypotheses
- Interview cases using trawling (hypothesis-generating) questionnaires of all relevant exposures

### 8. Test the hypotheses
- Analytical epidemiology: case-control or cohort studies
- Microbiological tests
- Food and veterinary trace-back
- Compare results with established facts

### 9. Implement control & prevention
- All relevant actions (e.g. product recall)

### 10. Communicate findings
- Writing and disseminating full reports
- Meetings and discussions with relevant stakeholders
- Local and mass media

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*Fig. 1 The ten steps of an outbreak investigation*
1–4 are a collaboration between all parties in the group. During the active case finding, descriptive, and hypothesis generation stages (5–7), public health representatives are primarily responsible for gathering most of the results which are then evaluated and discussed by the whole group. Prevention and control measures are mainly undertaken by the veterinary and food safety sectors (e.g., withdrawing contaminated food from the market, following up on restaurants with poor kitchen hygiene, cleaning of slaughterhouses). Finally, and importantly, communication of findings to stakeholders at all levels, from the public to specific authorities, should be correlated within the group to ensure that all communications are clear and concise and convey the same message. There are two primary types of communication: (1) “acute” in which the primary goal is to halt the outbreak and (2) “long term” which aims to summarize the lessons learnt from the outbreak in order to prevent a similar situation occurring again.

3 Methods

In many outbreak settings, the descriptive epidemiology alone or in combination with hypothesis-generating patient interviews will have generated evidence to suggest a likely source of the outbreak and general mode of transmission. However, the specific exposure that caused the disease may still be unknown or insufficiently substantiated and prevention and control measures cannot be initiated. More robust evidence from analytical epidemiology is needed and this is often undertaken in a cohort or a case-control study. A cohort study is used for point-source outbreaks where a well-defined group of people (the cohort) have been exposed to the same food at the same place and time and then subsequently evaluated with respect to outcome and exposures. In contrast, a case-control study is used for dispersed outbreaks and aims to assess whether a specific exposure is associated with illness, firstly by identifying cases (persons known to have been ill) and controls (persons who have not been ill, used as a reference group), then retrospectively determining specific exposures for all persons, and comparing the frequency of these exposures between the ill and the non-ill (Fig. 2). In an outbreak situation, case-control studies allow for examination of multiple exposures or risk factors, but they are prone to biases (see Note 1) and often hampered by the difficulty of identifying an appropriate control group. Incidences and relative risks cannot be calculated from case-control studies (see Note 3), but this is considered irrelevant in an outbreak setting where the objective is not to estimate risk but to identify the (often single) exposure which made people ill.
3.1 Steps Within the Case-Control Study

The general design of an outbreak case-control study consists of six steps as highlighted below. While undertaking the various steps, it is important to keep in mind that speed and timing are crucial. Firstly to interview cases while their memory of specific exposures is still clear and secondly to stop the outbreak by identifying and potentially recalling the source as quickly as possible.

1. Identify and Select Cases. A case definition has already been developed during stages 3–4 of the outbreak investigation, but it may be necessary to revisit this when designing the case-control study. The solid definition covers (a) clinical and laboratory criteria, i.e., confirmed infection with the *Salmonella* Typhimurium outbreak strain; (b) a period of time during which the person must have had symptoms or a confirmed diagnosis; (c) restriction by “place,” for instance working in the same place, living in the same town or region, or having visited the same restaurant; and, less often, (d) restriction by “characteristics,” for instance being of a certain age and having special comorbidities. During the outbreak investigation, a line list of cases is constructed. New cases are added to this list as the outbreak continues. These new confirmed cases (according to the definition) should be included in the case-control study prospectively as they are identified through the surveillance system. If no specific laboratory criteria exist, cases may be
included based on symptoms alone. If possible, it is preferable to exclude those cases who have already been interviewed during the initial steps of the investigation, focusing on including newly identified cases in order to reduce information bias (see Note 1).

2. **Identify and Select Controls.** This is an important step as inaccurate selection of controls can lead to bias (see Note 1). Controls should represent a “healthy” sample of the population from which the cases arose; they should be randomly selected and provide a reliable estimate of the expected exposure in the population. To increase the likelihood of statistically valid results, more controls than cases (often in the ratio of 1:2 or 1:3) may be used. Selection of controls should always reflect the scope and characteristics of the outbreak. In an outbreak of *Salmonella* Typhimurium, controls can be randomly selected from the same workplace or community, among relatives or friends of the cases, from phone registers, or using “random digit dialing” (a widely used method for selecting participants in surveys by generating telephone numbers randomly).

3. **Matching.** Cases and controls are often matched on one or several variables to control the effect of confounding (see Notes 1 and 2). Matching is at the individual level or by frequency. In individual matching, each case is matched to one or several controls, based on possible confounding factors (often age, gender, and geographical location). For instance, if a case is a 25-year-old female, living in the capital city, then 2–3 controls of the same gender and age and living in the capital city are selected. The result is pairs of individuals belonging to the same study population and sharing common characteristics. Matching by frequency is for groups of subjects where a number of controls are matched to the same number of cases, taking into account possible confounders. For example, a group of 20 cases (15 men and 5 women) living in the capital city could be matched to a group of controls with a male-to-female ratio of 3:1 living in the capital city.

4. **Collect Data.** After defining cases and controls, it must be decided which data to collect. Some exposure data have already been collected during the descriptive epidemiology phase. Outbreak case-control studies therefore use short and concise, tailored questionnaires focusing on the specific exposures hypothesized to be the vehicle of the outbreak (for instance: “did you eat hotdogs?”). Information should always be collected in a defined period before the onset of disease (for cases) or interview (for controls) (see “Analyze Data”). Also consider exclusion questions for cases and controls. For instance, in a domestic outbreak, all cases and controls who have traveled abroad before the interview are excluded. Controls who
experienced symptoms of gastrointestinal illness (i.e., diarrhea and/or vomiting and/or fever) would normally also be excluded. These questions should appear early in the questionnaire in order not to undertake a thorough interview of a person who will be excluded. Exposure data are traditionally collected by phone interviews with cases and controls; however with the advent of electronic tools consider using online questionnaires when feasible. This eliminates the need for a long phone interview and reduces the inconvenience to participants. With this approach, ensure that relevant general data protection regulations for collecting data online are followed.

5. **Analyze Data.** Following data cleaning, calculate the frequency of each exposure in the case and the control groups separately. Then measure the strength of the association between the exposure and the outcome (illness or no illness) by calculating an odds ratio (OR). An OR represents the odds that an outcome will occur, given a particular exposure, compared to the odds of the outcome occurring if the exposure is absent (see Note 3). The most simple formula for calculating the OR uses a classic $2 \times 2$ table. If the OR equals 1, then the exposure and the outcome are not associated. If the OR is greater than 1, the exposure is associated with the illness. All presented ORs must as minimum include a confidence interval as this allows assessment of the significance of the association between exposure and outcome. A confidence interval that includes 1.0 (at the 5% significance level) indicates that the association could have arisen by chance alone and that the association is not statistically significant. Calculations of ORs and confidence intervals are frequently undertaken in a statistical software program, and in this case a p-value (probability value) is also given with the confidence interval. Matched data are analyzed by calculating matched odds ratios (mORs) for which there are different methods but the primary approach is using conditional logistic regression analysis in a statistical software program. If there is a likelihood of confounding or different exposures being linked to the outcome, the analysis can be adjusted for these in a multivariate analysis.

6. **Draw Conclusions.** Firstly, conclude the results of the data analysis by evaluating the calculated ORs. In general, the most likely source of the outbreak is the exposure, which has the highest OR by far (rather than just the highest among a group of high ORs). Several exposures with high ORs can indicate several sources caused for instance by cross-contamination of fresh produce (e.g., raw meat juice contaminating salad and fruit) or statistical confounding (see “Notes”). To conclude that a specific exposure is the source of the outbreak, the majority of cases should of course also have
been exposed to this source. Further, the exposure should make sense in relation to what else is known about the outbreak such as initial hypotheses, patient demography, and previous outbreaks with the same pathogen. The outbreak investigation team will report the findings and conclusions in firstly a verbal report to the other active partners in the investigation (it is crucial to notify food safety authorities if a specific food source has been identified) and ultimately in a written report to other stakeholders and interested parties. Also here, the importance of communicating across the One Health chain should be emphasized. The timing and content of information targeted at the public should be agreed between all partners in the investigation team.

3.2 Two Salmonella Typhimurium Outbreaks Investigated by Case-Control Studies

3.2.1 A Small Localized Outbreak Linked to Consumption of Smoked Salami

In April 2010, the national Salmonella reference laboratory at Statens Serum Institut (SSI, the national institute for infectious disease control) in Denmark reported an increase in diagnosed infections of S. Typhimurium [32]. A number of these infections had the same bacterium-type profile and an outbreak was declared. The profile of the S. Typhimurium outbreak strain was verified by multilocus variable-number tandem repeat analysis (MLVA, see Note 4). A case was defined as a person residing in Denmark, who became ill with symptoms of gastroenteritis after first April 2010, whose culture results yielded the outbreak strain and who had not traveled abroad in the week before developing symptoms. Following hypothesis-generating telephone interviews of cases using a trawling questionnaire, a salami product was suspected as the source of the outbreak and an individually matched case-control study was initiated to confirm this. Controls were selected from the Danish population register and matched to cases on date of birth, gender, and municipality of residence. Three controls were selected for each case, and ultimately 17 cases and 79 controls were included. All participants were interviewed by phone using a tailored questionnaire focused on consumption of meat, cold cuts, and salami products as well as supermarkets frequented. Both cases and controls were asked if they had symptoms of gastroenteritis or had traveled abroad in the week before interview, and controls who answered yes to these questions were excluded. Matched odds ratios (mORs) were calculated for each exposure. These showed that consumption of a smoked deer and pork salami, purchased in a specific supermarket, was associated with a 150 times increased odds of developing illness. This result was statistically significant and no other food products were associated with elevated ORs. Further, 88% of cases reported having eaten the salami, a specific supermarket chain was most often used for purchases, and the salami had been on sale in this chain in the same period as cases appeared. Thus it was concluded that the outbreak was likely caused by this salami. By the time the case-control study concluded, the
salami batch had passed its expiry date and was no longer sold in the supermarket. Therefore, it was too late to recall the product and too late to sample the salami for microbiological examination. The salami was produced in another European country, and the Danish One Health outbreak investigation team corresponded with the relevant authorities in this country to trace the salami origin and identify the contamination point. Unfortunately, this was not possible. However, the suspicion based on the epidemiological investigation, in particular the case-control study result, was so strong that the Danish food authorities issued a warning to consumers to dispose of any packages of the salami with a specific use-by date. This outbreak exemplifies how a cluster of patients with a MLVA similar \( S. \) Typhimurium infection can lead to a rapid declaration of an outbreak. The descriptive epidemiology identified a likely source, which was confirmed by analytical epidemiology in the shape of an individually matched case-control study. Prevention and control measures consisted of advising consumers to dispose of the product. The conclusions of the outbreak were communicated to the public in a common statement by the SSI, the Danish Veterinary and Food Administration, and the National Food Institute at the Technical University of Denmark.

3.2.2 A Long-Lasting National Outbreak Caused by Several Pork Products

In March 2010, the National Food Institute in Denmark reported an increase in the number of multiresistant \( S. \) Typhimurium isolates from pork meat and pork products, primarily originating from a single pig slaughterhouse [33]. Although no human cases had been confirmed at this time, a cluster investigation was initiated to trace contaminated foods. One month later, human cases with the same type of \( S. \) Typhimurium were reported, and the “food cluster” was now considered an outbreak involving humans. The outbreak strain was defined by the phage-type U323 and MLVA profiles. The MLVA profile was common among \( S. \) Typhimurium and could not alone verify a specific enough laboratory diagnosis (see Note 4), and therefore phage typing was important for defining cases. A case was defined as a person residing in Denmark from whom the outbreak strain U323 was isolated and who had not traveled abroad in the week before disease onset. In the following months, an increasing number of cases throughout Denmark fulfilling the case definition were reported. These cases were interviewed by telephone using a hypothesis-generating questionnaire. There was no apparent common exposure for the cases; however all consumed pork and many had consumed ready-to-eat pork products, including a special smoked spreadable sausage called \( \text{teewurst} \), in the days before symptom onset. In July, an increasing number of cases reporting eating \( \text{teewurst} \) and a case-control study was initiated to assess whether this product could be the source of the outbreak. Following the case definition, 19 cases were included in the study. The study also included 41 controls selected from the Danish
population register and matched to cases by date of birth, gender, and municipality of residence. Cases and controls were interviewed using a tailored questionnaire focused on consumption of meats, cold cuts, and other ready-to-eat products. The results of the case-control study confirmed that not all 133 S. Typhimurium U323 infections during the outbreak could be explained by consumption of teewurst, but that this sausage had most likely caused the cases notified during July and August. The company that produced the teewurst had pork supplied by the slaughterhouse from which the first food isolates originated. The teewurst was recalled from the market and consumers were advised to dispose of purchased packages. Because of the many S. Typhimurium U323 isolates which were traced back to the specific pig slaughterhouse, the outbreak investigation group concluded that the outbreak source was pork or pork products and that teewurst in particular had caused a sub-outbreak in the latter part of the outbreak period. This outbreak demonstrates the importance of communication and collaboration across the One Health chain. The outbreak strain was identified in the food sector before human cases arose, and this allowed public health authorities to be vigilant for human infections with the same strain. When these were confirmed, the outbreak setup had already been initiated and the investigators were able to quickly assess hypotheses. The case-control study was instrumental in confirming a specific food item as a source and also highlighted that, although pork was the main source of the outbreak, cases were most likely infected through different products.

4 Notes

1. Although all studies can be affected by bias, case-control studies are particularly susceptible because they are retrospective in nature and because the validity of the results relies on control persons forming an unbiased sample of the population. Several types of biases need to be considered:

   - **Selection bias.** Arises when cases and controls are noncomparable, most importantly when controls are unrepresentative of the population which produced the cases. In an outbreak situation, this could be an outbreak of S. Typhimurium among school-aged children where the majority of selected controls are adults. Selection bias is also introduced when exposed cases are more likely to be selected than unexposed cases. Selection bias is reduced or eliminated by careful adherence to case and control definitions and by ensuring that controls are randomly selected from the best possible background population. Particular care should be taken when interpreting results where controls have been
drawn from a particular subgroup of the source population, such as patients, family members, or similar as these may not eat or do the same as the general population which will then lead to skewed results when comparing with cases.

- **Information bias.** Arises through systematic differences in the way exposure data are obtained from cases and controls. Information bias can be related to the **observer** (the investigator has previous knowledge of the hypothesis and collects information to reflect this), the **interviewer** (asking leading questions), or the **participants** which may not remember in detail what they ate or did (this may lead to recall bias if cases tend to report exposure experiences differently than controls for instance because of a preconception of how they were infected). The best method for reducing observer and interviewer bias is proper training of observers and interviewers and use of standardized, calibrated questionnaires, the latter of which is of course not always possible in an outbreak situation where questionnaires are often set up for that particular occasion. Blinding observers and interviewers to the exposure and disease status of the individual is also efficient but rarely possible in an outbreak setting. Recall bias can be reduced by blinding study participants to the hypothesis regarding the outbreak source.

- **Confounding.** Provides an alternative explanation for an association between an exposure and an outcome. Confounding occurs when an association between the exposure and outcome is clouded because the exposure is also correlated with another exposure. The association between the “real” exposure/risk factor is therefore not correctly estimated. This is likely to happen in an *S.* Typhimurium outbreak where cases have eaten many different dishes, for instance in a buffet setting. If cases who ate the contaminated pork chops were also more likely to eat potatoes, then the association between pork chops and potatoes confound the true result, making it appear as if potatoes could also be the source of the outbreak. Confounding can be addressed either at the study design stage (see **Note 2**) or when analyzing results.

2. Matching involves selecting controls so that the distribution of potential confounders is as similar as possible to that among the cases. Matching is primarily used in case-control studies as described above. The main reason for matching is to obtain the highest possible degree of estimate precision, given that outbreak case-control often has a small sample size. As highlighted, matching also helps reduce the effect of confounding
in the study. The primary disadvantage of matching is that it is not possible to assess the effect of the matching variables on the outcome. In some instances, it is also difficult to find exact matches to a given case, and the matching criteria therefore need to be re-evaluated. Finally, the effects of matching are irreversible and the analysis has to be performed on a matched basis and never by considering the data as non-matched.

3. When referring to diseases, most people think of “risk” rather than odds. The relative risk (or risk ratio, RR) of disease is the probability of disease in an exposed group divided by that of a nonexposed group. The exposure can be any factor such as smoking or taking a certain medication. Risks are measured in cohort studies where the exposed and nonexposed population are followed for a defined period of time to assess where the outcome (disease) occurs. A case-control study cannot estimate risks of disease because we study highly selected samples of the population and because the two study groups are already defined on the basis of the outcome that has occurred. Although the base for the calculation of the OR and the RR is similar, the two ratios convey different estimates. In an outbreak setting, identification of the source from the OR and RR is, however, the same: a high, statistically significant OR or RR for the exposure to which the majority of cases have been exposed.

4. An outbreak of S. Typhimurium can only be confirmed with reference to microbiological diagnostics. Because S. Typhimurium is in itself a very common serotype, the outbreak strain should be confirmed with reference to subtype. Traditionally, this has been undertaken using MLVA as described above or pulse-field gel electrophoresis (PFGE). More recently, the advent of whole-genome sequencing (WGS) [34] has allowed very detailed mapping and comparison of genetic profiles between S. Typhimurium isolates from patients and food sources [35, 36]. This has also resulted in detection of more outbreaks and, consequently, a greater need for resources and trained personnel to investigate the outbreaks [31, 36]. Overall, WGS has the potential for better distinction between outbreak, sporadic, and suspected isolates rather than the traditional methods. Further, WGS results are obtained in a time frame which is better suited for outbreak detection and investigation activities.
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