Section 4
Investigation of foodborne disease outbreaks

4.1 General

Foodborne disease outbreaks are investigated to prevent both ongoing transmission of disease and similar outbreaks in the future. Specific objectives include:

- control of ongoing outbreaks;
- detection and removal of implicated foods;
- identification of specific risk factors related to the host, the agent and the environment;
- identification of factors that contributed to the contamination, growth, survival and dissemination of the suspected agent;
- prevention of future outbreaks and strengthening of food safety policies;
- acquisition of epidemiological data for risk assessment of foodborne pathogens;
- stimulation of research that will help in the prevention of similar outbreaks.

The scale of an outbreak may range from a local outbreak of a small number of linked cases with mild disease to a nationwide or international outbreak of severe disease involving the mobilization of public health resources from all levels. Irrespective of the scale, a full investigation of a foodborne disease outbreak will normally include:

- epidemiological investigations;
- environmental and food investigations;
- laboratory investigations.

4.2 Epidemiological investigations

Preliminary assessment of the situation

Investigation of a potential outbreak starts with the assessment of all available information; this should confirm or refute the existence of an outbreak and allow a working case definition to be established. This assessment must be initiated quickly and completed promptly in order to prevent further illnesses, and should include:

- checking the validity of the information;
- obtaining reports of applicable laboratory tests that have been performed;
- identifying cases and obtaining information about them;
- ensuring the collection of appropriate clinical specimens and food samples.

Once the validity of the reporting source has been verified, a group of the initial cases – perhaps 5–10 persons – should be identified and interviewed as soon as possible. This critical step helps to provide a clearer picture of the clinical and epidemiological features of the affected group. Delays in conducting these interviews can lead to recall bias or to people’s inability to remember what they ate or what they did. The interviews should be open and comprehensive and include questions about:

- demographic details, including occupation;
- clinical details, including date of onset, duration and severity of symptoms;
- visits to health care providers or hospitals;
- laboratory test results;
– contact with other ill persons;
– food consumption history;
– the respondent’s thoughts on what caused their illness;
– whether the respondent knows others with the same or a similar illness;
– potential common exposures among those who have the same or a similar illness;
– date of exposure to suspected foods.

Clinical specimens (e.g. faecal samples, vomitus) from cases should be collected at the time
of first contact: many of the pathogens and toxins that cause foodborne disease remain in the
intestinal tract for only a short time after the onset of illness. If any of the foods that are
suspected or were eaten during the potential incubation period remain available, they should
be sampled for laboratory examination. Laboratory confirmation of these initial cases is
essential to guide further investigation. If there is any doubt about the source of
contamination, it may be reasonable to collect and store many samples, with subsequent
testing determined by epidemiological data as they become available. Information on the
collection of clinical and food samples can be found in Section 4.4.

If the vehicle of infection is thought to be food, the premises where the suspect food was
produced, processed or handled should also be visited. It is important to visit these premises
as early as possible – the amount of physical evidence of what may have caused the outbreak
will diminish with time. If the food premises are located outside the jurisdictional zone of the
local responsible authority, it may be necessary to contact other authorities/agencies. Relevant
food and environmental samples should be collected, and it may also be appropriate to collect
clinical specimens from food-service workers at this time.

Form preliminary hypotheses and plan further action

With the initial information from case interviews, the laboratory and the environmental
inspection, it is often possible to describe the event in simple epidemiological terms and to
form preliminary hypotheses about the cause of the outbreak. Apparent “outliers” or unusual
cases – for example, the only case who resides in a different town, the oldest case, the
youngest case – can often provide useful clues for generating hypotheses. General control and
precautionary measures may be implemented at this stage. For example, suspect foods can be
removed from sale or from the premises, ill food-handlers should be excluded from work, and
the public may be advised to avoid a certain food product or to seek appropriate medical
treatment (see Section 5). While obvious control measures must never be delayed at this early
stage simply because investigations are still under way, it is important to proceed with caution
and to acknowledge that initial hypotheses have yet to be proved. Failure to exercise this
cautions may result in the wrong food being implicated and the credibility of both investigators
and the food producer being damaged.

At the end of this first phase, a decision must be taken on whether to continue with the
investigation. When it is obvious that the outbreak is over or that there is no continuing public
health risk, the value of further investigation needs to be weighed against local priorities and
resources. However, it is often difficult to be certain that an outbreak is indeed over.
Generally, specific control measures can be implemented only when the source and the mode
of transmission are unknown – which provides a convincing argument for continuing with the
investigations. Other likely reasons for continuing may include the following:

– The outbreak poses an immediate health hazard to the local population.
– There are many cases.
– The disease is important in terms of its severity or its rapid spread.
– Cases have occurred over a widespread area without an obvious point source.
– Cases have occurred in high-risk establishments (schools, day-care centres, hospitals, housing or long-term care facilities for the elderly, food premises, etc.).
– There is a high level of public concern.
– There are potential legal implications.
– An investigation would generate new knowledge, e.g. in the area of food safety and risk assessment.
– An investigation would provide valuable learning opportunities for investigators.

If, on the other hand, a decision is taken to halt the investigation, the reasons for this decision should be carefully documented and included in the final investigation report.

**Descriptive epidemiological investigations**

Careful description and characterization of the outbreak is an important first step in any epidemiological investigation. Descriptive epidemiology provides a picture of the outbreak in terms of the three standard epidemiological parameters – time, place and person. This can direct immediate control measures, inform development of more specific hypotheses about the source and mode of transmission, suggest the need for further clinical, food or environmental samples, and guide the development of further studies.

The steps of descriptive epidemiology include:

– establishing a case definition;
– identifying cases and obtaining information from them;
– analysing the data by time, place and person characteristics;
– determining who is at risk of becoming ill;
– developing hypotheses about the exposure/vehicle that caused the disease;
– comparing the hypotheses with the established facts;
– deciding whether analytical studies are needed to test the hypotheses.

**Establishing a case definition**

A case definition is a set of criteria for determining whether a person should be classified as being affected by the disease under investigation. As such, it is an epidemiological tool for counting cases – it is not used to guide clinical practice. A case definition should be simple and practical and should include the following four components:

– clinical and laboratory criteria to assess whether a person has the illness under investigation; the clinical features should be significant or hallmark signs of the illness;
– a defined period of time during which cases of illness are considered to be associated with the outbreak;
– restriction by “place” – for example, limiting the group to patrons of a particular restaurant, employees of a particular factory or residents of a particular town;
– restriction by “person” characteristics – limiting the group to, for example, persons over one year of age, persons with no recent diarrhoeal disease, etc.

Ideally, a case definition will include all cases (high sensitivity) but exclude any person who does not have the illness (high specificity). A sensitive case definition will detect many cases but may also count as cases individuals who do not have the disease. A more specific case definition is more likely to include only persons who truly have the disease under investigation but also more likely to miss some cases.
There are no rules about how sensitive or specific a case definition should be. In the early stage of an outbreak investigation the aim is to detect as many cases as possible; this requires a sensitive case definition (e.g. a person with three or more loose stools in a 24-hour period). At a later stage, the clinical picture is often clearer and the diagnosis is laboratory-confirmed; this allows the use of a more specific case definition (e.g. laboratory-confirmed Salmonella infection), which may then be used to conduct further analytical studies. Criteria included in a case definition cannot be tested as risk factors in subsequent statistical analyses.

Because a single case definition that suits all needs is rare, it is quite common for case definitions to change during an investigation or for different case definitions to be used for different purposes. Many investigators use the following (or similar) case definitions in parallel:

- **Confirmed** cases – have a positive laboratory result (isolation of the causative agent or positive serological test). This case definition has high specificity.
- **Probable** cases – have the typical clinical features of the illness but without laboratory confirmation.
- **Possible** cases – have fewer or atypical clinical features. This case definition has high sensitivity.

<table>
<thead>
<tr>
<th>Box 1. Example of case definition used in investigation of an <em>Escherichia coli</em> O157 outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>A case is defined as gastrointestinal illness in any resident of Area A within five days of attending the Area A Fair in June, 2003. Cases may be further categorized as:</td>
</tr>
<tr>
<td><strong>Confirmed case:</strong> gastrointestinal illness with microbiological confirmation of <em>E. coli</em> O157</td>
</tr>
<tr>
<td><strong>Probable case:</strong> bloody diarrhoea or haemolytic uraemia syndrome without microbiological confirmation</td>
</tr>
<tr>
<td><strong>Possible case:</strong> non-bloody diarrhoea without microbiological confirmation</td>
</tr>
</tbody>
</table>

*Identifying cases*

The cases that prompt an outbreak investigation often represent only a small fraction of the total number of people affected. To determine the full extent of the problem and the population at risk of illness, an active search for additional cases should be undertaken.

Methods for finding additional cases will vary from outbreak to outbreak. Many foodborne disease outbreaks involve clearly identifiable groups (for example, persons all attending the same wedding party), so that case-finding is relatively straightforward. In other outbreaks, particularly those involving diseases with a long incubation period and/or with mild or asymptomatic illness, case-finding may be quite difficult. Directly contacting physicians, hospitals, laboratories, schools or other populations at risk may help to identify unreported cases.

In some cases, public health officials decide to alert the public directly. For example, in outbreaks caused by a contaminated commercial food product, announcements in the media can alert the public to avoid the implicated product and to see a medical practitioner if they have symptoms typical of the disease in question.
Cases themselves may know other people with the same condition – particularly among household members, work colleagues, classmates, friends or neighbours.

If an outbreak affects a restricted population (e.g. students in a school or factory workers) and if a high proportion of cases are unlikely to be diagnosed, a survey of the entire population can be conducted. Questionnaires may be administered to determine the true incidence of clinical symptoms.

Finally, a review of laboratory surveillance data can help to find people with similar infections, assuming the cause of the outbreak is known. Cases that may be epidemiologically linked to an outbreak can often be identified through a unique subtype or biochemical or molecular feature of the causative organism, which may be particularly helpful in an outbreak caused by a widely distributed food product that crosses jurisdictional or even international boundaries.

**Interviewing cases**

Once cases are identified, information about them should be obtained in a systematic way by use of a standard questionnaire. This is in contrast to the preliminary phase of the investigation during which the interviews may be more wide-ranging and open-ended to allow for generation of hypotheses.

Questionnaires may be administered by an interviewer (face-to-face or by telephone) or may be self-administered. Sometimes patients themselves will not be interviewed but their parents, spouses or caregivers may provide data; the sources of information should always be recorded on the questionnaire. Self-administered questionnaires may be distributed in person or by mail, e-mail, fax or internet. Annex 4 outlines the advantages and disadvantages of the various methods and provides information on the design of questionnaires.

Regardless of the disease under investigation, the following types of information should be collected about each case:

- **Identifying information** – name, address, contact details (e.g. daytime telephone number, work address) – to allow patients to be contacted with additional questions and to be notified of laboratory results and the outcome of the investigation. Names will be helpful in checking for duplicate records, and addresses may allow mapping of cases. When identifying information is recorded, issues of confidentiality must always be addressed in accordance with prevailing laws and regulations.

- **Demographic information** – age, date of birth, sex, race and ethnicity, occupation, residence, etc. – to provide the “person” characteristics of descriptive epidemiology that help to define the population at risk of becoming ill.

- **Clinical information** – to identify cases, verify that the case definition has been met, define the clinical syndrome or manifestations of disease, and identify potential etiologies:
  - date and time of first signs and symptoms;
  - nature of initial and subsequent signs and symptoms;
  - severity and duration of symptoms;
  - medical visits and hospital admission;
  - treatment;
  - outcome of illness.

- **Risk factor information** – to allow the source and the vehicle of the outbreak to be identified. This type of information will need to be tailored to the specific outbreak and
the disease in question. Generally, the questionnaire will address both food-related and personal risk factors.

Food-related risk factors:
- detailed food history (see below);
- sources of domestic food and water supply;
- specific food-handling practices, cooking preferences;
- eating away from home.

Personal risk factors:
- date and time of exposure to an implicated food or event (if known);
- contact with people with similar clinical signs and symptoms;
- information on recent travel (domestic and international);
- recent group gatherings, visitors, social events;
- recent farm visits;
- contact with animals;
- attending or working in a school, child-care facility, medical facility;
- working as a food handler;
- chronic illness, immunosuppression, pregnancy;
- recent changes in medical history, regular medications;
- allergies, recent immunizations.

Depending on the suspected etiology and local patterns of food consumption and availability, enquiries should be conducted about any foods that could be a potential source of contamination in the outbreak. It is important to collect a thorough history of food consumption for the entire suspected incubation period (which is often 3–5 days before illness for many common foodborne pathogens). An accurate and thorough food history will often require direct questions about specific foods as well as open-ended questions. Data should also be collected on the number and size of meals eaten, and the source and handling of suspected foods should be noted. Some sample questionnaires are provided in Annex 5.

If the pathogen is known, questions can focus on foods and other risk factors known to be associated with the particular pathogen. For information about the types of foods that are commonly associated with certain pathogens, see Section 6 and Annex 8. Knowledge of the incubation period of the pathogen can point to the most likely period of exposure or identify an unusual event or a suspect meal. If certain foods are known to be associated with the pathogen, specific questions should be asked about them (although enquiries should not be limited to these foods).

If the pathogen is not known but the clinical details suggest a short incubation period, information should be gathered about all meals eaten during the 72 hours before the onset of illness. Most people cannot remember all foods eaten over a 72-hour period: add a calendar, the menu of a suspect meal, or a list of foods to the questionnaire may help their recall of relevant items.

In protracted outbreaks, when investigating illnesses with incubation periods longer than 72 hours (e.g. hepatitis A, typhoid fever, listeriosis) or when a person does not remember specific foods eaten, questions should be asked about food preferences, i.e. foods usually eaten or routine dietary habits. Information should also be obtained about foods purchased during the incubation period of the disease under suspicion.
Collating data

Once the first questionnaires have been completed, the information they contain should be collated promptly to provide insight into the distribution of clinical symptoms and other factors among cases. The data can be summarized in a line listing, with each column representing a variable of interest and each row representing a case. New cases can be added conveniently to the list and updated as necessary (see Table 1). A line listing can be created directly by copying relevant information from the questionnaires or from a computerized database into which case data have been entered. Many types of computer software are available for this purpose, some of which are available free of charge, including Epi Info™ (www.cdc.gov/epiinfo/) and EpiData (www.epidata.dk/).

While entering data, their consistency and quality should be critically evaluated. If feasible, the respondents may be re-contacted to clarify illegible or ambiguous responses on the questionnaire.

Table 1. **Example of a line list for summarizing case data**

<table>
<thead>
<tr>
<th>ID</th>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Date &amp; time of illness onset</th>
<th>Major signs and symptoms</th>
<th>Laboratory tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MT</td>
<td>34</td>
<td>f</td>
<td>10/05, 22:00</td>
<td>+ – + + ND</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TG</td>
<td>45</td>
<td>f</td>
<td>11/05, 08:00</td>
<td>+ – dk + ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>SH</td>
<td>23</td>
<td>m</td>
<td>11/05, 05:00</td>
<td>+ – + + faeces</td>
<td>E. coli O157</td>
</tr>
<tr>
<td>4</td>
<td>RF</td>
<td>33</td>
<td>f</td>
<td>10/05, 18:00</td>
<td>+B + + + faeces</td>
<td>Pending</td>
</tr>
<tr>
<td>5</td>
<td>SM</td>
<td>23</td>
<td>m</td>
<td>11/05, 12:00</td>
<td>+ – – + faeces</td>
<td>Pending</td>
</tr>
</tbody>
</table>

a diarrhoea, B = bloody  
b vomiting  
c fever, dk = unknown/can' t remember  
d anorexia  
e ND = not done

Analysing data

Clinical details

The percentage of cases with a particular symptom or sign should be calculated and arranged in a table in decreasing order (see Table 2). Organizing the information in this way will help in determining whether the outbreak was caused by an intoxication, an enteric infection or a generalized illness. For example,

- If the predominant symptom is vomiting without fever and the incubation period is short (less than 8 hours), intoxication by, for example, *Staphylococcus aureus*, *Clostridium perfringens* or *Bacillus cereus* is likely.
- Fever in the absence of vomiting and an incubation period of more than 18 hours points to an enteric infection such as *Salmonella*, *Shigella*, *Campylobacter* or *Yersinia* (see Section 6 for clinical features of foodborne pathogens).
Table 2. **Frequency of signs and symptoms among cases (n = 296)**

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>No. of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea</td>
<td>260</td>
<td>88</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>122</td>
<td>41</td>
</tr>
<tr>
<td>Fever</td>
<td>116</td>
<td>39</td>
</tr>
<tr>
<td>Nausea</td>
<td>105</td>
<td>35</td>
</tr>
<tr>
<td>Headache</td>
<td>68</td>
<td>23</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>56</td>
<td>19</td>
</tr>
<tr>
<td>Vomiting</td>
<td>42</td>
<td>14</td>
</tr>
</tbody>
</table>

**Time**

The time course of an outbreak is usually shown as a histogram with the number of cases on the y-axis and the date of onset of illness on the x-axis. This graph, called an [epidemic curve](#), may help in:

- confirming the existence of an epidemic;
- forecasting of the further evolution of the epidemic;
- identifying the mode of transmission;
- determining the possible period of exposure and/or the incubation period of the disease under investigation;
- identifying outliers in terms of onset of illness, which might provide important clues as to the source.

To draw an epidemic curve, the onset of illness must be known for each case. For diseases with long incubation periods, day of onset is sufficient. For diseases with a short incubation period – such as most foodborne diseases – day and time of onset are more suitable.

The unit of time on the x-axis is usually based on the apparent incubation period of the disease and the length of time over which cases are distributed. As a rule of thumb, the x-axis unit should be no more than one-quarter of the incubation period of the disease under investigation (although this rule may not apply if the outbreak has occurred over a prolonged period of time). Thus, for an outbreak of salmonellosis, with an average incubation period of 24 hours and cases confined to a few days, a 6-hour unit on the x-axis would be appropriate (see Figure 5).

If the disease and/or its incubation time are unknown, several epidemic curves with different units on the x-axis can be drawn to find one that portrays the data best. The pre-epidemic period on the graph should be shown to illustrate the background or “expected” number of cases or the index case. If the outbreak has a known source (e.g. a particular food served at a common event such as a wedding), the epidemic curve can also be labelled with this information.

The shape of an epidemic curve is determined by:

- the epidemic pattern (point source, common source or person-to-person spread);
- the period of time over which persons are exposed;
- the incubation period for the disease.
In **common-source outbreaks**, a single source of pathogen results in exposure of persons at one point in time (point source), at several points in time (intermittent common source) or over a continuous period (continuous common source). An epidemic curve with a steep upslope, a more gradual downslope and with a width approximating the average incubation period of the pathogen indicates a **point-source outbreak** (see Figure 6A).

If there is a single source of pathogen but exposure is not confined to one point in time, the epidemic is either an **intermittent common-source** or a **continuous common-source outbreak**. In both these types of epidemic, onset will still be abrupt but cases will be spread over a greater period of time than one incubation period, depending upon how long the exposure persists (Figure 6B, 6C).

A **propagated epidemic** is caused by the spread of the pathogen from one susceptible person to another. Transmission may occur directly (person-to-person spread) or via an intermediate host. Propagated epidemic curves tend to have a series of irregular peaks reflecting the number of generations of infection. The time between the peaks may approximate the average incubation period of the pathogen (Figure 6D).

A **mixed epidemic** involves both a common source epidemic and secondary propagated spread to other individuals. Many foodborne pathogens (such as norovirus, hepatitis A, *Shigella*, and *E. coli*) commonly exhibit this mode of spread.

**Calculate incubation periods**

The incubation period is the interval between ingestion of food contaminated with enough pathogens or toxins to cause illness and the first sign or symptom of the illness. Incubation periods will vary with individual resistance and with the different amounts of pathogens/toxins ingested and their uneven distributions in food.
It is often best to characterize outbreaks using the *median* incubation period. Unlike the mean (or average), the median is a measure of central tendency which is not influenced by very short or very long incubation periods. For details of how to calculate the median, see Annex 7.
If the time of exposure and the time of onset of illness are known, individual incubation periods can be calculated directly and summarized by calculating the median.

If only the time of onset of illness is known and the shape of the epidemic curve suggests a point-source outbreak, inferences about the average incubation period and thus the suspected time of exposure may be drawn from the epidemic curve:

- Identify the median time of onset of illness.
- Calculate the time between occurrence of the first and last case (width of the epidemic curve).
- Count back this amount of time from the median to obtain the probable time of exposure (see Figure 7).

Figure 7. **Determining the median incubation period and probable time of exposure in a point-source outbreak**

If the organism and the time of onset of illness are known and the shape of the epidemic curve suggests a point-source outbreak, the probable time of exposure may be determined from the epidemic curve as shown in Figure 8.

Figure 8. **Determining the probable period of exposure in a point-source outbreak with known pathogen**
If the pathogen and onset of illness are known, the range of time during which the exposure probably occurred can be calculated as follows:

- Look up the minimum and the maximum incubation period for the disease (see Section 6).
- Identify the last case of the outbreak and count back on the $x$-axis one maximum incubation period.
- Identify the first case of the epidemic and count back the minimum incubation period.
- Ideally, the two dates will be similar and represent the probable period of exposure.
- Alternatively, the probable time of exposure can be determined by identifying the peak of the epidemic and counting back one average incubation period. This method is useful in ongoing outbreaks in which the last cases have not yet appeared.
- These methods cannot be used if secondary spread is involved or exposure is prolonged.

**Place**

Assessment by “place” provides information on the geographical extent of the outbreak and may reveal clusters or patterns that provide important clues about its cause. Geographical information is best displayed by the use of maps: the types most commonly used in outbreak situations are spot maps and area maps. These can be produced by hand or by using sophisticated geographical information systems.

A **spot map** is produced by placing a dot or other symbol on the map showing where a case lives, works or may have been exposed. Different symbols can be used for multiple events at a single location. On a spot map of a community, clusters or patterns may reflect water supplies or proximity to a restaurant or to a grocery (see Figure 9). On a spot map of a hospital or a nursing home, clustering of cases is consistent with a focal source or person-to-person spread, while scattering of cases throughout the facility may be more consistent with a widely disseminated vehicle or a source common to all residents.
Figure 9. Spot map showing the occurrence of 578 fatal cases of cholera, clustering around a shared well, London

*Source: Snow, 1854.*

If the size of the population varies between areas, a spot map that shows only numbers of cases can be misleading. In such instances, an area map (or density map) should be used. An area map takes differences in population size into consideration by employing rates (cases/population) rather than absolute numbers (see Figure 10).

**Person**

The purpose of describing an outbreak by “person” characteristics is to identify features that are common to cases as a clue to etiology or sources of infection. Age, sex, ethnicity and occupation are among the numerous characteristics that can be used to describe the case population. If a single or specific characteristic emerges, this often points towards the population at risk and/or towards a specific exposure. For example, it may be apparent that only certain students in a school became ill, or only workers in a single factory or a group of people who attended a local restaurant were involved. Nevertheless, even if it appears that only a single group of people was at risk, it is important to look carefully at the entire population to be sure that no other groups are affected. Certain groups of people may be more susceptible to disease or more likely to seek medical attention for their symptoms, for example people who live in a city where medical care is readily available. Sometimes cases in a particular group are more likely to be detected and reported than cases in other groups, and premature conclusions about the population affected could therefore be misleading.
Determining who is at risk of becoming ill

A measure of disease frequency is important in characterizing an outbreak, and the commonest such measure in epidemiology is a rate. Rates adjust for differences in population size and thus allow comparison of the occurrence of disease in various subgroups (see Table 3). Calculating rates of disease requires knowledge both of the number of cases and of the number of people in the population group(s) in which the disease may occur in a given period of time (often referred to as the denominator). This population group is called the population at risk and is usually defined on the basis of general demographic factors. For example, if the disease affects only children aged 5–14 years, the population at risk is the children in this age group living in the area of the outbreak.

Excluding population groups in which the disease does not occur helps the investigation to focus only on those affected, leading to clearer findings and allowing more effective intervention and control activities. If only a certain ethnic group within a region is involved, for example, the investigation may focus on food items specific to that group.
Table 3. Cholera attack rate by age group, Mankhowkwe Camp, Malawi, March–May 1988, showing the highest rates of disease among persons aged 15 years and above

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>No. of cases</th>
<th>Population</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>131</td>
<td>5 303</td>
<td>2.5</td>
</tr>
<tr>
<td>5–14</td>
<td>261</td>
<td>12 351</td>
<td>2.1</td>
</tr>
<tr>
<td>&gt;15</td>
<td>392</td>
<td>12 091</td>
<td>3.2</td>
</tr>
<tr>
<td>Total</td>
<td>784</td>
<td>29 745</td>
<td>2.6</td>
</tr>
</tbody>
</table>

*Source: Reproduced with permission of the publisher, from Moren et al., 1991.

The *attack rate* is commonly used in disease outbreak investigations and is a key factor in the formulation of hypotheses. It is calculated as the number of cases in the population at risk divided by the number of people in the population at risk (see Annex 7).

Sometimes it may be impossible to calculate rates because the population at risk is not known. In such situations, the distribution of cases themselves may help in formulating hypotheses.

**Developing explanatory hypotheses**

At this stage of the investigation the data need to be summarized and hypotheses formulated to explain the outbreak. Hypotheses should address the source of the agent, the mode and vehicle of transmission, and the specific exposure that caused the disease. They should also be:

- plausible;
- supported by the facts established during the epidemiological, laboratory and food investigations;
- able to explain most of the cases.

While it is important to consider what is already known about a disease, an unlikely or unusual hypothesis should not be automatically discarded. In 1985, for example, when epidemiological data incriminated horse meat as the source of a trichinosis outbreak in France, the hypothesis that consumption of horse meat caused this outbreak seemed unlikely. Before then, it had always been assumed that only carnivores were a source for *Trichinella* infection. However, this proved not to be the case, and since 1985 several trichinosis outbreaks have been traced back to horse meat (Ancelle, 1988).

Formal testing of a hypothesis may be unnecessary if it is strongly supported by epidemiological, laboratory or food data, but if such support is lacking or important questions remain unanswered, further studies may be needed. For example, descriptive epidemiology will often explain the source of the outbreak and the general mode of transmission but not reveal the specific exposure that caused the disease. Analytical epidemiological studies are then used to test the hypotheses.

**Analytical epidemiological investigations**

Analytical epidemiological studies frequently involve comparisons of the characteristics of a group of well persons with those of ill persons in order to quantify the relationship between...
specific exposures and the disease under investigation. The two types of analytical studies most commonly used in outbreak investigations are **cohort studies** and **case–control studies**. When investigating outbreaks a rapid result may be required to assist in control efforts, and it may be advisable to conduct a limited analytical study initially. More thorough investigations can be conducted later, for example to increase the knowledge of a particular food pathogen.

The value of a comparison group for identifying specific exposures is illustrated by the example of a school outbreak of gastroenteritis, in which 30 cases are identified. Interviewing all 30 cases about their food consumption shows that all ate vanilla ice cream purchased from a street-vendor one day before illness. Enquiries about consumption of other foods show that no other food item was consumed by as many cases as vanilla ice cream.

Comparing the 30 cases with a group of 60 healthy students from the same school reveals that all the healthy students also ate vanilla ice cream purchased from the same street-vendor. Comparison of other exposures, however, reveals that most of the 30 cases had lunch in the school canteen the day before illness while most of the healthy students did not. This difference indicates that food from the school canteen is the more likely vehicle for the outbreak than vanilla ice cream: the finding that all cases had eaten vanilla ice cream merely reflects its popularity among the students.

**Retrospective cohort studies**

Retrospective cohort studies are feasible for outbreaks in small, well-defined populations in which all exposed and all non-exposed persons are identifiable. These studies compare the occurrence of disease among those who were exposed to a suspected risk factor with occurrence among those who were not (Box 2). For example, all persons attending a wedding reception (the “cohort”) may be interviewed to determine whether they became ill after the reception, and to identify what foods and drinks they had consumed. After collecting information from each attendee, attack rates for illness are calculated for those who ate a particular food and for those who did not eat that food (see Table 4).

### Table 4. **Cohort study**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Ill</th>
<th>Not ill</th>
<th>Total</th>
<th>Attack rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate food “A”</td>
<td>48</td>
<td>20</td>
<td>68</td>
<td>71%</td>
</tr>
<tr>
<td>Did not eat food “A”</td>
<td>2</td>
<td>100</td>
<td>102</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>120</td>
<td>170</td>
<td>29%</td>
</tr>
</tbody>
</table>

In this example, of a total of 68 persons who ate food “A”, 48 fell ill (attack rate 48/68 or 71%). The attack rate for those who did not eat food “A” was 2/102 or 2%. Food "A" is a likely risk factor for illness because:

- the attack rate is high among those exposed to food “A” (71%);
- the attack rate is low among those not exposed to food “A” (2%), so the difference (risk difference) between the two attack rates is high (69%);
- most cases (48/50 or 96%) were exposed to food “A”.

---

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In addition, a ratio of the two attack rates, known as the relative risk (RR), can be calculated in the following way:

\[
\text{relative risk (RR)} = \frac{\text{Attack rate for those who ate food “A”}}{\text{Attack rate for those who did not eat food “A”}} = \frac{71\%}{2\%} = 35.5
\]

A relative risk has no units and is a measure of the strength of association between the exposure and the disease. In the above example, the relative risk associated with eating food “A” is 35.5. This means that persons who ate food “A” were 35.5 times more likely to develop disease than those who did not. Statistical significance tests are used to determine the probability that this relative risk could have occurred by chance alone. For information about statistical significance testing, see Annex 7.

**Case–control study**

In many circumstances, no clearly defined “cohort” of all exposed and non-exposed persons can be identified or interviewed. In such situations – when cases have already been identified during a descriptive study and information has been gathered from them in a systematic way – a case–control study can be an efficient study design (Box 3).

In a case–control study, the distribution of exposures among cases and a group of healthy persons (“controls”) are compared with each other (see Table 5). The questionnaire used for the controls is identical to that administered to the cases, except that questions about the details of clinical illness my not pertain to the controls.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Cases</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate food “A”</td>
<td>48</td>
<td>20</td>
<td>68</td>
</tr>
<tr>
<td>Did not eat food “A”</td>
<td>2</td>
<td>100</td>
<td>102</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>120</strong></td>
<td><strong>170</strong></td>
</tr>
</tbody>
</table>

| Percentage exposed | 96% | 17% | 40% |

In this example, 96% of all cases had consumed food “A” compared with only 17% of the controls. This suggests that consumption of food “A” is associated with illness in one way or another. In contrast to a cohort study, attack rates (and therefore relative risk) cannot be calculated since the total number of persons at risk is unknown. Instead, a different measure of association – odds ratio (OR) – is used in case–control studies. The odds ratio is calculated as the “cross-product” of a two-by-two table (see Table 6).
Table 6. **Example of a two-by-two-table from a case-control study**

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate food &quot;A&quot;</td>
<td>48</td>
<td>20</td>
<td>54</td>
</tr>
<tr>
<td>Did not eat food &quot;A&quot;</td>
<td>2</td>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>46</td>
<td>29</td>
<td>75</td>
</tr>
</tbody>
</table>

Odds ratio = \( \frac{48 \times 100}{20 \times 2} = 120 \)

Chi-square 92.6, \( p \)-value <\(6 \times 10^{-22}\)

The odds ratio is calculated as the cross-product from a two-by-two table (the number of cases exposed times the number of controls not exposed, divided by the number of controls exposed times the number of cases not exposed). For rare conditions (i.e. less than 5% in the general population are affected), the odds ratio is a good estimate of the relative risk. Thus, in this example, an exposure odds ratio of 120 for food “A” can be interpreted as: the odds of having been exposed to the contaminated food in those who developed the disease was 120 times that of people who did not eat food “A”. This odds ratio means that there is a very strong association between being a case and consumption of food “A”. As in a cohort study, statistical significance can be calculated to determine the probability that such an odds ratio could have occurred by chance alone. For the example above, this probability is extremely small (\(1/6 \times 10^{22}\)). Box 3 gives a calculated example of a case–control study.

**Choosing controls**

An important decision in the design of a case–control study is defining who should be the controls. Conceptually, controls must not have the disease in question but should represent the population from which the cases come. In this way, controls provide the level of background exposure that might be expected among cases. If cases have a much higher exposure than controls, exposure may be associated with disease.

Often it is difficult to know who the controls should be. Practical matters need to be taken into consideration, such as how to contact potential controls rapidly, gain their permission, ensure that they are free of the disease under investigation (and not just asymptomatic), and get appropriate exposure data from them. In a community outbreak, a random sample of the healthy population may be the best control group. Sometimes such community controls are identified by visits to randomly selected homes in the community of interest or by telephone calls to randomly selected telephone numbers within the area.

Other common control groups consist of:

- neighbours of cases;
- patients from the same physician practice or hospital who do not have the disease in question;
- family members or friends of cases;
- people who attended an implicated event but did not become ill;
people who ate at an implicated food service facility during the time of exposure but did not become ill.

While controls from these groups may be more likely to participate in the study than randomly identified population-based controls, they may not be as representative of the population. This kind of bias in the control group can distort the data in either direction masking an association between the exposure and disease or producing a spurious association between an innocent exposure and disease. However a group of controls is chosen substantial efforts should be made to interview all those selected. Making only a single attempt to contact randomly selected controls, for example, could result in a biased sample of people who are most likely to be available at a certain time of the day rather than being representative of the entire population of interest.

When designing a case–control study, the number of controls must be considered. While the number of cases is limited by the size of the outbreak the number of potential controls will usually be greater than is needed. In general, the more subjects are included in a study, the easier it will be to find a statistical association between exposure and disease.

In an outbreak of 50 or more cases, 1 control per case will usually suffice. In smaller outbreaks, 2, 3 or 4 controls per case can be used. Increasing the number of controls beyond 4 per case, however, will rarely be worth the effort.
Box 2. **Example of a cohort study**

Table A is based on an outbreak of gastroenteritis following a church supper. Of the 80 persons attending the supper, 75 were interviewed. Forty-six met the case definition. Attack rates were calculated for those who did and did not eat each of the 14 food items.

**Table A. Attack rates by food items served at church supper, Oswego, New York, April 1940**

<table>
<thead>
<tr>
<th>Number of persons who ate food item</th>
<th>Number of persons who did not eat food item</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ill Total Attack rate (%)</td>
<td>Ill Total Attack rate (%)</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Baked ham</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>63</td>
</tr>
<tr>
<td>46</td>
<td>59</td>
</tr>
<tr>
<td>Spinach</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>60</td>
</tr>
<tr>
<td>43</td>
<td>62</td>
</tr>
<tr>
<td>Mashed potatoes</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>62</td>
</tr>
<tr>
<td>37</td>
<td>62</td>
</tr>
<tr>
<td>Cabbage salad</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>64</td>
</tr>
<tr>
<td>28</td>
<td>60</td>
</tr>
<tr>
<td>Jello</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>70</td>
</tr>
<tr>
<td>23</td>
<td>58</td>
</tr>
<tr>
<td>Rolls</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>57</td>
</tr>
<tr>
<td>37</td>
<td>66</td>
</tr>
<tr>
<td>Brown bread</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>67</td>
</tr>
<tr>
<td>27</td>
<td>58</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
</tr>
<tr>
<td>Coffee</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>61</td>
</tr>
<tr>
<td>31</td>
<td>61</td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>54</td>
</tr>
<tr>
<td>24</td>
<td>65</td>
</tr>
<tr>
<td>Cakes</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>67</td>
</tr>
<tr>
<td>40</td>
<td>54</td>
</tr>
<tr>
<td>Vanilla ice cream</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>80</td>
</tr>
<tr>
<td>54</td>
<td>14</td>
</tr>
<tr>
<td>Choc. ice cream*</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>53</td>
</tr>
<tr>
<td>47</td>
<td>74</td>
</tr>
<tr>
<td>Fruit salad</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
</tr>
</tbody>
</table>

*Excludes one person who was unsure of consumption.

Looking at this table the most likely vehicle is vanilla ice cream. It has the highest attack rate (80%) for those who ate vanilla ice cream and the lowest for those who did not. Forty-three of the 47 cases can be “explained” by having eaten vanilla ice cream. The attack rates for the other 13 food items do not display the same characteristics.

Table B shows the same data for vanilla ice cream in the format of a two-by-two table which makes the calculation of attack rates, relative risks and statistical significance easier to visualize:

**Table B. Two-by-two-table for consumption of vanilla ice cream (cohort study)**

<table>
<thead>
<tr>
<th>Ill</th>
<th>Well</th>
<th>Total</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate vanilla ice cream</td>
<td>43</td>
<td>11</td>
<td>54</td>
</tr>
<tr>
<td>Did not eat vanilla ice cream</td>
<td>3</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>29</td>
<td>75</td>
</tr>
</tbody>
</table>

RR = 79.6/14.3 = 5.6

The relative risk (RR) for eating vanilla ice cream is 79.6/14.3 or 5.6. This means that persons who ate vanilla ice cream were 5.6 times more likely to become ill than those who did not.

To determine the probability that the relative risk of 5.6 could have occurred by chance alone a statistical significance test can be calculated. This shows that the probability of obtaining a relative risk of 5.6 or even higher is 1/5 000 000 and therefore very unlikely to have occurred by chance alone. For details of how this calculation was obtained see Annex 7.

1 Source: Reproduced with permission of the publisher, from Goss, 1976.
Box 3. Example of a case-control study

Table A. Odds ratios for exposure to foods served in hospital “X”, Dublin, Ireland, 1996

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 65)</th>
<th>Controls (n = 62)</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ate (n)</td>
<td>Did not eat (n)</td>
<td></td>
</tr>
<tr>
<td>French onion soup</td>
<td>8</td>
<td>51</td>
<td>15</td>
</tr>
<tr>
<td>Baked ham</td>
<td>21</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>Parsley sauce</td>
<td>18</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>Cold salads</td>
<td>5</td>
<td>54</td>
<td>8</td>
</tr>
<tr>
<td>Creamed potatoes</td>
<td>23</td>
<td>35</td>
<td>23</td>
</tr>
<tr>
<td>Turnips and cabbage</td>
<td>30</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Chicken curry rice</td>
<td>15</td>
<td>44</td>
<td>7</td>
</tr>
<tr>
<td>Sandwiches</td>
<td>6</td>
<td>53</td>
<td>3</td>
</tr>
<tr>
<td>Danish pastries</td>
<td>1</td>
<td>58</td>
<td>6</td>
</tr>
<tr>
<td>Chocolate mousse cake</td>
<td>42</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Ice cream</td>
<td>10</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>Scones</td>
<td>1</td>
<td>58</td>
<td>4</td>
</tr>
</tbody>
</table>

Persons who were uncertain about consumption of a particular food item are excluded.

Table A is based on a salmonellosis outbreak in a hospital. Sixty-five patients and staff members met the case definition. Their exposures to specified foods were compared to those of 62 healthy patients and staff members. To determine the most likely vehicle of the outbreak, odds ratios were calculated for a total 56 food items served during breakfast, lunch and dinner over a three day period (Table A shows only food items served during one lunch). The highest odds ratio was found for consumption of chocolate mousse cake.

Table B. Two-by-two table for consumption of chocolate mousse cake (case control study)

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate chocolate mousse cake</td>
<td>42</td>
<td>5</td>
<td>47</td>
</tr>
<tr>
<td>Did not eat chocolate mousse cake</td>
<td>16</td>
<td>53</td>
<td>69</td>
</tr>
</tbody>
</table>

Odds ratio (OR) = \( \frac{42 \times 53}{5 \times 16} = 27.8 \)

The odds ratio for being exposed to chocolate mousse cake was 27.8. As salmonellosis is infrequent in the general population (and even in hospital) this odds ratio can be taken as a relative risk estimate, i.e. the risk of developing illness was much higher among persons who ate chocolate mousse cake than among those who did not.

1 Source: Reproduced with permission of the publisher, from Grein et al., 1997.
**Dose response**

A dose response is present if the risk of illness increases with increasing amount or duration of exposure. For example, if individuals who ate two portions of a stew were more likely to become ill than people who ate only one portion, this would suggest a “dose response”. Finding a dose response supports the hypothesis that a particular exposure caused illness.

Looking for a dose response is particularly important in outbreaks where cases and the comparison group (i.e., controls in case-control studies and unaffected persons in cohort studies) were exposed to the same risk factors. When the entire study population has been exposed to the same risk factors, demonstrating a dose response can be particularly helpful in assessing a situation.

Careful attention to study design is important to ensure that dose response can be evaluated. The first and most important step in looking for a dose response is to include questions about exposure levels in the questionnaire (e.g., how often or how much of a food was eaten). Once data on exposure levels have been collected, odds ratios (in case-control studies) or relative risks (in cohort studies) are calculated for each level of exposure and compared with the unexposed group or the group with the lowest exposure (the “reference” group). Statistical tests such as the chi-square test for trend can be employed to assess the statistical significance of the dose response. Table 7 gives an example of a dose-response calculation for a case control study, in which people eating more than 12 oysters were much more likely to become ill than people eating 7–12 oysters, who in turn were more likely to become ill than those eating fewer than 7 oysters.

Table 7. *Number of oysters eaten among oyster-eating patients and controls, Hepatitis A outbreak, Florida, 1988*

<table>
<thead>
<tr>
<th>Number of raw oysters eaten</th>
<th>Cases (n = 51)</th>
<th>Controls (n = 33)</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>percentage</td>
<td>number</td>
</tr>
<tr>
<td>1–6</td>
<td>6</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>7–12</td>
<td>20</td>
<td>39</td>
<td>11</td>
</tr>
<tr>
<td>&gt;12</td>
<td>25</td>
<td>49</td>
<td>4</td>
</tr>
</tbody>
</table>

*a Source: Reproduced with permission of the publisher, from Desenclos et al., 1991.

Chi-square for trend 20.0, $p < 0.001$

This chi-square value indicates that there is less than a 1 in 1000 chance that the increased odds of becoming ill after eating a larger quantity of oysters could be due to chance alone.

Table 8 gives an example of a similar calculation for a cohort study in which illness was increasingly likely among persons eating more éclairs.
Table 8. *Number of éclairs eaten among sport day attendees, Thailand, 1995*^a^  

<table>
<thead>
<tr>
<th>Pieces of éclair eaten</th>
<th>Number ill</th>
<th>Total number</th>
<th>Attack rate</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15</td>
<td>285</td>
<td>5.3</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>0.5–1</td>
<td>51</td>
<td>105</td>
<td>48.6</td>
<td>9.2</td>
</tr>
<tr>
<td>2–4</td>
<td>299</td>
<td>524</td>
<td>57.1</td>
<td>10.7</td>
</tr>
<tr>
<td>&gt;4</td>
<td>105</td>
<td>171</td>
<td>61.4</td>
<td>11.6</td>
</tr>
</tbody>
</table>

^a^ Source: Thaikruée et al., 1995.

Additional information on these and other topics pertaining to epidemiological and statistical aspects of investigating outbreaks is available free of charge on the internet (WHO, 2002; Dicker, 1992).

**Addressing additional research issues**

Outbreaks provide unique opportunities to address scientific questions above and beyond the immediate requirements of the investigations. While the rapid control of an outbreak must remain the primary objective for the investigator, additional research questions or collection of additional data related to the pathogen or to the food under investigation may be addressed without jeopardizing this objective. Outbreak investigations can be an important opportunity to learn about a pathogen, the emergence of drug resistance, and other important aspects of the epidemiology of foodborne disease.

Data derived from epidemiological studies can be used in risk assessment, a process of evaluating known or potential adverse health effects resulting from human exposure to foodborne hazards. Risk assessments for foodborne pathogens have become an important tool for responding to increasing scientific, legal and political demands in the area of food safety. Epidemiological data derived from foodborne disease outbreaks can be valuable in risk assessments for foodborne pathogens, particularly if data collection follows a standardized protocol. For the type of data useful in risk assessment of a particular pathogen, see Annex 6.

### 4.3 Environmental and food investigations

**General**

Environmental investigations (often also referred to as food or sanitary investigations) are conducted in parallel with epidemiological and laboratory investigations to find out how and why an outbreak occurred and, most importantly, to institute corrective action to avoid similar occurrences in the future. The specific objectives of an environmental investigation during a foodborne disease outbreak include:

- identifying the source, mode and extent of the food contamination;
- assessing the likelihood that pathogens survived processes designed to kill them or to reduce their numbers;
- assessing the potential for growth of pathogens during food processing, handling or storage;
- identifying and implementing corrective interventions.
Because environmental investigations will differ according to the nature and size of the outbreak, the type of establishments involved, the resources available, local priorities, political and legal concerns, and many other factors, only general aspects can be outlined in this manual.

An environmental investigation performed in the context of a foodborne disease outbreak differs significantly from a routine regulatory inspection carried out to identify regulatory violations. Outbreak-related environmental investigations should be guided by data as it becomes available from other components of a multi-disciplinary investigation. Such investigations should endeavour to clarify the actual conditions at the time the suspected foods were prepared (i.e. before the outbreak) rather than simply observe the current conditions. Each suspect food item that has been (or could be) implicated in the outbreak should be thoroughly investigated.

Examples of records that may be useful in an investigation include:

- menus, recipes or product formulations;
- processing records;
- purchasing and inventory records;
- shipping records and other documentation relating to the source of an implicated product;
- hazard analysis and critical control points (HACCP) plans and records;
- records of corrective action;
- flow diagrams;
- floor plans of the establishment;
- complaint records;
- cleaning records;
- food laboratory testing results;
- past inspection records;
- personnel records (including who was working when, and absenteeism).

The amount of physical evidence may diminish rapidly with time after an outbreak has been identified, and associated food investigations should therefore be carried out as soon as possible. In a small, well-defined outbreak (e.g. a point-source outbreak originating in a restaurant), the site of the outbreak may be easily identified, and an environmental investigation can be launched promptly. In more complex outbreak investigations, in which there may be delays in linking cases to a particular food establishment or event, the food investigation may be particularly challenging – or even impossible.

**Investigation of food establishments**

During a foodborne disease outbreak, investigation of a food establishment will often require:

- interviewing managers;
- interviewing any employees who may have had a role in the processing or preparation of suspected foods;
- a review of employee records (to determine whether some were out ill during the period of interest);
- a review of the overall operations and hygiene;
- a specific assessment of procedures undergone by a suspect food;
- food and environmental sampling;
– a review of food worker health and hygiene, including specimens for analysis;
– an assessment of the water system and supply;
– measurement of temperatures, pH and water activity \((a_w)\) with appropriate equipment.

Investigations should be guided by what is already known about an outbreak from epidemiological and laboratory investigations and about known reservoirs for the suspected agent. If a food has been incriminated epidemiologically, efforts should focus on how this particular food became contaminated. If laboratory investigations have identified a pathogen, efforts may focus on foods and conditions known to be associated with the particular pathogen (see Section 6). Food investigations that lack this kind of clear focus can be expensive, time-consuming and of limited value. The following questions may help to focus an efficient food investigation:

– What are the known reservoirs or common sources of the suspected pathogen?
– What type of environment does it survive in?
– Where and how could the food have been contaminated?
– What environmental conditions support the growth and spread of the suspected pathogen?
– Where are the opportunities for cross-contamination, survival or growth of the pathogen in this environment or establishment?

One of the goals of an environmental investigation is to identify “contributing factors” – the factors that probably played a role in the occurrence of the outbreak. These are often classified into factors related to contamination, proliferation or amplification of a pathogen, and survival of a pathogen (Bryan, Guzewich & Todd, 1997).

**Investigation of a suspect food**

When the role of a suspect food is investigated, the complete processing and preparation history should be reviewed, including sources and ingredients, persons who handled the specific foods, the procedures and equipment used, potential sources of contamination, and time-and-temperature conditions to which foods were exposed.

**Product description**

The suspect food should be fully described in terms of:

– all raw materials and ingredients used (menus, recipes, formulations);
– sources of the ingredients;
– physical and chemical characteristics, including pH, water activity \((a_w)\);
– use of returned, reworked or leftover foods in processing;
– intended use (e.g. home use, catering, for immediate consumption, for vulnerable groups).

**Observation of procedures from receipt to finish**

Observations must cover the entire range of procedures, focusing on actual processes and work practices and including cleaning methods, schedules, personal hygiene of food-handlers and other relevant information. The temperature history (temperature and duration) of the suspect food should be recorded as completely as possible, including the conditions in which the food was stored, transported, prepared, cooked, heat-processed, held warm, chilled or re-heated. Observation of food-handling practices may be valuable for small-scale operations and in the domestic setting as well as in commercial operations.
**Interviewing food-handlers**

All food-handlers who were directly involved in producing, preparing or handling suspect foods should be interviewed. Information should be obtained about the exact flow of the suspect food, its condition when received by each food-handler, the manner in which it was prepared or handled, and any unusual circumstances or practices prevailing during the relevant period. Recent illnesses of food-handlers (before, during or after the date of the outbreak exposure) and times of absence from work should also be noted. Specimens for microbial analysis should be obtained from any food-handlers who are ill. If any employee is found to be infected with the agent of concern, it is essential to determine whether he or she is a potential source of the problem or is infected because of having eaten the same food. At every step of the process, data should be evaluated with respect to contamination, growth/proliferation and survival factors associated with the suspected pathogen(s).

Employees should be interviewed regarding their observations and recollections of specific days implicated in the outbreak. Examples of such questions are:

- What were each employee’s specific duties that day?
- Were there any unusual working conditions that day?
- Were deliveries arriving on time?
- Was all equipment working properly?
- Was anyone out ill?
- Was the establishment short-staffed?
- Were unusual quantities of food being prepared?

**Taking appropriate measurements**

An effort should be made to estimate food processing conditions at the time the implicated foods were produced. Product temperatures during processing and storage and time sequences of operations should be measured and recorded as appropriate. This includes:

- time and temperature conditions to which suspect foods were exposed;
- water activity ($a_w$), water content and pH of suspect foods;
- size of containers used in procedures, depth of food in containers, etc.

Again, attempting to understand actual conditions at the time that implicated foods were prepared is paramount.

**Drawing a flowchart of the operations**

All information and measurements should be entered on a flowchart to facilitate assessment of factors that may have contributed to the outbreak. The flowchart should be based on actual practices at the time of the outbreak and, as applicable, should show:

- exact flow of operations for the suspect food(s);
- name of persons performing operations;
- equipment used;
- results of measurements taken;
- other relevant information.

If practices at the time of the outbreak can no longer be reconstructed, a flowchart of current practices may be useful.
Conducting an outbreak hazard analysis

Hazard analysis in an outbreak situation should address the following questions at each step of the processing of potentially implicated foods:

- Could pathogens have been introduced at any stage?
- Could pathogens already present have been able to grow at any stage?
- Could pathogens have survived processes designed to kill them?

This analysis also includes observation of the food-handling environment, assessing such factors as the location and availability of sinks and appropriate hand-washing facilities, and determining whether separate areas are maintained for the preparation of raw and ready-to-eat foods.

**Food and environmental sampling**

If laboratory facilities are available, appropriate food and environmental samples should be taken as early as possible since the amount of physical evidence will diminish with time. The laboratory should be alerted in advance of sample collection and can provide sampling materials appropriate to the type and quantity of specimens to be collected, their storage, packing and transport.

**Food samples**

Laboratory analysis of foods for microbial or chemical contamination is time- and resource-intensive and liable to a number of sampling and handling errors. Targeted sampling and laboratory analysis of foods should be directed by epidemiological and environmental investigations. If an implicated food has not been identified at the time of sampling, a large number of specimens may be collected and stored for subsequent laboratory testing as additional information becomes available.

Food samples that may be appropriate for collection and testing include:

- ingredients used to prepare implicated foods;
- leftover foods from a suspect meal;
- foods from a menu that has been implicated epidemiologically;
- foods known to be associated with the pathogen in question;
- foods in an environment that may have permitted the survival or growth of microorganisms.

If a packaged food item is suspected of being involved in an outbreak, it is particularly important to collect unopened packages of that food – ideally, from the same lot. This can help to establish whether the food was contaminated before its receipt at the site of preparation. If no foods are left from a suspect meal, samples of items that were prepared subsequently but in a similar manner may be collected instead, although findings from these tests must be interpreted with care. Any ingredients and raw items that are still available should also be sampled. Storage areas should be checked for items that may have been overlooked; even food retrieved from garbage containers may provide information useful in an investigation.

The circumstances in which samples were collected, the names of the suppliers and distributors, and coding information on packaged foods should be recorded so that the distribution channels of the product can be determined if necessary.
Environmental samples

The purpose of collecting environmental samples is to trace the sources of, and evaluate the extent of contamination that may have led to, the outbreak. Samples may be taken from work surfaces, food contact surfaces of equipment, containers, and other surfaces such as refrigerators, door handles, etc. Environmental samples may also include clinical specimens (such as faecal specimens, blood or nasal swabs) from food workers and water used for food processing.

Raw poultry, pork, beef and other meats are often contaminated with *Salmonella*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli* O157 and other pathogens by the time they come into kitchens. If any of these agents is suspected in an outbreak, meat scraps, drippings on refrigerator floors and deposits on saws or other equipment can be helpful in tracing the source of contamination. Swabs can also be taken from tables, cutting boards, grinders, slicing machines and other utensils that had contact with the suspect food. However, as these pathogens are often present in such raw products, their detection does not automatically imply that they were the cause of the outbreak.

Food-handlers

Food-handlers can be a source of foodborne contamination. Stool specimens or rectal swabs may be collected from food-handlers for laboratory analysis to identify potential carriers or sources of contamination. Toxin-producing strains of *S. aureus* are carried in the nostrils, on the skin and occasionally in the faeces of many healthy persons. If *S. aureus* intoxication is suspected, the nasopharynx of food-handlers can be swabbed. Swabs should also be taken from skin lesions (pimples, boils, infected cuts, burns etc) on unclothed areas of the body. Arrangements should be made for workers to be examined by a medical practitioner as appropriate. If hepatitis A virus (HAV) is suspected, blood from food-handlers can be tested for IgM antibodies against HAV, which are an indication of acute infection (Heymann, 2004).

If ill food-handlers are identified, an immediate decision is needed on whether to exclude those people from work until their symptoms have resolved or until additional investigations have been completed. Local jurisdictions may have different policies and rules regarding exclusion of food-handlers, and different criteria for allowing them to return to work, although guidelines have been established (Heymann, 2004, and Section 6.3).

Food traceback

If a food investigation fails to identify a source of contamination at the place of preparation (e.g. infected food-handler or cross-contamination), attention should be drawn to the possibility that contamination may have occurred before the food or ingredient arrived at the establishment (Box 4). The simultaneous occurrence of multiple outbreaks due to the same pathogen at different sites is often evidence of primary contamination. It is generally recognized that many raw foods may commonly be contaminated (primary contamination). Primary contamination may be more or less ubiquitous (e.g. *Bacillus cereus* in grain) or so common (e.g. *Salmonella* in poultry) that food safety measures will rely on subsequent procedures such as thorough cooking to ensure that food is fit for consumption. In such instances, investigation of the place of primary contamination will depend on the available resources, priorities and the epidemiological situation with regard to the outbreak.
Box 4. Factors contributing to contamination of foods

- Raw foods may be contaminated at their source with *Salmonella*, *Campylobacter*, *Clostridium perfringens*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Staphylococcus aureus* or other pathogens. In some regions, raw fish are often contaminated with *Vibrio parahaemolyticus* and non-O1 *Vibrio cholerae*. Rice and other grains often harbour *Bacillus cereus*, and herbs and spices may harbour *C. perfringens*.
- Foods were obtained from unsafe sources (shellfish, raw milk, raw eggs, mushrooms, etc.).
- Non-potable water was used in food preparation.
- Infected persons (e.g. nasal carriers of *Staphylococcus aureus*, persons in the incubatory phase of hepatitis A, persons infected with norovirus and intestinal carriers of Shigella); contaminated foods that were not subsequently heat-processed.
- Contaminants were spread, by worker’s hands, cleaning cloths or equipment, from raw foods of animal origin to cooked foods or to foods that were not subjected to further heat treatment.
- Equipment (slicers, grinders, cutting boards, knives, storage containers) was not properly cleaned.
- Contaminated food or ingredients were eaten raw or insufficiently heat-processed.
- High-acid foods were stored in containers or conveyed through pipelines that contained toxic metals (antimony, copper, cadmium, lead, zinc), causing leaching or migration of the toxic substance into the food.
- Poisonous substances such as pesticides reached foods as a result of carelessness, accidents or improper storage or because they had been mistaken as food ingredients.
- Substances were added to foods in excess of culinary needs (e.g. monosodium glutamate) or processing needs (e.g. sodium nitrite).
- Food became contaminated during storage, e.g. through exposure to leaking or overflowing sewage.
- Contaminants penetrated cans or packages through seam defects or breaks.
- Food was contaminated by sewage during growth or production.

Factors affecting survival

- Food was cooked or heat-processed for an insufficient time or at an inadequate temperature.
- Previously cooked food was reheated for an insufficient time or at an inadequate temperature.
- Food was inadequately acidified.

Factors affecting microbial growth

- Cooked food was left at room temperature for an excessive time.
- Food was improperly cooled (e.g. stored in large pots or other large containers in refrigerator).
- Hot food was stored at a temperature that permitted multiplication of bacteria.
- Fermentation (and thus acid formation) was inadequate or slow.
- Inadequate concentrations of curing salts were added or curing time was too short.
- Low- and intermediate-moisture foods had elevated water activity, or there was condensation on these foods.
- By inhibiting competing organisms and providing favourable conditions (e.g. vacuum packing), the environment selectively permitted certain pathogens to multiply.
Other situations in which tracing contamination to raw foods may be important and should be considered include:

- The pathogen is uncommon, newly emerging or re-emerging or causes serious disease (e.g. *E. coli* O157).
- It can be expected that foods will be eaten raw or lightly heated (e.g. shellfish, fresh vegetables, shell eggs).
- Little is known about a pathogen and there is a need to advance knowledge about its ecology.
- Unlicensed or illegally sold foods were involved.
- It is suspected that foods were adulterated.
- The source of contamination is unusual.
- A new or unusual vehicle is involved.

In such situations, a “traceback”, or tracing of the implicated food backwards through its distribution and production channels to its place of origin, is commonly performed. The purposes of such tracebacks include:

- identifying the source and distribution of foods in order to alert the public and remove the contaminated product from the marketplace;
- comparing the distribution of illnesses and distribution of product in order to strengthen an epidemiological association (sometimes referred to as an “epi” traceback);
- determining the potential route or source of contamination by evaluating common distribution sites, processors or growers.

Food tracebacks are often resource-intensive investigations requiring the coordination of many investigators from different agencies and organizations, often spread across different jurisdictions. Such investigations frequently require the review of detailed data on dates, quantities, sources and conditions of foods received, collection of original shipping containers and labels or other documentation, and information on lot numbers, facilities involved, production dates and the like. Traceback investigations can result in irreparable damage to food firms. It is therefore critical that each part of the investigation (epidemiological, laboratory and environmental) is thorough, complete and accurate.

An investigation at a farm or dairy will follow the same principles as the investigation of a food establishment. However, depending on the type of food product or animal involved, specific knowledge and skills may be needed to carry out the actual investigations. Most commonly, veterinarians, agriculturists, microbiologists and water supply experts will conduct these investigations in collaboration with epidemiologists.

Traceback investigations may lead to the identification of an ongoing public health threat and a consequent need to take appropriate actions, such as recall of foods, closing of a facility, confiscation of foods, or warning consumers of a potential risk. Investigators should be prepared to coordinate activities closely with other appropriate agencies and organizations to ensure a prompt and effective response as necessary.

### 4.4 Laboratory investigations

**General**

Most outbreaks of foodborne disease are microbiological in origin and their investigation will usually require a microbiology laboratory. Outbreaks caused by chemically contaminated
food also occur, although they are much less common than microbiological events. Symptoms resulting from both microbiological and chemical contamination can be similar and may be difficult to distinguish, even by laboratory tests. While the general principles of investigation apply to both types of incident, it is important to involve a chemical laboratory from the beginning if a chemical cause seems likely.

The role of the clinical laboratory in foodborne disease outbreak investigations includes:

- ensuring that appropriate clinical specimens are collected;
- arranging appropriate laboratory investigations of clinical samples;
- working with other members of the investigation team to identify and characterize the pathogen involved in the outbreak.

The role of the food laboratory in foodborne disease outbreak investigations includes:

- advising on appropriate samples to be taken from food;
- performing appropriate laboratory investigations of the food to identify the suspect pathogens, toxins or chemicals;
- advising on further sampling when a specific agent is found in the food (e.g. guiding collection of clinical specimens from food-handlers);
- working with the clinical laboratory to arrange for typing or additional characterization of organisms (e.g. serotyping, phage typing, molecular subtyping, antibiograms) as appropriate;
- supporting epidemiological and environmental investigations in detecting the pathogen in the implicated food and understanding how the outbreak occurred.

**Microbiological analyses**

In any outbreak of suspected foodborne disease, a microbiologist should be consulted as soon as possible. This person should be a member of the OCT.

**Clinical samples**

Diagnosis of most infectious diseases can be confirmed only if the etiological agent is isolated and identified from ill persons. This is particularly important when the clinical diagnosis is difficult to make because signs and symptoms are nonspecific, as is the case with many foodborne diseases. Faecal samples are the most commonly collected specimens; others include vomitus, urine, blood and clinical specimens (e.g. swabs from rectum, nostrils, skin or nasopharynx) obtained from food-handlers during the food investigations. If a disease has already been diagnosed, specimens should be collected according to Section 6.2. If a disease has not yet been diagnosed, specimen collection should be informed by clinical and epidemiological observations. Information on the collection, storage and transport of clinical specimens is provided in Annex 9.

If there is doubt about appropriate methods for collection, preservation (including selection of appropriate collection material) and shipment of specimens, guidance should be sought from the clinical laboratory. An indication should be given of how many samples are likely to be sent for analysis and whether the laboratory has sufficient resources to deal with them.

Clinical specimens should be taken from ill persons as soon as possible. Whenever possible, they should be taken from individuals who have not received antibiotic treatment for their illness. In large outbreaks, specimens should be obtained from at least 10–20 individuals (ideally 15–20% of all cases) who manifest illness typical of the outbreak and from some
exposed, but not ill, persons. Once the diagnosis has been confirmed, there is usually no need to obtain additional samples if individuals manifest characteristic symptoms. In smaller outbreaks, specimens should be collected from as many cases as practicable.

Specimens should be collected from persons who have been interviewed so that a link can be made between the laboratory and the epidemiological investigations. A unique identifier on the laboratory request form and the questionnaire will allow linkage of laboratory results with epidemiological information.

All containers should be labelled with a waterproof marking pen before or immediately after collection with the patient’s name, identification, date and time of collection, and any other information required by the laboratory.

**Molecular typing**

Recent advances in laboratory methods have contributed substantially to improvements in the detection and investigation of foodborne disease outbreaks. Molecular microbiology technology has markedly changed the nature of many acute disease epidemiology investigations. Polymerase chain reaction (PCR) technology is increasingly being used for the rapid identification of pathogens and in many cases allows determination of subtypes that previously required time-consuming and resource-intensive methods.

Pulsed-field gel electrophoresis (PFGE) can provide “DNA fingerprints” of bacterial isolates; if the PFGE patterns of clinical and food specimens are the same, the investigators have additional evidence that the suspected food item is implicated in the event. PFGE can also help investigators to include related cases and exclude concurrent cases that are epidemiologically unrelated to an outbreak. Such subtyping can be particularly useful when a pathogen implicated in an outbreak is very common and its presence in related specimens (e.g. cases, food and farm animals) may be purely coincidental.

Genetic sequencing technology has become more readily available and has been useful for assessing the relatedness of various pathogens involved in outbreaks of foodborne and waterborne disease. For example, sequencing of hepatitis A viruses collected during three large outbreaks associated with green onions demonstrated that similar virus strains caused all three outbreaks and were related to hepatitis A strains commonly isolated from patients living in the region where the green onions were grown. Sequencing of noroviruses is also becoming increasingly useful in identifying relatedness among potential outbreak-associated viruses.

Many subtyping and molecular microbiology tests are available only at specialized reference laboratories, and may require coordination with the primary laboratory involved in an outbreak investigation.

**Chemical investigations**

The features of important chemical foodborne illnesses are summarized in Section 6.2. In acute chemical exposures, most toxins or their metabolites are rapidly cleared from easily accessible specimens such as blood; prompt collection and shipment of specimens is therefore of critical importance.

When collecting samples for chemical analyses it is important to closely collaborate with the analytical laboratory, make arrangements in advance for chemical samples to be analysed and to seek advice about what specimens should be collected and how. The types of specimens to
be collected will depend on the suspected chemicals (Annex 9). In an emergency where it is impossible to contact the laboratory, biological specimens (whole blood, serum, urine, vomitus) should be collected as soon as possible, sealed in a clean container and sent to the laboratory promptly. Substances from the ambient air, the collector's skin or clothes, or interfering substances in collection and storage supplies may be concentrated and measured along with the specimens, yielding inaccurate results. Because care must be taken to avoid cross-contamination, contaminant-free materials (such as specialized collection containers) may be provided by the laboratory to ensure that extraneous contamination is kept to a minimum. Consultation with the testing laboratory is important in accurately interpreting results.