Nordic Workshop addressing case-control studies and food borne infections

Report from a workshop in Stockholm, Swedish Institute for Infectious Disease Control (SMI), Sweden, 12 October 1998

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The Nordic Food Policy Co-operation

The Nordic Committee of Senior Officials for Food Issues is concerned with basic Food Policy issues relating to food and nutrition, food toxicology and food microbiology, risk evaluation, food control and food legislation. The co-operation aims at protection of the health of the consumer, common utilisation of professional and administrative resources and at Nordic and international developments in this field.

The Nordic Council of Ministers

was established in 1971. It submits proposals on co-operation between the governments of the five Nordic countries to the Nordic Council, implements the Council's recommendations and reports on results, while directing the work carried out in the targeted areas. The Prime Ministers of the five Nordic countries assume overall responsibility for the co-operation measures, which are co-ordinated by the ministers for co-operation and the Nordic Co-operation committee. The composition of the Council of Ministers varies, depending on the nature of the issue to be treated.

The Nordic Council

was formed in 1952 to promote co-operation between the parliaments and governments of Denmark, Iceland, Norway and Sweden. Finland joined in 1955. At the sessions held by the Council, representatives from the Faroe Islands and Greenland form part of the Danish delegation, while Åland is represented on the Finnish delegation. The Council consists of 87 elected members - all of whom are members of parliament. The Nordic Council takes initiatives, acts in a consultative capacity and monitors co-operation measures. The Council operates via its institutions: the Plenary Assembly, the Presidium and standing committees.

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1 Preface

Each year, about 15% of all Europeans are reported to be affected by food borne diseases. No etiological agent or source of infection is found in the majority of cases. Food borne diseases, like salmonellosis and campylobacteriosis, comprise the majority of culture-confirmed cases, and the reported cases are increasing in several European countries.

The most common method of establishing the source of a food borne infection has been to take samples of different food items and from sick persons. Several sources of food borne outbreaks have been identified by this method. However, in many outbreaks, there is no food left to sample or there is no laboratory method available to find the organism in the food which caused the outbreak. A case-control study is an alternative approach to identify the source and the vehicle and therefore of great value when conducting an acute outbreak investigation. Furthermore, a case-control study could be very useful when investigate the source of infection of sporadic cases and could contribute to establishing the risk factors and their significance, which would create a basis for a specific control strategy.

In order to start Nordic co-operation in case-control studies and to learn from each other's experiences, a workshop was organised by Yvonne Andersson of the Swedish Institute for Infectious Disease Control, Sweden (project leader), Georg Kapperud of the National Institute of Public Health, Norway, Jakob Neimann of the Danish Zoonosis Centre, Petri Ruutu of the National Public Health Institute, and Asmunder Torkelsson of the Environmental and Food Agency, Iceland. Funds were granted by the Nordic Committee of Senior Officials for Food Issues (ÄK-livs), under the Nordic Council of Ministers.

In addition, Professor Stephen Palmer of the Public Health Laboratory Service, Wales, United Kingdom, and Dr. Jean-Claude Desenclos of the Réseau National de Santé Publique, France, were invited to present one lecture each (see workshop programme, p. 6). The participants from the Nordic countries who presented papers and/or took part in the discussions are listed below.

The contributions to the workshop have been set out according to the sessions in the workshop programme. The contents have been summarised and the appropriate abstracts and overheads are presented in this report.

Denmark

Hanne-Dorte Emborg, Danish Zoonosis Centre

Tine Hald, Danish Zoonosis Centre

Kåre Mølbak, Statens Serum Institut

Jakob Neimann, Danish Zoonosis Centre

Sören Aabo, Danish Veterinary and Food Administration

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Finland

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Pekka Nuorti, National Public Health Institute

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Iceland

Asmundur Thorkelsson, Environmental and Food Agency

Norway

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Christer Wiberg, National Food Administration

Ivar Vågsholm, Swedish Zoonosis Centre, National Veterinary Institute

Yvonne Andersson, Swedish Institute for Infectious Disease Control

Malin Arneborn, Swedish Institute for Infectious Disease Control

Johan Carlson, Swedish Institute for Infectious Disease Control

Birgitta de Jong, Swedish Institute for Infectious Disease Control

Johan Lindbäck, Swedish Institute for Infectious Disease Control

Reinhild Strauss, Swedish Institute for Infectious Disease Control

2 Summary

Representatives of the Nordic countries and two invited speakers from the United Kingdom and France gave presentations about different case-control studies conducted in their respective countries. The invited speakers were also asked to take up if possible, different questions relevant to case-control studies. The questions were sent out before the workshop to the speakers. The presentation and discussions at the meeting are summarised below as a number of conclusions and recommendations.

It is important to conduct case-control studies and it is also important that they should be well designed. It may initially be difficult to introduce case-control studies, but a few success stories make it much easier.

Try to get the local authorities to start case-control studies. It is always best to start with outbreak investigations; it is then easier to get funding for retrospective, case-control studies of sporadic cases.

There was much discussion about matching or not. A carefully prepared selection of controls may be better than a strict matching, as done does not need to analyse the case and controls in pairs. If matching is done, it should only be done on important confounding variables.

Caution should be exercised in interpreting the result of “protective” factors in a case-control study.

It is possible to perform a case-control study even with rather few cases. It is better to start early, because one never knows how many cases one will be able to include in the study.

If there are a large number of cases that will be interviewed, one or two controls are enough. In a small outbreak, three or four controls might be better. However, more than four controls will not strengthen the power of the study.

It is crucial to have a good study protocol before conducting a case-control study.

It is often easy to find the cases but it is more crucial to get the controls right.

To get more opportunities for Nordic epidemiologists to collaborate in outbreak investigations, there should be created opportunities to take part in outbreak investigations in another Nordic country.

More co-operations in conducting case-control studies between the Nordic countries is requested. Examples are a listeriosis case-control study in Norway and Sweden and a case-control study that involved most Nordic countries and some other European countries after an outbreak of *Salmonella* Paratyphi B in Turkey.

One suggestion for moving forward with case-control studies was to have a meeting every second year between Nordic epidemiologists, public-health doctors, veterinarians, environmental inspectors and others engaged in the investigation of food and waterborne outbreaks. The meeting should review the outbreak investigations of the last two years and thereby all participants may learn from these outbreaks. This will also encourage people to conduct more case-control and cohort studies.

3 Sammanfattning

Representanter från de Nordiska länderna och de två inbjudna föredragshållarna från Storbritannien och Frankrike höll föredrag om de erfarenheter av fall-kontrollstudier i deras respektive land som de vunnit under dessa studier. De inbjudna föredragshållarna hade också tillfrågats om de var villiga att ta upp olika metodfrågor vad gällde fall-kontroll studier. Metodfrågorna hade sänts ut tidigare.

Presentation och diskussion av mötet är sammanfattat som ett antal konklusioner och rekommendationer.

Det är viktigt att göra fall-kontroll studier vid livsmedelsburna utbrott.

Det är också viktigt att fallkontrollstudierna är väl utformade. Det kan i början vara svårt att få gehör för att utföra fall-kontrollstudier, men efter några lyckade utbrottsutredningar med fall-kontrollstudier brukar det gå lättare att få gehör för denna typ av utredningsteknik.

Försök att få lokala hälsovårdsmyndigheter att göra fall-kontrollstudier.

Det är alltid bäst att börja med utbrottsutredningar. Då metoden är känd är det lättare att få medel för sporadiska fall-kontrollstudier.

Det var mycket diskussioner om fall-kontroller skulle matchas eller ej. En väl utvald kontrollgrupp kan vara bättre än att strikt matcha då man slipper att utföra analyserna med matchade par. Om matchning görs skall det bara utföras på variabler där det finns stor risk för konfounding.

Det är viktigt att vara försiktig med tolkningen av ”skyddande” faktorer i fall-kontrollstudier.

Det går att utföra fall-kontrollstudier även med relativt få fall. Det är bättre att starta tidigt med få fall, då man aldrig vet hur många fall det egentligen kommer att bli i en studie.

Om studiepopulationen är stor räcker det med en eller två kontroller. I ett litet utbrott kan 3 eller 4 kontroller behövas. Det är ingen ide att ha mer än 4 kontroller då det inte ger mer styrka åt studien.

Det är viktigt att ha ett bra studieprotokoll innan man börjar en fall-kontrollstudie. Det är oftast lättare att hitta fallen än att få bra kontroller.

För att ge mer möjligheter åt Nordiska epidemiologer att samarbeta i utbrottsutredningar borde det skapas möjligheter att kunna delta i utbrottsutredningar i andra Nordiska länder.

Mer samarbete vad gäller fall-kontrollstudier i de Nordiska länderna är önskvärt. Goda exempel är en fall-kontrollstudie om sporadiska listeriafall utförd i Norge och Sverige och en fall-kontrollstudie som utfördes mellan flera nordiska och andra europeiska länder angående ett *Salmonella* Paratyphi B utbrott i Turkiet.

Ett förslag att ytterligare initiera fall-kontrollstudier var att ha ett möte vartannat år mellan Nordiska epidemiologer, folkhälsointresserade läkare, veterinärer, miljö och

hälsoskyddsinspektörer och andra personer engagerade i utredningar av vatten och livsmedelsburna utbrott. Mötet skulle t.ex. diskutera de två senaste årens utbrott och lärdomar av dem. Det skulle även uppmuntra andra att utföra fler fall-kontroll och kohort studier.

4 The objectives of the project

The objectives of this project were as follows:

1. To exchange experience of different case-control studies and food borne infections.
2. To enhance the knowledge of case-control studies and food borne infections.
3. To discuss different methodological tricks and hints.
4. To establish a Nordic mode of action for the different types of food borne infections.
5. To encourage co-operation between the Nordic countries in conducting case-control studies.

Programme of the Nordic Workshop addressing case-control studies and food borne infections, 12 October 1998

The workshop was divided into three parts. Three lectures were given about how to conduct case-control studies and the methodological difficulties in outbreak situations and sporadic cases. The invited lecturers had received 11 questions about the methodological difficulties involved in case-control studies that they had agreed should be the baseline for their lectures and the following discussion (see below).

Experiences gained from case-control studies in the Nordic countries, both of sporadic cases and of outbreak situations were reported. There was a discussion about how to promote the co-operation between the Nordic countries in case-control studies.

The workshop was held at Smittskyddsinstitutet, Solna, Sweden.

09.30-09.45	Introduction	Johan Carlson, Swedish Institute for Infectious Disease Control, Sweden
09.45-10.15	Study design	Georg Kapperud, National Institute of Public Health, Norway
10.15-11.15	Methodological difficulties in case-control studies (solitary cases)	Jean-Claude Desenclos, Réseau National de Santé Publique, France
11.30-12.30	Methodological difficulties in case-control studies (outbreak investigation)	Stephen Palmer, Public Health Laboratory Service, Wales, United Kingdom
12.30-13.30	LUNCH	
13.30-14.00	Discussion	
14.00-14.30	Campylobacter case-control study in Denmark	Jakob Neimann, Danish Zoonosis Centre, Denmark

14.30-15.00	Outbreak investigation (case-control study) in Denmark	Kåre Mølbak, Statens Serum Institut, Denmark
15.00-15.30	Coffee	
15.30-16.00	Study of yersiniosis in Finland	Petri Ruutu, National Public Health Institute, Finland
16.00-17.00	Co-operation between the Nordic countries, discussion	Yvonne Andersson, Swedish Institute for Infectious Disease Control, Sweden Georg Kapperud, National Institute of Public Health, Norway,

5 Introduction

Before the workshop, the working group discussed different issues that must be decided in the protocol before conducting and analysing case-control studies and also some fundamental issues about epidemiological investigations. The invited speakers were asked, if possible, to consider these questions (see below) in their presentations. The lectures were also followed by a group discussion about these issues.

1. **Time covered by the interview.** In our studies of campylobacteriosis, yersiniosis and salmonellosis, this period has invariably been two weeks, even though most of the patients have an incubation period of less than one week. Does this pose a problem or do we actually increase the probability of recording a relevant risk factor by enlarging the period of interview, since many of the exposures are general habits?
2. **Problem with case-control studies and Enterohaemorrhagic E. coli (EHEC).** Normally the interview will be conducted with the first case in a family that fell ill. But the small child in the family that fell ill might be a secondary case to a symptomless mother or father. How can this problem be solved?
3. **Statistical estimate for matched pairs, should one always match?** Is it better not to match when one has a small study? Normally we will match for age, sex and geographical area. What kind of problems can arise when one matches, compared with not matching?
4. **If a significant proportion of the population was immune, for example, to Campylobacter, some of them may appear as controls resulting in a selection bias which will underestimate the true risk factors.** Will this potential problem significantly influence the estimated risk? Is this only a problem in highly endemic areas where the prevalence of immunity in the general population is very high after repeated exposure to a dominant risk factor? What problem will this raise in the case-control study if only some age-groups are immune?
5. **How few cases can one have and still have a valid case-control study?** In an outbreak situation, how few cases should one have before one starts the case-control study? One to six controls? Can we make rules for the number of controls?
6. **In a case-control study about yersiniosis, it was found that eating fish was “protective” (i.e. more common exposure in controls than in cases).** In another study, eating a lot of beef was protective. Of course, not really protective, but people choose these types of food instead of eating chicken, for example. Should we report or reject them? If we include them in the multivariate analysis, it may give rise to a problem; what is the correct way to do it?
7. **Sequential, case-control studies; what is the advantage or disadvantage?**
8. **When should one stop a case-control study in an outbreak situation? Versus sporadic cases, how strong does the proof need to be?** If done does not find a suspected source, how long should one continue?

9. **Does one lose more in "unknown" bias when, to avoid recall bias, one asks the controls what they were eating in the last week before the interview and the cases what they were eating in the week before onset of symptoms?**
10. **Why does one not always find the associated food risk from case-control studies?** See the two examples attached to the outbreak investigations of bean sprouts.
11. **Is it possible to detect through a case-control study the risk factors for a disease with a low, infective dose?**

6 Study design

Georg Kapperud

National Institute of Public Health, Norway

Hand-outs of the lecture are enclosed, (see the appendix).

Design, conduct and analysis

The following steps should be followed in a prospective case-control study:

- Design
 - Define research hypotheses
 - Develop study protocol
 - Create the questionnaire
- Conduct
 - Test feasibility in small-scale, pilot study
 - Collect data
 - Enter and validate data
- Analysis
 - Perform statistical analyses
 - Report the results

In a study of sporadic cases, care should be taken to ensure that the enrolled cases are not part of a recognised outbreak. Suspected secondary cases should be excluded (e.g. only one case from each household). Preferably, a case should be laboratory-confirmed and identified by routine examination of clinical specimens within the study period. The case should also inhabit the geographically defined study area. If the study focuses on indigenous risk factors, the case should not have been travelling abroad prior to the onset of illness. Healthy carriers should not be included in the study.

Selection of cases

All eligible cases should have an equal chance of being enrolled. Depending on the expected number of cases in the study area, all cases could be selected during the study period or a random sample of cases or a systematic sample (e.g. every n^{th} patient with the study disease) could be made. It is important to have a predetermined sampling scheme. Cases may be identified from any of the following sources:

- Medical microbiological laboratories performing examinations of stool specimens.
- Reference laboratories receiving isolates for confirmation.
- Surveillance or notification systems that record culture-confirmed cases.

- Primary health service, hospitals or private practitioners.
- The best source is screening of the population

Selection of controls

Ideally, all potential controls should have an equal chance of being enrolled. Controls may be chosen from the general population (i.e. from a population registry), the patient's physician, or neighbours or by random digit-dialling. Household members and friends should not be used as controls, as this may lead to underestimation of risk factors.

Cases and controls should have an equal chance of being exposed to the disease or developing it; controls should be "at risk".

Exclusion criteria, case-control study

Cases should be excluded if they are not residents of the study area, have been travelling abroad in the two weeks prior to the onset of illness (if indigenous cases are studied) or are unable to complete the questionnaire or interview.

Controls should be excluded if they have a present or past history of the study disease or other diarrhoeal disease in the past month. They should also be excluded as controls if they are unable to complete the questionnaire or interview, are non-residents of the study area or have travelled abroad in the last two weeks. Also factors influencing the development of the studied disease, such as immunity and vaccination, may exclude them as controls.

Matching or not matching

A common subject of discussion in case-control studies is whether the controls should be matched or not. The purpose of matching is to control confounders, to eliminate of biased comparisons and to control of unpreventable risk factors (i.e. age, sex and geography). Some problems with matching are the following:

- 1) The risk-factor status of matching variables cannot be assessed
- 2) Factors closely associated with the matching variables (overmatching) may be underestimated
- 3) Loss of statistical power in matched multivariate analysis. In small studies, matching may nevertheless offer an advantage

Timelines

The cases are interviewed for the period before the illness. The length of this period is dependent on the incubation period of the study disease. Ideally, controls should be questioned in the same time period as the cases. This approach, however, may generate a recall bias, since cases, unlike their controls, are sensitised to the recall of exposures that occurred in their incubation period. Moreover, controls are often identified and contacted several days after the cases. To reduce recall problems, controls may be questioned for the time period before the date of interview. On the other hand, this approach may underestimate exposures that are seasonal, such as special food items eaten at Christmas or Easter and during barbecuing in the warm season.

Collecting data

Face-to-face interview, telephone interview, self-administered questionnaire, mailed questionnaire followed by interview.

Creating the questionnaire

A questionnaire for a case control study should include the following information:

Personal and demographic data

- Clinical-impact data (cases)
- Economic-impact data (cases)
- Exposure to potential risk factors
 - Consumption of specific food items
 - Drinking untreated water
 - Animal contact
 - Foreign travels
 - Eating outside the home
 - Eating raw, rare, or undercooked meat
 - Kitchen hygiene, food-handling practices, preferences

Interview

Approval by an ethical committee is usually required before a case-control study of sporadic cases can be conducted. Such approval is not necessary in outbreak investigations. In some countries, permission from the patient's health-care provider is needed. There is also a need to obtain informed consent from the persons enrolled. Those enrolled should be informed about the confidentiality and safety of the information they have given.

The interviewers should:

- Be motivated and trained for the study
- Be routinely monitored
- Not disclose the research hypotheses
- Not encourage positive responses from cases
- Not encourage negative responses from controls
- Same interviewer for each matched, case-control set

Number of cases and controls (sample size)

The number of cases and controls in a retrospective case-control study of sporadic cases is determined by the following factors:

- Anticipated risk (OR)
- Anticipated, statistical, exposure frequency
- Desired statistical power

- Control-to-case ratio
- Resource limitations, practical considerations

Data entry and validation

Enter the questionnaire data in a computer database and after that validate the consistency and the quality of the material.

The essential variables are:

- The dependent variable (case-control status)
- The set identifier (case-control group)
- The unique identifier

7 Methodological difficulties in case-control studies (solitary cases)

Jean-Claude Desenclos, Réseau National de Santé Publicque, France

Hand-outs of the lecture are enclosed, (see the appendix).

Introduction

To be able to perform a case-control study of sporadic food borne infections the requirements are that the sporadic occurrence is frequent and the prevention of sporadic infections is cost-effective. In France, there are about 40,000 reported, human cases of salmonellosis each year and about 700-800 reported, food borne outbreaks. Sporadic infections are related to continuous or intermittent contamination, low-level contamination of food items, changes in consumer behaviour and vulnerable populations, e.g. persons with an immunodeficiency or chronic disease.

To be able to prevent sporadic disease, it is crucial to know the risk factors, and therefore information from sporadic cases is essential. The goal is to keep food hygiene at a high and safe level, to prevent contamination at each level of the food chain (HACCP) and to educate the general public. For salmonellosis, the most common, risk items identified in France are eggs, poultry and beef, with facilitating factors of high-risk behaviour in cooking or storing food and host factors.

Why the case-control study approach?

- It is quick and easy
- Cases are usually easy to find
- The hypotheses may be multiple
 - Exploratory: explore several risk factors
 - Hypothesis-testing: specific hypothesis
 - A mix of both will give: “real life”
- What are the questions addressed
- Risk of bias
- Many technical issues
- Need for a well-prepared protocol and a good questionnaire

The case definition is very specific:

- Laboratory-verified cases, with the same serotype and, when necessary phage type
- Cases from outbreaks and secondary cases should be excluded
- The case finding is laboratory-based

Control selection

- Controls from the same population
- Controls should not be cases (silent infection)
- Control selection does not mean matching
- Fifteen cases and 30 or 45 controls may be enough in an outbreak investigation. However, the goal is about 40 cases. But most important is a well-designed study.
- For a study of sporadic cases, depending on the hypothesis, up to several hundred cases and controls are necessary

The hypothesis is of crucial importance. In outbreaks, one should not perform a case-control study if there is no hypothesis.

Outbreak versus sporadic approach

Outbreaks	Sporadic cases
Large relative risk	Smaller relative risk
High attack rate	Lower attack rate
Large inoculum	Lower inoculum
Source and vehicle	More cases in risk groups
Specific hypothesis	Hypothesis more complex, e.g. source and vehicle but also other factors, behaviours and host factors
Be quick	
Small sample size	Large sample size
Adjusting not an issue	Adjust for confounders
No research questions	There is a research question
General protocol	Specific protocol
	Need of additional resources

1. Time covered by the interview

By enlarging the time period, there will be an increase of exposed persons, both among cases and controls and this will also increase the number of persons exposed to a food item that is not contaminated. This will cause a dilution of the exposures among cases and controls. An enlarged time period will also result in a difference between cases and controls due to recall bias.

2. Problem with case-control studies and Enterohaemorrhagic E. coli (EHEC)

There are different tasks and the problem is that there is sometimes not enough knowledge to answer the question of introduction or transmission into the household. If foods have been introduced into the family, include the index case only. If there are co-primary cases, choose one of the cases at random.

3. Statistical estimate for matched pairs, should one always match?

One important thing that was discussed was that it is not always that clear about what is meant by matching; “control selection does not mean matching”. Matching is not the way of selecting controls. Controls should belong to the population from which the cases come. Matching is used for controlling confounding. Confounding is a third variable associated with the exposure and the outcome that distorts the measure of association between exposure and illness. Match only if there is a reason for doing so (confounding). A matched case-control study means matched analysis. Pertinent matching can be very beneficial, but matching also has drawbacks. Sometimes matching is necessary, but there may be a risk of overmatching. The exclusion of cases without matched controls may change the power of the study. Never match in exploratory studies and avoid matching, whenever possible, because it is often possible to adjust for a potential confounder during the analysis by multivariate analysis.

4. If a significant proportion of the population was immune, for example, to Campylobacter, might not some of them appear as controls, resulting in a selection bias which would underestimate the true risk factors?

An answer to this question was not established in the group.

5. How few cases can one have and still have a valid, case-control study?

That depends on the Odds Ratio (OR) level to be detected, risk α (5%), the power, the prevalence of exposure among controls and the number of controls (most efficient 3 or 4). In one legionellosis outbreak in Paris 8 cases and 24 controls were sufficient to find an association with staying in a specific part of Paris and contracting the disease. In a case-control study with sporadic infection, the OR to be detected is much smaller. Therefore the sample size usually requires several hundred cases and controls.

6. In a case-control study about yersiniosis, it was found that eating fish was “protective”, i.e. more exposure in controls than in cases. Should we report or reject this? If we take this into the multivariate analysis it may give us a problem; what is the correct way to do it?

When there are too many variables, significant risk or protecting factors will be found by chance in 5% of the variables. If this was part of the hypothesis, report it; otherwise, be very careful in interpretation.

7. Sequential case-control studies, what is the advantage or disadvantage?

From the general to the specific. Not used in case-control studies of sporadic cases. Can be used in outbreak situations when they are complex. The first step should then be to

discover the type of food and the second step the brand name, lot number or place of sale.

8. When should one stop a case-control study in an outbreak situation? Versus sporadic cases, how long does the proof need to be?

Sporadic cases. The protocol should have stated the hypotheses to be tested, the OR to be detected and the sample size to be reached. Stop when the sample size is reached, whatever the results. If there is a lack of cases, stop when the largest possible number is reached. A specific, independent, scientific committee may be useful in helping the investigation to resolve this question.

Outbreak investigation. There is a need to form a hypothesis. If negative (OR different from 1) after 30 to 40 cases and controls, the hypothesis was probably wrong. Stop and reconsider the hypothesis.

9. Does one lose more in "unknown" bias when, to avoid recall bias, one asks the controls what they were eating in the last week before the interview and the cases what they were eating in the week before the onset of symptoms?

Information should be collected among cases and controls in the same way. If you collect information for the last week before the interview among the controls, it should be the same for cases.

10. Why does one not always find the associated food risk from the case-control studies?

There may be several reasons for this. The appropriate variable was not included in the questionnaire. The exposure was not specific enough; it may have been due to a specific brand or lot number or a very short period of contamination. Another explanation may be very common foods item, so that most people were exposed, e.g. tap-water. A solution to this problem is to quantify the dose response, normally a larger amount for cases than controls. It may also be due to a different cooking practice, food habits and hygienic procedures among cases, compared with controls. Also a bias may have been introduced.

11. Is it possible to detect by a case-control study the risk factors for a disease with a low infective dose?

A low inoculum implies a low attack rate and case-control studies are very efficient for low attack rates. One example of this is outbreak investigations involving *Salmonella* and raw shell eggs, which normally have a low inoculum.

8 Methodological difficulties in case-control studies (outbreak investigation)

Stephen Palmer, Public Health Laboratory Service, Wales, United Kingdom

Some of the introduced points (hand-outs of the full paper are enclosed, see the appendix)

Wales conducted 18 case-control studies between 1988 and 1998. A case-control study should be quick and clean. If you are in doubt, do it; if the source of the outbreak is absolutely clear, there are no reasons to conduct a case-control study. It is important to publish well-investigated outbreaks, so that others can learn from the experience. In dealing with community outbreaks, case-control studies should be used and, if there was a closed party (e.g. weddings or birthdays parties) cohort studies are normally the best method. It is better to do a case-control study or a cohort study than not to do any epidemiological study. Good descriptive work is needed. Case-finding is not so difficult, use laboratory-verified cases.

Water as a suspected source, may be a problem in a suspected outbreak investigation, because most people have been drinking tap-water. Dose response may be a way of solving this. However, it may be difficult to recall how much water people have consumed.

Case-control studies may be selected as a method when one is unsure about the source of infection. Case-case studies comparing one outbreak e.g. a *S. Enteritidis* outbreak with other salmonellosis outbreaks, is another possibility.

Few cases in an outbreak investigation is no problem, but matching is needed.

However, about 20% of the answers are wrong (recall bias). That was studied by using a video camera when people were eating at a buffet and 3 days later they were asked what they had eaten from the buffet. However, there were answers from people saying that they had eaten dishes that had not been served at the buffet.

The hypothesis cannot be tested in the right way if the cases have been interviewed knowing the hypothesis. There is no need to have more than two controls to each case.

Outliers are always good to find. They may sometimes reveal the source of the outbreak

8.1 Discussion

The lectures were followed by a discussion.

In dealing with investigations of water borne outbreaks, there may be a problem with matching. In a large study, it was found that about 80% of all the people used tap-water,

which means that it might have been difficult to find unexposed people. As regards tap-water; focus on where patients are living and where they have been. Even if water is suspected, check other food sources. Sometimes, in dealing with water borne or vegetable borne outbreaks, it is better to ask about food habits instead of what food or water they have been exposed to. Try to quantify the amount of tap-water that people have been drinking.

The optimal investigation of a food borne outbreak indicates that there is both epidemiological and microbiological evidence for the incriminated food. This is not always the fact, often there is no food left to investigate. It is a great responsibility to stop a food plant if the evidence is not absolutely clear; the food plant may be declared bankrupt. A law court will not accept a case-control or a cohort study if the microorganism is not isolated from the food.

It is important that more case-control studies are conducted. Train more people and have more epidemiological courses in outbreak investigation. By conducting more case-control studies, people will be better trained and studies will be of a higher standard and will be completed in less time. In Wales, where case-control studies are more common than in the Nordic countries, they first complete a case-control study and after that an investigation of the food items that the studies have indicated as risk factor. All case-control studies must be well prepared to be reliable. With greater experience in performing case-control studies, there will be less bias. Colleagues must be persuaded to do more case-control studies. One should not conduct a case-control study in an outbreak situation if done does not have a hypothesis. It has been demonstrated several times that it is crucial to have a valid hypothesis. Should we not conduct more case-control studies of sporadic cases? The problem may be that it is difficult to get resources for sporadic, case-control studies. But it is important to conduct both kinds of case-control studies. One success story at an outbreak is important as regards receiving funding for case-control studies for sporadic cases. It could reveal a new source of food borne outbreaks that one would not have been aware of if one had not conducted case-control studies or other outbreak investigations.

You need at least one year for a retrospective, case-control study of sporadic cases. Experience in England has shown that it took a very long time to persuade local health authorities to conduct case-control studies. To be able to control for bias, more case-control studies are needed to get more experience.

9 Presentations from the Nordic countries

9.1 Campylobacter case-control study in Denmark

Jakob Neimann, Danish Zoonosis Centre, Denmark

Hand-outs of the lecture are enclosed,(in the appendix).

The number of campylobacteriosis cases has increased in Denmark since 1993. The objective of this one-year case-control study was to identify the risk factors for sporadic cases in Denmark. Sporadic Campylobacter cases were selected from samples taken from the two-thirds of the Danish population that were investigated and infected with Campylobacter. Controls, matched for date of birth, sex and municipality, were selected from the Danish central population register. There was a postal questionnaire with a diary included, then a telephone interview was conducted and the answers were recorded directly into the computer. The period of exposure was 14 days. All cases and controls were interviewed, using a standard questionnaire. However, cases with a history of foreign travel were only subjected to a shorter interview, leaving out information on, for example, food and animal contacts. In total, 979 questionnaires were sent out, and 60% answered the questionnaire. After exclusion, 282 cases and 319 controls were included in the case-control study. The risk factors were travel, underdone chicken, ground beef, barbecuing and water of poor quality. In the multivariate analysis, underdone chicken, barbecuing meat and water of poor quality were still significant. There was also a risk in having another underlying disease or having consumed antibiotics, laxatives, hormones, or antacids.

9.1.1 Discussion

How should one define travelling abroad in case-control studies? One way may be travelling in the last 2 weeks before onset. Exclude short visits (up to 8 hours). In Norway travelling inside the country was a risk factor. The risk is in where and what one eats while travelling, since there may be bad food hygiene at premises serving travellers.

Should protective factors be published in the results of the case-control study? A long discussion followed about protective factors; the different view.

Why are the protecting factors normally not published? There may be a lot of information included in them? The protective factors reflect more food habits than protective factors; if there are more studies that show the same thing, this may be important information. This is something problematic. Risk factors are associated with behaviour; we know nothing about protective factors. Protective factors should be reported.

Protective factors do not have the same estimate in the test. Daily contact with dogs was a risk in three studies of campylobacteriosis, not living with a dog. Protective factors

show the food habits of the cases but not those of the controls. Protective factors and vaccine efficiency should both have been exposed to the contagious pathogen. Protective factors should not be reported as interesting findings; instead, it is better to design a study to ascertain the protective factors.

9.2 Outbreak investigation (case-control study) in Denmark

Kåre Mølbak, Statens Serum Institut, Denmark

In Denmark, since 1996, case-control or cohort studies have been part of several outbreak investigations. The size of outbreaks has decreased since case-control studies were started. One of the breakthroughs was a *Salmonella* Manhattan, outbreak investigation with 16 cases and 45 controls; the source of the outbreak was smoked fillet of pork. It was traced back to the production line and the product was recalled on the basis of the result of the case-control study. Later, salmonella of the outbreak type was found in the product. This was the first time that smoked meat had been shown to be the cause of an outbreak in Denmark, and it had never before been associated as a risk factor with food borne infections. On the basis of this outbreak, the standards of production and monitoring were changed.

It is important that the source of the outbreak is not common knowledge during the case-control study. In this outbreak, the epidemiologists managed to perform the case-control study before the media became aware of the outbreak and the suspected food items. In some situations, the result of the analytical study is not available until the outbreak is over; nonetheless it is of value to conduct the study, as one is likely to obtain more detailed knowledge of the cause of the outbreak. This knowledge may be used to improve food safety and prevent the next outbreak.

9.2.1 Discussion

Can we reach consensus about a standard p-value that could be used as a cut off-value for a variable that is significant in analysis and will be used in the multivariate analysis?

9.3 Studies of Yersiniosis in Finland

Petri Ruutu, National Public Health Institute, Finland

Hand-outs are enclosed in the appendix.

Yersinia enterocolitica is a heterogeneous group, with at least 57 different serotypes. It's reservoir is: in pig tonsils (40% in Finland), pig intestines and faeces. The implicated food might be pork items,; particularly raw ground meat, sausage, chitterlings, unpasteurised milk and untreated drinking water. This was an example of a not yet analysed, case-control study, which is why only the method is presented.

To obtain information on the risk factors for acquiring *Y. enterocolitica* O:3 in children younger than 6 years, a prospective postal case-control study was conducted. Questions included related to symptoms and treatment, exposure 4 weeks prior to positive stool sample, different food items, tap-water source, pet animals and/or contacts with other domestic animals. No decision was made before hand on how many cases should be involved in the study; 24 cases and 37 controls were involved .

10 Co-operation between the Nordic countries, final discussion

Yvonne Andersson, Swedish Institute for Infectious Disease Control, Sweden and Georg Kapperud, National Institute of Public Health, Norway

How can we get more experience of conducting case-control studies and get the local health authorities to do more investigations of food and waterborne outbreaks? There is an interest in Denmark in more epidemiological courses on the part of medical officers and microbiologists. They would like to learn more about case-control studies and how to use them in local outbreaks. In this kind of course it is better to use data from outbreaks in one's own country or in a neighbouring country than to use the data from for example. Epi-Info. There was also agreement on the importance of teaching local people to conduct case-control studies. One way may be to have a group of central advisers to help local authorities with evaluation. In Finland, local authorities do cohort studies in outbreak investigations. The Finns have very good experience of giving courses to the local authorities and these courses are very popular. The community has the responsibility, but it is crucial that the epidemiologists should push the importance of case-control or cohort studies. Local outbreak investigations with cohort studies are a very good way to start with epidemiological studies.

Sometimes, there is no food left for sampling and one has to intervene on epidemiological information. After outbreaks it is important to trace the course of events back to the source of the outbreak. It is important to have field investigations and withdraw also of food items.

It is important to have a Nordic base and further co-operation in case-control studies. One way to increase the co-operation between the Nordic countries could be to have a workshop every second year. During this workshop all case-control and cohort studies carried out in the Nordic countries in the previous years should be discussed, in order to gain the experiences of others, (learning by doing). It is important to remember that there are both similarities and differences between the Nordic countries. There should be more opportunity for intra-Nordic collaboration in epidemiological studies. More, mutual, Nordic case-control studies should also be performed than the two already completed (*Listeria* and *S. Paratyphi B*, with also other European countries). Mutual studies can show similarities and dissimilarities that could be useful in outbreak investigations. In large outbreaks, people from the other Nordic countries could help.

10.1 Conclusion remark

It is important to move forward in this type of co-operation. This workshop was a good start. Now every one knows who to get into touch with to be able to discuss any epidemiological issues.

Appendix

Case-control studies: Possibilities and problems

Georg Kapperud

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Section of Food Hygiene, Norwegian College of Veterinary Medicine, Oslo Norway.*

During the past decades the world has experienced the emergence of a series of foodborne infectious diseases. In addition, several infections which we thought were under control, have re-emerged and sometimes reached pandemic proportions. There is an increased awareness that foodborne infectious diseases may cause serious and sometimes chronic sequelae. In order to meet the challenges, it is of primary importance to identify the sources of infection to enable implementation of specific control and preventive measures. To achieve this, two different methodological approaches are available: (1) laboratory methods based on microbiological or serological examination of samples from suspected sources of infection, and (2) analytic epidemiological methods which entail statistical analysis of epidemiological data, often in the form of questionnaires. The purpose of this lecture is to present some of the possibilities and problems associated with case-control studies of outbreaks and sporadic cases of foodborne infections.

The case-control study

In a case-control study patients who have recently been ill with the disease under study, are compared with healthy control persons regarding past exposures to potential risk factors in the incubation period. The patients are interviewed about their food consumption and exposure to other suspected risk factors in the period prior to onset of their illness. The control persons are interviewed about the same exposures, and the patterns of food consumption in cases and controls are compared to reveal statistically significant differences. This design is particularly relevant for studies of rare diseases. Compared to a cohort study, it is relatively quick to mount and conduct, relatively inexpensive and requires few subjects. These characteristics make the case-control design well suited for the study of both outbreaks and sporadic cases of foodborne diseases.

The aim of a case-control study is to identify the most important risk factors and determine the relative importance of these factors in terms of prevention. The results therefore provide a scientific basis for a specific control and prevention strategy aimed at reduction of those risk factors that are responsible for the greatest number of cases. Thus, compared to a microbiological approach which identifies risk factors through isolation of the agent from suspected sources of infection, a case-control study provides: (1) quantitative estimates of the risks of developing disease when exposed to various factors under study (e.g. food consumptions, cooking preferences, kitchen hygiene practices), (2) estimates of exposure frequencies - the proportion of the population exposed to the factors, and (3) attributable fractions, - the proportion of cases in the study population attributable to the

exposures of concern (the proportion of cases that could potentially be prevented by elimination of exposure). Multivariate analysis of the data collected enables an assessment of whether the risk factors are independently related to an increased risk of disease and may reveal interactions between the factors. Univariate analysis of relationships between variables can be explored by means of simple cross-tabulations and calculations of measures of association (odds ratios).

The design, conduct, and analysis of such a study consist of the following steps: (1) defining the research hypotheses, (2) developing the study protocol, which includes the procedures for case-finding, control selection, and data collection, (3) creating a standardized questionnaire based on the study hypotheses, (4) test the feasibility of the protocol and the questionnaire in a small-scale pilot study to allow for last-minute alterations, (5) collecting the questionnaire data, entering the data into a computer database, and validating the consistency and quality of the data, (5) performing univariate and multivariate statistical analyses, and (6) reporting the results.

Bias

When developing the study protocol, much of our efforts are focused on the avoidance of biased comparison of cases and controls. Bias may be defined as systematic errors in design, conduct, or analysis resulting in mistaken estimate of risk, as compared with the true values. Bias may be due to misclassification or under- or overrepresentation of the following categories: diseased, nondiseased, exposed, and unexposed. In other words: errors in the figures entered in the two-by-two table as compared with the true values. Biased comparison may be prevented if the following basic rules are obeyed: (1) cases and controls should be correctly classified (cases should have the study disease, and controls should not have this disease), (2) all eligible cases and all eligible controls should have an equal chance of being enrolled, (3) the cases and their controls should all have an equal chance of being exposed to the factors of interest, (4) cases and controls should have the same possibility of developing the study disease, (5) all enrollees should be given the same chance to recall exposures, and finally (6) data should be collected, entered and evaluated equally regardless of the case-control status of the enrollees. Unfortunately, it is seldom possible to follow all of these rules. Errors and inaccuracies are inevitable. However, by definition, bias is created only if the errors are unevenly distributed among the study groups (cases, controls, exposed, nonexposed). In this lecture, I will give a more detailed description of this problem and discuss how they may be solved.

Selection of cases

Cases may be identified from any of the following sources: (1) medical microbiological laboratories performing routine examination of stool specimens, (2) reference laboratories receiving bacterial isolates for confirmation and typing, (3) surveillance or notification

systems, or (4) the primary health service or hospitals. All of these sources are based on diagnosed cases where the etiological diagnosis have been confirmed by bacteriological analysis of clinical specimens. However, to ensure that all true cases have an equal chance of being enrolled, the optimal procedure would be to identify cases through screening of the general population. The reason for this is that the likelihood of being diagnosed may be associated with exposure, thus creating a potential selection bias. The decision to seek medical attention, the decision to take a sample, as well as the ability to isolate the causative agent may all be associated with exposure.

One example is campylobacteriosis: *C. coli* is common in pigs, while *C. jejuni* is frequently encountered in poultry. Since *C. coli* may produce milder symptoms, such patients are less likely to see a doctor, and the doctor is less likely to submit a stool from them. Hence, the exposures associated with *C. coli* will be underestimated. This problem is increased at the laboratory stage because *C. coli* do not grow well at the agar media used routinely for cultivation of *C. jejuni*. As a result, *C. coli* risk factors may be underestimated or even overlooked at several stages if only diagnosed cases are enrolled. A parallel problem arises with a disease like toxoplasmosis where the patient may be more likely to seek medical attention and the doctor may be more likely to take a sample if the patient has been exposed to a well-known risk factor, like living in a household with a cat. Consequently, suspected risk factors may be overemphasized. It is quite conceivable that false associations may be created in this way, unless the cases are identified through a screening procedure.

These problems should be addressed in the case definition which includes clinical information as well as information related to time place and person. The following case definition was used in a case-control study of *Campylobacter* infections in Norway. It is based on: (1) Acute culture-confirmed clinical cases from whom *Campylobacter* was isolated as the sole pathogen during the one-year study period, a criterion which assures that the patients actually had campylobacteriosis and not a different disease. Unfortunately, we were not able to identify cases through screening of the general population. (2) The patients were identified by routine examination of clinical stools samples from persons with acute gastroenteritis, which means that were not selected by any means at the stage of the laboratory. (3) They were recently diagnosed to ensure that they were able to remember exposures. (4) They were not part of a recognized outbreak, or if an outbreak occurred, only the first identified case was enrolled. This was done to ensure that factors involved in outbreaks were not overemphasized. (5) The cases were also inhabitants of a geographically defined study area, and (6) in addition, we decided to restrict the investigation to infections acquired in Norway by excluding cases who had travelled abroad in the last two weeks before onset of illness.

Selection of controls

Sources

One of the major rules of case-control studies is that all potential controls should have an equal chance of being enrolled in the study. The optimal way to achieve this is by selecting controls at random from the general population in the study area using an updated population registry. If a population registry is not available, any of the following approaches may be used: (1) Asking the patients' physicians to nominate eligible controls from their practice. (2) Selecting eligible controls by going into the patient's neighborhood selecting households using a predetermined search strategy, or asking the patients to nominate controls among their friends. While this method is easy to accomplish, this type of friend control often has more similar eating habits and other potential risk factor habits than the general population, and may therefore decrease the ability to detect important risk factors. This problem is even more accentuated when household members are used as controls, and this type of controls is not recommended. (3) Finally, in an area with extensive use of telephones in households, controls may be selected by dialing a random selection of telephone numbers within the patient's exchange area.

Exclusion criteria

In general, a person should not be used as a control for any of the following reasons: (1) Microbiological, serological or clinical evidence of the disease, (2) conditions which preclude exposure, for example non-residence of the study area, (3) factors influencing development of the disease, like immunity or insusceptibility, and (4) inability to complete the questionnaire or interview. In a practical situation, it is difficult to fulfill all of these criteria. One problem is exclusion of immune controls.

Immune controls

A basic requirement is that cases and controls should have an equal chance of developing the study illness. Immune persons are not at risk of developing this disease, and inclusion of such subjects as controls may lead to underestimation of risks, since they may have increased exposure to legitimate risk factors by virtue of the fact that they have already been exposed and infected. However, exclusion of immune persons is not possible unless the immunological status of the potential controls is tested. This is rarely done in studies of foodborne infections. Nevertheless, immune controls may pose a potential problem in areas where the prevalence of immunity is high, either due hyperendemicity or vaccination.

Matching

In all case-control studies the question arises whether the controls should be matched to the cases. In general, the purpose of matching is to control confounders and assure that the cases and controls are comparable with respect to the factors used for matching so that no large imbalances occur and biased comparison is avoided. Secondly, by matching on unpreventable risk factors like age, sex and geography, the effect of these factors are controlled in the study design. Without matching these factors must be controlled in the multivariate analysis. The problems associated with matching are: (1) The risk factor status

of the matching variables cannot be assessed because by definition cases and controls are similar on these factors. (2) Unfortunately, matching may lead to underestimation of factors closely associated with the matching variables. For example, geographical matching may underestimate the effect of drinking water as a risk factor, since people living in the same area are likely to have the same or a similar drinking water supply. (3) Finally, a matched design requires an analysis which takes the matching into account. Such matched analysis have lower statistical power than its unmatched counterpart. However, in small studies statistical power is also lost when many variables are included in the multivariate analysis.

Some epidemiologists feel that there are indeed good reason to match cases and controls in many studies of sporadic as well as outbreaks of foodborne infection. The purpose is to identify risk factors that may be subject to prevention. This requires that other factors that may be associated with an increased risk but are not amenable to prevention, such as age and sex, must be controlled for. Unless one wants to demonstrate once again that age or sex are risk factors, then one can control for these factors by matching. Although this could potentially be achieved by the multivariate analysis, such a strategy would be difficult when low number of enrolled cases are anticipated. Too many factors will lead to breakdown of multivariate models. Therefore, in small studies it is an advantage to get rid of unpreventable factors by matching on them.

Selection bias

At this point, I would like to summarize some of the problems associated with selection bias. Such problems may arise if: (1) The cases or controls are misclassified. This problem may be minimized by using a case-definition based on a culture-confirmed diagnosis, and excluding potential controls with a recent or present history of gastrointestinal complaints. (1) The likelihood of being diagnosed is associated with exposure. (2) The willingness to participate is associated with exposure. (3) The chance of exposure differs between cases and controls. (4) The susceptibility to disease differs between cases and controls, for example due to immunity among the controls. (5) The enrollees are not representative for the study population. (6) Overmatching may lead to underestimation of factors closely related to the matching variables.

Data collection

Interview

Once the questionnaire and study protocol have been developed and tested in a pilot study, the relevant information may be obtained from the cases and their controls through any of the following approaches: (1) Face-to-face interview. (2) Telephone interview. (3) Self-administered questionnaire, mailed or delivered to the enrollees. The self-administered questionnaire often produces less reliable information than one administered by an interviewer. It eliminates the ability to ensure that the questions are understood and to probe for subtleties and additional information. On the other hand, this approach eliminates

interview bias, reduces costs, and enables easy questioning of large numbers of persons. One combined approach which may improve the quality of interview data, is to forward the questionnaire to the respondents and subsequently conduct the interview, after they have read the questionnaire and refreshed their memory.

Period covered by the interview

Ideally, the cases and their controls should be questioned about the same time period to ensure that they had the same chance of being exposed to risk factors. However, this approach may introduce a systematic recall bias since controls usually have greater problems recalling past food consumption than cases. This is particularly critical if controls are identified and contacted several weeks after the case. To reduce recall problems, the controls may therefore be questioned about the period before the date of interview, while the cases are interviewed for the period before the onset of their illness. Such a strategy, however, may create new problems because some exposures may vary seasonally, for example eating at barbecues or factors related to outdoor activities. Moreover, since risk factors may vary seasonally, the study period should comprise at least one full year to enable estimation of the relative contribution of the different risk factors. For example, if a seasonal activity like camping is associated with risk, this factor may be overlooked if the study is limited to seasons where this activity does not take place.

Recall bias

In general, problems related to recall bias may occur because: (1) The time interval between exposure and interview is often longer for controls than for cases. (2) Cases are more likely than controls to remember any exposure which occurred around the onset of illness. (3) Cases may be sensitized to their recall of suspected risk factors, in a situation where the study hypotheses have been revealed or is well known to the public. Although the patients do not necessarily falsely report an exposure that did not occur, they may search their memory more thoroughly than control persons. (4) Finally, the problem of differential recall may be enlarged if controls suppress their recall of suspected risk factors. If possible, the enrollees should be blinded to their case-control status, but this is often not possible for practical and ethical reasons.

Interviewer bias

Some common sources of interviewer bias are: (1) Interviewers disclose the study hypotheses leading to differential recall of exposures, and thereby creating a recall bias. (2) They encourage and reward positive response from the cases on suspected risk factors. (3) Interviewers encourage and reward negative response from the controls on the same factors, or (4) they record answers differently. If possible, the interviewers should be blinded about the case-control status of the respondents.

Analysis

After the questionnaire data have been entered into a computer database and their

consistency and quality have been critically evaluated, the study results are ready for analysis. It is recommended to start with simple descriptive analyses and summaries. Next, associations between the study disease and potential risk factors can be assessed using univariate analysis which corresponds to the matched design. Finally, multivariate methods should be applied. It is recommended that conditional logistic regression is implemented in the multivariate analysis. Logistic regression may also be employed for univariate analysis of continuous variables. To address the issue of representativeness, the enrolled cases should be compared to non-enrollees with respect to age, sex and geographic distributions.

Statistical significance vs. causal relationship

Finally, a few words should be said about the limitation of case-control studies. The finding of a statistically significant association between a potential risk factor and the study disease may be due to a multitude of factors. Although we hope that the observed effects are due to a causal relationship, it is necessary to emphasize that associations may also appear by chance. A statistically significant association may also be due to bias, as detailed earlier, or confounding: co-variation with a true risk factor which has not been controlled for neither in the multivariate analysis nor in the design. In general, a causal relationship is reasonable if the association is biologically plausible, consistent with previous and subsequent studies, stable in the multivariate analysis, if there is a dose-response relationship, or if there is a logical sequence from exposure to effect.

In conclusion, the design, conduct, and analysis of a case-control study is a long and laborious climb to the summit. The importance of careful planning, protocol development and attending to details at the outset cannot be overemphasized. Likewise, good management and organizing over the course of the study are critical for its success. I would like to draw your attention to a recent publication from the WHO entitled: "Suggestions for the design, conduct and analysis of an epidemiological study aimed at identification of risk factors for *Campylobacter* infections in humans" (WHO/EMC/ZOO/98.3). Although this document focuses on case-control studies of *Campylobacter* infections, it may also be helpful in designing similar studies for identification of risk factors for other foodborne infections.

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Identify Sources of Infection

Laboratory methods:

Analytic epidemiology:

Case-Control Study

Effect to cause

Patients (cases) are compared with healthy control persons regarding past exposure to potential risk factors

Aim

- Identify the most important risk factors
- Determine their relative importance
- Provide a basis for a specific control strategy
 - Adequate health information
 - Reduce level in food and domestic animals

Case-Control Studies Provide:

- An estimate of risk (Odds ratio, OR)
- An estimate of exposure frequency
- An estimate of attributable fraction
- An assessment of independence and interaction

Design - Conduct - Analysis

- Define research hypotheses
- Develop study protocol
- Create the questionnaire
- Test feasibility in small-scale pilot study
- Collect data
- Enter and validate data
- Perform statistical analyses
- Report the results

Design

Conduct

Analysis

Bias

Systematic error in design, conduct or analysis resulting in mistaken estimate of risk

THIS MAY BE DUE TO:

- Misclassification
- Over- or under-representation

- Diseased
- Nondiseased
- Exposed
- Unexposed

A	B
C	D

Common Types of Bias

- Classification bias
- Detection bias
- Selection bias
- Recall bias
- Interviewer bias
- Analysis bias

Bias May Be Prevented

CASES AND CONTROLS:

- Should be correctly classified
- All eligible subjects should have an equal chance of being enrolled
- Should have the same chance of being exposed
- Should have the same possibility of developing disease
- Should be given the same chance to recall exposures
- Data should be collected, entered, and evaluated equally

Selection of Cases

SOURCES:

- Medical microbiological laboratories
- Reference laboratories
- Notification systems
- Primary health service, hospitals

Diagnosed cases

- Best: Screening of the population

Diagnosed Cases

THE LIKELIHOOD OF BEING DIAGNOSED MAY BE ASSOCIATED WITH EXPOSURE:

- Patient: The decision to seek medical attention
- Physician: The decision to take a sample
- Laboratory: The ability to isolate the agent

IDEAL SOLUTION: Screening of the population

Case Definition Campylobacter Case-Control Study

- Acute culture-confirmed campylobacteriosis
- Identified by routine examination of clinical specimens
- Recently diagnosed within study period (incident cases)
- Not part of a recognized outbreak (sporadic cases)
- Inhabiting a geographically defined study area
- Not travelled abroad prior to illness onset

Selection of Controls

SOURCES:

- The general population, population registry
- The patient's physician
- Neighbours, friends, household members
- Random digit dialing

All potential controls should have an equal chance of being enrolled

Exclusion Criteria

- Microbiological, serological, or clinical evidence of disease
- Conditions which preclude exposure
- Factors influencing development of disease (immunity, susceptibility)
- Inability to complete questionnaire or interview

Immune Controls

Inclusion of immune controls may lead to underestimation of risk:

- Are not susceptible to the study disease
- Have already been exposed and infected
- May have increased exposure to legitimate risk factors

Problem if prevalence of immunity is very high:
- hyperendemic area
- vaccination

Matching

PURPOSE:

- Control of confounders, elimination of biased comparison
- Control of unpreventable risk factors (age, sex, geography)

PROBLEMS:

- Risk factors status of matching variables cannot be assessed
- Underestimates factors closely associated with matching variables (overmatching)
- Suboptimal analysis strategy? Not in small studies!

Rational for Matching

- PURPOSE: Identify preventable risk factors
- PROBLEM: Unpreventable risk factors must be controlled
- SOLUTION: Matching!

With few cases, it is difficult to control many factors in the multivariate analysis (model instability, non-convergence)

Selection Bias

PROBLEMS MAY ARISE IF:

- Misclassification of cases or controls
- Likelihood of being diagnosed is associated with exposure
- Willingness to participate is associated with exposure
- Chance of exposure differs between cases and controls
- Susceptibility to disease differs between cases and controls
- Enrollees are not representative for the population
- Overmatching

Collecting Data

ALTERNATIVES:

- Face-to-face interview
- Telephone interview
- Self-administered questionnaire
- Mailed questionnaire followed by interview

Period Covered by Interview

Recall bias !

- CASES: Period before illness
- CONTROLS: Period before interview

PROBLEM: Exposures may vary seasonally !

Recall Bias

- Time interval between exposure and interview is often longer for controls than for cases
- Cases are more likely to remember any exposure
- Cases are sensitized to their recall of suspected risk factors
- Controls may suppress their recall of suspected risk factors
- Cases exaggerate, controls underemphasize suspected risks

Blinding ?

Interviewer Bias

- Interviewers disclose the study hypotheses leading to differential recall of exposures (recall bias)
- Interviewers encourage and reward positive response from cases on suspected risk factors (probe more deeply)
- Interviewers encourage and reward negative response from controls on suspected risk factors
- Interviewers interpret or record information differently

Blinding ?

Statistical Analyses

- Descriptive analyses and summaries
- Univariate analysis of risk factors
- Multivariate analysis of risk factors

- Independence ?
- Interactions ?

Representativeness

Compare enrolled and non-enrolled cases:

- Age distribution
- Sex distribution
- Geographic distribution

Statistical Significance

MAY BE DUE TO:

- Causal relationship (cause - effect)
- Chance (random effect)
- Systematic error (bias)
- Association with a true risk factor (confounding)

Causal Relationship

REASONABLE IF:

- Biological plausible
- Consistent with other investigation
- Strong association
- Dose-response relationship
- Logical sequence: Exposure - disease

The use of the case-control study design for studying risk factors of sporadic foodborne infection

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Goals

- Answer Yvonne's questions !
- Three examples in France :
 - *Salmonella* Enteritidis : 1995
 - *Salmonella* Typhimurium : 1996
 - *Listeria monocytogenes* : 1997

Why case-controlling sporadic foodborne infections ?

- Sporadic occurrence is frequent
 - France : $\approx$ 40 000 isolates per year of *Salmonella*
 - 700 - 800 foodborne outbreaks
- Sporadic infection is related to :
 - continuous or intermittent low level of contamination
 - change in consumer behaviours
 - vulnerable populations
- How to prevent ?
 - Need to know risk factors
 - information from outbreaks may not be relevant

Objectives of a case control study

- Goal : prevention at each level of the food chain
 - food production, hygiene...
 - education
- What is (are) the vehicle(s) ?
 - eggs
 - beef, poultry...
- Facilitating factors :
 - high risk behaviours ?
 - cooking practices
 - storing practices...
 - host factors
- Protecting factors
- Evaluate prevention measures

Why the case control approach ?

- Faster and easier to go
- Cases are usually easy to find
- Hypothesis may be multiple :
 - exploratory : explore several risk factors
 - hypothesis testing : specific hypothesis
 - a mixture of both : « real life »
- What is (are) the questions addressed ?
- But risk of biases+++
- Many technical issues !
- Need for a well prepared protocol

Outbreak vs sporadic approach

• Outbreaks	• Sporadic cases
– large relative risk	– smaller relative risk
– high attack rate	– lower attack rate
– large inoculum	– lower inoculum
– source and vehicle	– high risk groups
– specific hypothesis	– source, vehicle but also other factors : behaviours, host...
– be fast	– hypothesis • more complex• several
– small sample sizes	– much larger sample size
– adjusting not an issue	– adjust for confounding
– no research questions	– specific protocol
– general protocol	– need of additional resources

Outbreak vs sporadic cases: time interval for interview

- Common source outbreak

- Sporadic cases

Time period interview

- Outbreak investigation be specific +++

- hypothesis : brand or lot contaminated for a specific period
- exposure period :
 - cases : incubation period from date of onset
 - controls : same period from date of onset of cases
 - if not, controls may have a zero opportunity to be exposed if the food was contaminated for a short time interval
- investigation need to be timely for cases and controls

- Sporadic occurrence

- interviews are done later
- low continuous and/or intermittent contamination
- food vehicle hypothesis is often less specific
- define a longer period of exposure (2 weeks) from onset

Consequence of enlarging time covered by interview

- By enlarging the time period
 - increase the % of exposed among cases and controls
 - increase the % of exposed to the food that is not contaminated
 - therefore dilute the exposure among cases and control and bias toward zero (if no big differences in recall between cases and controls)
 - in theory should not pick up a food that is not relevant
- But if exposure period is too small
 - cases and controls do not recall and answer anything
 - cases recall better than controls : risk of bias +++
- Compromise based on incubation period (2 to 4 weeks)

How to do when Several cases in a family !

- What is the question ? introduction vs transmission
- Foods that introduced the infection in the family
 - include the index case only
 - if co-primary cases : choose one at random
- Risk factors that facilitate transmission in household
 - compare households with one case only to households that have documented secondary cases
- Any factor that affect transmission
 - foods and person to person
 - choose one case at random
 - choose the or one of the confirmed case(s) if several

Case definition

- Case definitions :
 - be very specific to avoid missclassification
 - laboratory confirmed
 - same serotype ? same phage type ?
 - Which cases to exclude ?
 - cases associated with common source outbreaks
 - if secondary transmission
 - depend of the question
- Case finding :
 - laboratory based

Control selection

- Controls should be sampled from the population in which cases occur : define well this population
- Controls should become a case if exposed (past immunity)
- Controls should not be cases (silent infection)
- Controls should not have a zero opportunity of being exposed
- Control selection does not mean matching

Matching ?

- Matching is not the way to select controls
- Controls should belong to the population from which cases come from
- Matching is used for controlling for confounding
- Confounding is a third variable associated to the illness and to the exposure that distorts the measure of association between exposure and illness
- Match only if you have reasons for (confounding)
- Matched case control study = match analysis
- Pertinent matching can be very beneficial
- Matching have drawbacks

Confounding and matching

Analysis of matched pair data

- Analyse pairs of case and control
- Use of discordant pairs only in the analysis
- The contingency table of a matched pair case control study can be summarized as follow

	Controls				Cases		Controls
	exp+	exp-	total		exp+	exp-	
Cases	exp+ a	exp- b	exp+ a+b	→	exp+ c	exp- d	exp+ c+d
	exp- g	exp- h	exp- g+h		exp- e	exp- f	exp- e+f
	e+g	f+h					

OR = f/g : Mac Nemar OR

- $OR = \frac{\text{N° of pairs case exp+ / control exp-}}{\text{N° of pairs case exp- / control exp+}}$

Analysis of matched data

Sets of case and controls are decomposed in pairs

case	Control1	Control2	Control3	Control4	C+Ctrl-	C-Ctrl+
+	-	+	-	-	1	0
+	+	-	+	+	1	0
+	-	-	-	-	0	0
+	-	+	-	-	0	1
+	-	-	+	+	1	0
+	+	+	+	+	0	0
Total					6	1

OR = $\frac{\text{pairs case exposed / control unexposed}}{\text{pairs case unexposed / control exposed}} = \frac{6}{6} = 1$

Matching : drawbacks

- A matched analysis is necessary
 - more complex (pairs)
 - the matching variable can not be studied
 - dose response is more complex to analyze
 - other confounders ?
- Selection of controls may be more difficult
- Lose of efficiency if :
 - overmatching : matching on one or several variables only associated to the exposure (increases the number of concordant pairs)
 - Exclusion of cases without matched controls
 - No pair "control exposed/case unexposed"
 - Matching can be beneficial for one variable and not for others (exploratory studies)

Matching or not matching ?

- Matching is not the way to select controls
- Match on purpose only
- To control one or several known confounder(s) by preparing an efficient stratified analysis
- Confounders should have been identified "a priori"
- No need to analyze the matching variable (however, a true risk factor may have a confounding effect)
- Selection of matched controls is not too difficult and costly or it is less expensive
- Never match in exploratory studies
- Avoid matching whenever possible

Susceptibility of controls ?

- Controls should become cases if exposed
- If immune, they can not : should then be excluded
- If not, it biases the results towards zero
- If risk of infection and immunity associated to age, age may act as a confounder : match or stratify on age
- The question is how long is the person immune ?
- If possible, test controls for antibody (hepatitis A)
- Exclude patients with a recent history of diarrhea
- Avoid family controls : immunity and silent infection

How many cases and controls

- Depends of :
 - relative risk to detect (OR)
 - risk α (5%)
 - power $1 - \beta$ ($\beta = 20\%$)
 - prevalence of exposure among controls (smallest if 50%)
 - control to case ratio : most efficient if 3 or 4
- If OR to detect = 15, and 25% of controls are exposed : 14 cases and 14 controls are sufficient ($\alpha = 5\%$, $\beta = 20\%$)
- *Legionella* outbreak in Paris in June : 8 cases and 24 controls were sufficient to find an association between staying in the 9th district of Paris (OR = 15%)

Trichinosis cases and controls by meat consumed, Paris, December 1993

Meat	Cases (%) (N = 239)	Controls (%) (N = 177)	Odds ratio	95% C.I.
Porc	84.9	84.7	1.0	0.6-1.8
Wild pig	1.6	3.3	0.5	0.1-2.0
Beef	95.0	94.9	1.0	0.4-2.7
Sheep	68.2	66.1	1.1	0.7-1.7
Horse	99.6	72.3	91.1	5.1-330.4

Cases of trichinosis and controls by meat consumption, France, 1998

Meat	Cases (17)		Controls (34)		OR	IC 95%
	N	%	N	%		
Pork	13/16	81.3	28/33	84.8	0.78	0.1 - 5.8
Sangler	2/16	12.5	4/34	11.8	1.07	0.1 - 8.5
Beef	14/15	93.3	32/34	94.1	0.88	0.04 - 55.3
Sheep	9/16	56.3	13/33	39.4	1.95	0.5 - 7.9
Horse	13/17	76.5	6/34	17.6	14.09	3.1 - 82.5
Charcuteries	12/14	85.7	28/33	84.8	1.07	0.1 - 12.7

Usual sample size for community foodborne outbreaks, RNSP

- Community outbreak
- Detect a minimal relative risk
- Exposure among controls
- $\alpha = 5\%$, $\beta = 20\%$:
- 42 cases and controls
- If enough cases (listeriosis outbreak...)
- In fact true RR is often greater than 4

When to stop a case-control study ?

- For sporadic infection
 - the protocol should have stated the hypothesis to test, the OR to detect and the sample size to reach
 - Stop when the sample size is reached whatever the results
 - if lack of cases, stop when the largest possible number
 - if intermediate analysis : define the way to do in the protocol (avoid intermediate analysis)
- Outbreak investigation
 - need of an hypothesis+++
 - if negative after 30 to 40 cases and controls,
 - the hypothesis was probably wrong
 - do not continue
 - reconsider your hypothesis

Sequential case-control studies

- Sporadic infection : not used (specific protocol)
- Outbreak needed when complex situation
 - From the general to the specific
 - First step : type of food
 - Second step brand name and/or lot number or place of sale...
- Which cases and controls for the second step ?
 - Cases and controls exposed to the food

Brand	Cases	Controls	OR
A	a	b	13,0
B	c	d	0,6
C	e	f	0,4

- All initial cases and controls

Brand	Cases	Controls	OR
A	a	b	11,0
B	c	d	2,1
C	e	f	1,2
none	g	h	1,0

Low inoculum and case control studies

- Low inoculum imply low attack rate (AR)
- Case-control studies are very efficient for low AR
- Low inoculum associated with sporadic food contamination, such as shell eggs
- The key is to define at best :
 - exposure: define each food in a specific way
 - period of exposure : as specific as possible
 - quantify exposure to assess dose-response
 - use more vulnerable populations
 - reduce information bias : timely interview
- See Salmonella Enteritidis example

Multivariate analysis

- Consider variables that make sense
- And
- Have a p value < 20% in univariate analysis

Protective effect

- Does it make sense ?
- For example :
 - beef well cooked : OK
 - portable telephone : ???
- When too many variables : significant risk or protecting factors found by chance in 5% of variables
- If part of the hypothesis : report it, otherwise be very careful in interpretation

Negative results

- The appropriate variable was not included
- Exposure not specific enough
 - specific brand
 - specific lot number
 - very brief period of contamination
- Very common food :
 - almost every body is exposed
 - quantify
 - dose response
 - larger amount for cases than control (tap water)
 - cooking practices...
- Bias

Publication

- At least write a full report = institutional memory
- Share with public health colleagues : publish in Epidemiological Bulletin (national or European)
- Share with the scientific community : publish in peer review journals+++ and present in seminar and conference

***Salmonella enteritidis* infection : how young children get infected**

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² Centre National de Référence des *Salmonella* et *Shigella*

Rationale

- *Salmonella* Enteritidis still ranked first
- Despite control efforts on the food chain
- *Young children*
 - Greater risk
 - More severe
 - Easier to collect food history <5 years
- *Knowledge on risk factors was based on :*
 - data from local outbreak
 - not on data from sporadic cases in France
- Goal : improve primary prevention

Objectives

- **Mode of transmission**
 - person to person
 - foods implicated
 - eggs
 - poultry
 - facilitating behaviors
 - storage
 - preparation
- **Host factors**
 - underlying condition
 - prematurity, breast feeding...

Methods (1)

- *Study design*
 - incident matched case-control study
 - 1 control per case
- *Selection of cases*
 - *Case definition :*
 - child $\leq$ 5 years
 - stool or blood culture positive for *S enteritidis*,
 - gastro-enteritis or infectious syndrome between March 1 and September 30 1995 not associated to a common source outbreak
 - *Case Identification :*
 - National Reference Center (50 % of isolates for typing)

Methods (2)

- *Selection of controls*
 - Matched on age and place of residence
 - Age :
 - Same age group : $\leq$ 1, >1 to 5 years
 - Place of residence
 - same city
 - or same parish if rural area
- *Mode of selection*
 - case general practitioner
 - minitel telephone directory if not possible

Methods (3)

- **Data collection**
 - *Standardized questionnaire*
 - tested during a pilot phase (5 cases and controls)
 - by telephone
 - one investigator not blinded to case status
 - *Variables (1)*
 - social and demography
 - attendance of nursery and day care
 - breast feeding, underlying condition, treatment...
 - diarrhea among contacts
 - eggs and poultry: purchase, storage, preparation
 - parents food habits : control for information bias

Methods (4)

- Time period for data collection
 - during the month prior to onset in the case
 - delay in the interview
 - food consumption does not vary very much <5years
 - contamination is sporadic
- Sample size
 - OR to detect = 2.5
 - exposure among controls 25%
 - $\alpha = 5\%$, $\beta = 20\%$, 1 control matched for each case
 - 94 pairs
 - stratification on summer (yes/no) : 120 pairs to reach

Results (1)

Characteristics of cases included

	NRC Cases	Cases included	%
≤ 1 year	51	23	45.0
> 1 year	209	85	41.0
Summer	201	68	33.8*
Non summer	59	40	67.7*
Hospitalized	84	32	38.0
Non hospitalized	176	76	43.0

Salmonella enteritidis, France 1995 Cases included by region

Results (2)

- Controls : $n = 105$
 - 92 selected by case physician
 - 12 selected by minitel directory
- Number of pairs : 105
 - ≤ 1 years : 22 ; >1 year : 83 p
 - Cases and controls similar for
 - social characteristics
 - demographic characteristics
 - underlying conditions (chronic disease, treatment, prematurity, breast feeding...)

Results (3)

Cases and controls by contact to a person with diarrhea, France 1995

Results (4)

Cases and controls by consumption of chicken bought ready to eat and parent chicken cooking habit

Results (5)

Cases and controls by eggs cooking habits and duration of hard boiling eggs, France, 1995

Results (6)

Cases and controls undercooked egg consumption by age

Results (7)

Cases and controls duration of egg storage by season

Results (8)

Multivariate analysis by season

Conclusion (1)

- *Eggs*
 - Important role among children > year
 - Duration of storage > 2 weeks in summer
- *Poultry*
 - Chicken bought ready to eat
- *Person to person transmission*
 - Children $\leq$ 1 year

Conclusion (2)

- Control egg and poultry contamination ?
- Before it is achieved : prevention measures at the family household
 - Duration of storage
 - Duration of cooking
 - Cooking of chicken
 - Kitchen hygiene

Risk factors for sporadic *S. Typhimurium* among children <15 years, France 1996

- *Salmonella Typhimurium* ranked first in 1996
- Greatest incidence <15 years
- Risk factors for sporadic cases
 - beef related
 - person to person
 - antibiotic use
- To adjust prevention message

Method

- Similar to *S. Enteritidis*
- Matched case-control study
 - age group: <1 year, 1-5 years, 6-14 years
 - place of residence
- Cases identified at the NRC
- Nation-wide, 1996

RESULTS

- Case-control pairs = 101
- 86% selected by general practitioner
- 14% selected by Minitel telephone directory
- Age distribution
 - <1 years : 11 pairs
 - 1-5 years : 77 pairs
 - 6-14 years : 13 pairs
- No difference of case and controls by social and demographic characteristics

Salmonella Typhimurium, case-control study, France 1996 :
recent antibiotic use.

Salmonella Typhimurium, case-control study, France 1996 :
exposure to diarrhea in household

Salmonella Typhimurium, case-control study, France 1996 :
camembert and poultry consumption

Salmonella Typhimurium, case-control study, France 1996 :
beef consumption

Salmonella Typhimurium, case-control study, France 1996 :
multivariate analysis

CONCLUSION

- Minced beef meat not well cooked
- Recent antibiotic use = risk factor
- Person to person transmission among younger children

➡ Prevention measures

Risk Factors for Sporadic Listeriosis in France

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Methods (1)

Case definition

- Lm in a normally sterile site
- Confirmation by NRC
- Resident in France
- Between 01/01/97 - 31/12/97

Exclusion

- Hospital associated cases
- Epidemic cases

Methods (2)

Categories of cases

- Pregnancy associated cases: pregnant women or newborns < 7 days
- Non pregnancy associated cases
 - with predisposing condition: malignancy, corticosteroid or chemotherapy, HIV infection, organ transplant recipient, chronic hemodialysis, liver disease, alcoholism, diabetes, inflammatory bowel disease
 - without predisposing condition

Methods (3)

Controls

- One control for each case
- Matched for
 - pregnancy status
 - type of predisposing condition
 - age (+/- 10 years)
 - area of residence
- Selected through case physician

Methods (4)

Data collection

- Same interviewer for each pair
- Non-blinded telephone interviews
- Interview with surrogates
- Month prior to onset of symptoms: food history, conditioning and place of purchase of foods, food preparation and storage, travel, knowledge of listeriosis prevention.

Results (1)

Results (4)

Univariate analysis of food exposures, listeriosis patients and matched controls, France, 1997

Results (5)

Conditional logistic regression analysis of food exposures associated with sporadic listeriosis, France, 1997

Results (6)

Consumption of different types of brie cheese by cases of pregnancy associated listeriosis and controls, France, 1997

Results (7)

Cases of listeriosis and matched controls by consumption of food items previously found to have been associated with epidemic or sporadic listeriosis, France, 1997

Food item	Cases n=120 %	Controls n=120 %	Matched OR	p
Paté	53	59	0.8	0.4
Rillettes	33	30	1.2	0.6
Uncooked hot dogs	9	5	1.8	0.3
Undercooked chicken	8	5	1.8	0.5
Ready cooked chicken	21	24	0.8	0.6
Smoked fish	33	40	0.7	0.3
Shrimps	38	48	0.7	0.1
Seafood	43	53	0.7	0.2

Results (8)

Awareness of listeriosis and its prevention among persons with increased susceptibility, France, 1997

Discussion

- Soft cheese (possibly unpasteurised)
 - 49% of cases attributed to its consumption
 - Food surveillance: contamination with *L. monocytogenes*
 - Responsible for 2 latest outbreaks in France (1995, 1997)
- Previously identified 'high *Listeria* risk' foods
 - No association
 - Effect of intensified monitoring and industry actions?
 - Lack of statistical power?

Conclusion

Health education

- Avoidance of soft cheese and 'high *listeria* risk' foods
- Targeted at:
 - pregnant women
 - persons with a predisposing condition
- Potential to substantially reduce the burden of listeriosis

Methodological difficulties with case-control studies in outbreak investigations

Developing a hypothesis

The traditional distinction between descriptive (hypothesis generating) and analytical (hypothesis testing) studies has been criticised by Rothman,¹ a criticism which holds true in outbreak investigation. At the simplest level epidemiological investigation may be described in two stages: firstly, generating a hypothesis of causation from descriptive data analysed by time, place and person, and secondly, testing that hypothesis by a case-control or cohort study. But in almost all outbreaks competing a priori hypotheses are voiced at the outset by public health investigators, patients, employers, food producers, etc. Some of these hypotheses may be excluded by the "descriptive" data. These descriptive data in turn may stimulate alternative hypotheses. Indeed, as in clinical investigation of an individual patient, this process of hypothesis generation and refutation goes on continuously as new cases are being interviewed, and challenges the rigid dichotomy of descriptive versus analytic. For example, in an outbreak of *Salmonella ealing*, cases were mainly infants who were discovered to have been fed on a single brand of milk formula.² Comparison with a group of well infants showed a significant difference in use of this brand. Was this comparison testing the hypothesis, or could such a design only firm up the hypothesis - the hypothesis seemingly was generated on the same set of cases as it was "tested"? In such outbreaks a priori hypotheses are formulated on the prior knowledge of the epidemiology of salmonellas and, in this case, on the observation of the age distribution of cases. The search for a common milk formula fed to the infants followed a prediction based on the deduction that the most likely vehicle of infection in infants was formula feed.

In some outbreaks the descriptive epidemiology suggests hypotheses for which there are no biological explanations (eg a cluster of three cases of aplastic anaemia in children³) or which go against the accepted microbiological theory (eg an outbreak of shingles⁴⁻⁶). In the practice of infectious disease control, the framework of proven microbiological causes and modes of transmission enables statistical data to be interpreted confidently. Once outside known causal pathways statistical associations are difficult to interpret and public health action, like the clinical diagnosis of an individual patient, usually is taken on the basis of clinical experience and instinct.

Hypothesis testing

When hypotheses are formally tested the epidemiological techniques usually used are retrospective cohort and case-control studies. In certain outbreaks, eg, food poisoning outbreaks in institutions or following receptions, it is usually possible to identify retrospectively and follow up the whole population to identify simultaneously exposures and disease status. Comparison of attack rates in exposed and unexposed (eg food

specific attack rates) or exposures in cases and controls will often implicate the source. Paradoxically, when a vehicle is universal, eg tap water, it may be impossible to demonstrate an association with illness unless there is a dose response (which is less likely if the vehicle of infection is heavily contaminated). Another epidemiological paradox is the "widow of Hampstead" phenomenon, when one outlying case may give the most valuable information on the source of infection.

Strength of association

The case-control study begins by identifying people with and without infection and then retrospectively identifies factors associated with infection. There are no denominators from which risks may be estimated and compared. It is necessary therefore to work with the 'odds' of infection or of being exposed which do not require denominators. Data obtained may be summarised in a two by two table (Table 1). The odds of being exposed if a case is a/c . The odds of being exposed if a control is b/d . The odds ratio (OR), ad/cb , is also known as the cross-product ratio. Very conveniently this is also the odds ratio of being a case if exposed compared to not exposed. If there is no association between cases and exposure the odds ratio will be 1. It is generally taken that the bigger the odds ratio the more likely the association is causal. Weak associations ($OR < 5$) may be difficult to interpret and may reflect biases or confounding. For example, in an outbreak of campylobacter infection, 26 out of 32 cases had experienced bird attacks on doorstep- delivered milk bottles.⁷ The odds of this exposure for cases was $26/6$ (or 4 to 1 on). Of 64 controls only 5 had a similar exposure. The odds for controls was therefore $5/59$ (or 12 to 1 against). The odds ratio was 51. Simply explained, cases were 51 times more likely to have been exposed to bird attacks on milk bottles than controls. Such a strong association is difficult to explain if the milk bottles were not the source of infection. This explanation could be strengthened further if it could be shown that the more frequent the bird attacks, and therefore the more likely that contaminated milk was consumed the stronger the association with illness. And this was indeed the case. The number of days in the week before onset on which bird attacks were noted were divided into four categories, 0, 1-3, 4- 5, 6-7. Within each of these strata the odds ratio was calculated, by comparing each of the strata to the first. The odds ratio increased from 51 for those attacked 1-3 days per week to 70 and then 140 for the next two strata. This demonstrates a gradient of risk which can be interpreted as a dose response if the attached milk bottles were the source of infection.

It should be noted that a dose-response is not always found even when the cause is established. If the infecting dose to which all are exposed is high then the attack rate will not necessarily increase with increasing the exposure. It is helpful however to look for a dose-response especially when most or all people are exposed to the possible source, as in waterborne outbreaks.

Table 1 An example of a two by two table

	Cases	Control
Exposed	a	b
Not exposed	c	d

Sampling Bias

Sampling may not be required at all as the size of the population at risk or the number of cases is usually small. However the corollary of this is that the power of possible studies in outbreak investigations cannot usually be controlled by the investigators. Nevertheless, the speed at which such studies can be done by experienced investigators, and the relatively low cost of the studies, as well as the potential benefits of demonstrating an association between infection and a possible source, mean that studies of low power will usually still be worth carrying out.

Diagnostic suspicion bias⁸ in case selection is always a problem when a possible vehicle of infection is widely-publicised. For example, people who have eaten a food believed to be the source of an outbreak of botulism may present with neurological symptoms unrelated to botulism. When laboratory confirmation of diagnosis is not possible the only safeguard is to choose cases which meet the strictest clinical case definition (and therefore who are at the most severe end of the disease spectrum).

In case control studies the selection of controls always poses a problem of practicality versus validity. Sampling frames commonly used to select controls include general practitioner age/sex registers, hotel and reception guest lists, family members of cases, neighbours of cases, and persons investigated by the laboratory but who were negative for the disease in question. When diagnostic access bias is considered potentially important (e.g. cases of enteric infections are only recognised in the main if they have general practitioners who regularly recommend faecal sampling), it is important to match for neighbourhood of residence, or use patients investigated by laboratories but found to be negative, since failure to do so may lead to a spurious association with, say, a restaurant in the neighbourhood of the "keen" general practitioner. However, using general practitioner lists may result in overmatching. Obtaining controls from general practitioner registers is laborious and slow, and in our experience about 5-10% of general practitioners are reluctant to co-operate.

Increasingly, acquaintances nominated by cases are used as controls although the representativeness of such groups and possible biases they may introduce have not been evaluated systematically. It is possible, for example, that if cases know that a particular exposure is the source of the outbreak their nomination of controls will be influenced. Additionally, the case may know the exposure history of several acquaintances and reveal this with their names to the interviewer, so possibly influencing the interviewer's choice of controls. A protocol to avoid this may be derived, although in practice there is the danger of a conflict of priorities when local investigators, e.g. environmental health officers with their own local responsibilities for outbreak investigation, are called upon to carry out the national protocol.

Measurement Bias

In all but the smallest outbreaks data are connected by questionnaire, but often practicalities require groups to be interviewed by a combination of telephone, mail, face to face and proxy interviews. The sensitivity and specificity of questionnaires is seldom considered. In one study food consumption recalled by people 2-3 days after luncheon was compared with that observed at the time and recorded on video tape.⁹ Only four of 32 attendees made no errors in reporting. The sensitivity of the food consumption histories questionnaire was 87.6% and the specificity 96.1 %. Thirteen percent of the respondents reported eating "dummy" food items listed on the questionnaire but not served at the luncheon. Such errors in recall can be reduced by providing background details of events, good questionnaire design, and making use of other sources to check data such as diaries, menus, discussion with relatives, etc. Validation of patient histories is often not possible in practice due to the nature of the exposures, the lag between exposure and investigation and the urgency of investigation. However, random errors in recall are unlikely to give rise to false associations between illness and, for example, a food item. Rather they will reduce the power of a study to identify the true vehicle of infection.

However loss of cases or controls from the study because of refusal to be interviewed or failure to trace patients is a serious problem and may completely undermine the validity of a study. Non-responders are likely to have different exposure experience to responders and this may seriously bias inferences drawn from responders about the exposure status of the whole group. Once a group of cases and controls are identified as the study population every effort should be made to obtain a high response rate (> 85 %). In any event, the target population and response rate must be reported with the results of the study. The possible effect of non-response may be assessed by calculating 'worst case' scenarios. If it is assumed that all non-responders would have given answers which went against the hypothesis being tested then their effect on the overall odds ratio can be assessed. If the odds ratio is still significantly greater than 1 the conclusion drawn from the study is shown to be robust.

Throughout the course of their illness cases will have gone over in their minds possible exposures and their recall may be biased by their own preconceptions or by press and media speculations. Cases will often have been interviewed on many occasions (by general practitioners, environmental health officers, community physicians) before an analytical study is carried out, and this may have introduced bias from suggestions made by interviewers, as well as prompting a more detailed recall. Family members may have suggested likely sources of infection to the cases. Patients may have anticipated the view of the interviewer if the suspected cause of the outbreak has been learned from local media reports and they may alter their answers accordingly. These biases can often be anticipated and can sometimes be assessed in analysis. For example, in a suspected waterborne outbreak of giardiasis

in Reno, Nevada, considerable local publicity occurred before the case-control study was conducted.¹⁰ However, because of the long incubation period of giardia many new cases became ill several days after the water supply was treated and so media attention persisted and water was still considered suspect. However, increased water consumption was only associated with cases exposed before the water supply was treated. Bias from media reports was likely to have affected later cases also but no association was found.

A major methodological problem is the bias which may result from the interviewer knowing the disease status of the person and having his own suspicion or prejudice as to the source. Selective histories may be taken in which the patient with salmonella is only asked in detail about a particular food. Blinded interviewing is rarely possible since resources are sampling bias and measurement bias. Professionalism in the technique of interviewing, and use of a structured questionnaire are the only safeguards. Controls, who any way have no special reason to recall certain exposures, are usually interviewed several days after cases, which adds to the problem of accuracy and detail of recall. To help overcome this problem, questionnaires may include memory jogging points related to notable events.

Confounding and matching

If more than one possible vehicle or exposure is significantly associated with illness in a two by two table there are two broad explanations. Either there really was more than one vehicle of infection or there is confounding. For example, people who chose to eat the contaminated chicken at the banquet may also have been more likely to eat the seafood starter. Both foods may then be significantly associated with illness. This possibility can be tested by stratifying the data by food preference (Table 2).

Table 2 Stratification of data by food preference

	Group 1		Group 2	
	Ate chicken		Did not eat chicken	
	Case	Control	Case	Control
Ate seafood	a ¹	b ¹	a ²	b ²
Did not eat seafood	c ¹	d ¹	c ²	d ²

If seafood was not a vehicle of infection the odds ratio in Group 1 ($a1d1/b1c1$), will be similar to that in Group 2 ($a2d2/b2c2$). If, conversely, the data are stratified by seafood consumption then the odds ratio for chicken consumption in seafood eaters and non-seafood eaters would be similar and greater than 1. A summary odds ratio for stratified analysis can be calculated by the Mantel-Haenszel method or in more complex situations by logistic regression modelling methods.

If, as is usually the case, the need to allow for confounding factors such as age, sex and neighbourhood or residence or hospital speciality of patient can be foreseen, matching of controls to cases should be considered. When a matched study is done statistical analysis should preserve the matching using what is known as McNemar's test and the exact binomial probability, instead of X^2 and Fishers exact test, or by using logistic modelling methods.

Statistical tests and sample size

The presentation of study results in the form of odds ratios and relative risks is a growing trend and found to be more useful generally than just presenting p values from tests of hypotheses. The p value is the probability of obtaining a difference between proportions of cases and controls exposed as large or larger than that observed, if there is no association between infection and exposure. It will depend upon the size of the difference in proportions and on the sample size studied. In a large enough study a very small difference between groups, possibly due to bias, could be highly statistically significant. In a small study a big difference in observed proportions may not reach statistical significance. For these reasons it is unwise to base conclusions solely on the all or nothing significance tests. It is better to present quantitative estimates. Usual practice is therefore to present the odds ratio and the 95 % confidence intervals. If the lower end of the 95 % confidence interval is about 1 this means that the p value (ie the probability of a result this far from 1) is <0.05 .

A study may fail to show an odds ratio or relative risk significantly >1 even when a real difference exists because the study size is too small. This is the so called Type II error. When a large number of cases and controls are available and only a sample is recruited, the size of the sample has to be chosen according to how sensitive the study needs to be for detecting odds ratios specified distances from 1.

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Risk factors associated with sporadic campylobacteriosis in Denmark

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Annual incidence of human campylobacteriosis in Denmark, year 1980-1997

Objectives

- Identify risk factors for sporadic campylobacteriosis in Denmark
- One year case control study
- Travel and food related risk factors

Material and methods

- Sporadic cases from 2/3 Danish population
- Controls from Central Person Register
 - match on birthday, sex and municipality
- Mailed questionnaire and interview (CATI system)
- 14 days exposure period
- Exclusion criteria
 - unknown onset date or outbreak related
 - history with Campylobacter infection
 - subclinical infection
- Domestic cases and controls

Study population

- Study period May 1, 1996 to May 12, 1997
- 979 (46%) of 2136 cases selected
- 585 (60%) cases responded
 - 150 (26%) cases excluded
- 2200 controls contacted by letter
 - 566 (26%) controls responded
 - 72 (13%) controls excluded
- Totally 282 cases/319 controls in study
- Domestic pairs 234 cases/264 controls

Age distribution of all registered Danish Campylobacter cases (1993-1997) and cases from case control study (1996-1997)

Risk factors significant in univariate analysis

Exposure	P-value	OR
Travel	0.000	10.1
Undercooked poultry	0.016	6.38
Ground beef	0.055	2.37
Meat at a barbecue grill	0.012	1.85
Bad taste or smell of water	0.054	2.83
Private well	0.039	2.69

Protective factors significant in univariate analysis

Exposure	P-value	OR
Eating at a restaurant	0.058	0.67
Duck	0.042	0.44
Roastbeef	0.048	0.52
Offal from pork	0.008	0.34
Ham	0.052	0.64
Other types of pork	0.037	0.60
Game	0.009	0.27
Cake w/ "raw cream"	0.020	0.50
Raw carrots	0.006	0.57
Raw cabbage	0.022	0.50
Unpeeled apples/pears	0.001	0.49

Exposure variables significant in multivariate analysis

Exposure	P-value	OR
Undercooked poultry	0.006	5.5
Meat at a barbecue grill	0.002	2.3
Bad taste or smell of water	0.031	4.4
Unpeeled apples/pears	0.000	0.4
Other types of beef	0.005	0.4
Eat at a restaurant	0.013	0.5
Offal from pork	0.024	0.4
Ham	0.026	0.6

Travelling abroad

- Totally 46 (16.3%) of 282 cases
- Totally 7 (2.2%) of 319 controls
- Travelling abroad: OR=10.1, $p<0.0001$
- Swiss case control study (1991)
 - Southern Europe, Middle East and Asia increased risk of Campylobacter infection

Undercooked poultry

- Totally 16 (9.2%) of 172 cases
 - 10 (63%) chicken,
 - 2 (13%) turkey and
 - 4 (25%) duck or pigeons
- Totally 5 (2.6%) of 201 controls
 - 1 (20%) chicken,
 - 3 (60%) turkey and
 - 2 (40%) had duck or pigeons
- Eating undercooked poultry: OR=6.4, $p=0.016$

Poultry - a likely source?

- Campylobacter jejuni/coli at retail level
 - 24-54% of broilers
 - 18-32% of turkey
 - 19-40% of wild birds
- Norwegian case control study (1989)
 - eating poultry produced in DK or S associated with Campylobacter infection (OR=13.7, $p=0.014$)

Meat at a barbecue or grilled over open fire

- Totally 62 (27.1%) of 229 cases
 - 32 (52%) beef,
 - 50 (81%) pork and
 - 6 (10%) had lamb/mutton
- Totally 41 (16.1%) of 255 controls
 - 27 (66%) beef,
 - 36 (88%) pork
 - 5 (7%) lamb/mutton
- Eating meat at a barbecue: OR=1.9, p=0.012

Meat at a barbecue or grilled - a likely source?

- Campylobacter jejuni/coli at retail level
 - 0.5% of beef & beef products
 - 0.5-2% of pork & pork
- Norwegian case control study (1989)
 - Consumption of sausages at a barbecue associated with Campylobacter infection (OR=7.64, p=0.005)

Bad taste or smell of household potable water

- Totally 17 (7.5%) of 227 cases
 - bad taste or smell of household water
- Totally 9 (3.5%) of 260 controls
 - bad taste or smell of household water
- Potable water having a bad taste/smell: OR=2.8, p=0.054

Water supply in Denmark

- 1% of water supply is surface water
- In Norway and Sweden surface water 85-75%
- 5 of 32 private wells showed heavy coliform and *E. coli* contamination
- Waterborne *C. jejuni* outbreak in Denmark in 1996
 - sewage contaminated water supply
 - outbreak identified because of bad taste and smell

Conclusions

- External risk factors
 - travel to a foreign country
 - undercooked poultry
 - meat at a barbecue or grilled over open fire
 - drinking water i.e. bad taste or smell of water or having own well
- Certain underlying illnesses or medication show a tendency to predispose for Campylobacter infection
- Hygiene knowledge needs to be addressed in the further analysis

Population attributable fraction

Jakob Neimann

Underlying illness

Disease	Number (%)		Matched Odds Ratio	p-value
	Cases N: 282	Controls N: 319		
Arthritis	8/281 (2.8)	7/318 (2.2)	1.31	0.608
Disease in stomach	3/282 (1.1)	6/319 (1.9)	0.59	0.466
Disease in intestines	16/282 (5.7)	5/319 (1.6)	4.44	0.009
Disease in liver	6/281 (1.8)	2/319 (0.6)	2.79	0.223
Diabetes (ever diagnosed)	2/281 (0.7)	0/319 (0)	-	-
Other serious diseases	35/282 (12.4)	22/319 (6.9)	1.86	0.036

Premedication

Medication	Number (%)		Matched Odds Ratio	p-value
	Cases N: 282	Controls N: 319		
Penicillin/antibiotics	21/280 (7.5)	13/319 (4.1)	2.05	0.058
Antacids	11/282 (3.9)	8/319 (2.5)	1.43	0.415
Laxatives	8/282 (2.8)	1/319 (0.3)	8.00	0.050
For intestinal diseases	1/282 (0.4)	0/318 (0)	-	-
Hormone preparation (including contraceptive)	43/281 (15.3)	19/319 (6.0)	3.80	< 0.001
Medication treatment for cancer	0/282 (-)	0/319 (-)	-	-

Study of yersiniosis in Finland

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Yersinia enterocolitica

- ◆ Heterogenous grouping
 - at least 57 O-serogroups
 - six biogroups
 - most common serogroup O:3, biogroup 4 (O:3/4)
- ◆ Reservoirs
 - pig tonsils, ranging 20 - 80 % (in Finland 40 %)
 - pig intestines (and stools)
- ◆ Grows in refrigerator temperatures

Y. enterocolitica - transmission

- ◆ Food
 - pork 'items', particularly raw ground meat
 - sausage, chitterlings
 - with sensitive methods Y. enterocolitica found more frequently in pork products
 - direct contamination during slaughter ?
 - cross contamination during processing ?
- ◆ Untreated drinking water
- ◆ Milk

Y. enterocolitica in children

- ◆ Increasing in US
- ◆ Norway; 34 % were < 5 years of age
- ◆ Holland
 - among children, > 1/3 below 3 years of age
- ◆ Incubation period 3-7 days, rarely > 10 days

Y. enterocolitica in Finland

1995	903
1996	802
1997	618

Y. enterocolitica O:3 in children <6 years in Finland by 6-mo periods

1995	1	38
	2	48
1996	1	23
	2	21
1997	1	13
	2	14
1998	1	12

Y. enterocolitica O:3 in children - objectives

- ◆ To obtain information on risk factors for acquiring Y. enterocolitica O:3 infection in children < 6 years old

Yersinia in children - methods

- ◆ Prospective case-control study
- ◆ Questionnaire survey by mail
- ◆ Questions including
 - disease and treatment
 - exposures 4 weeks prior to positive stool sample
 - food
 - drinking water source
 - pet animals and their food, other domestic animals

Yersinia in children - definitions

- ◆ Case
 - age less than 6 years
 - Y. enterocolitica O:3 isolated from stool
- ◆ Control (two for each case)
 - attendance in the same healthy child clinic
 - age within 1 month of case
 - same sex
 - no gastrointestinal disease within previous month

Yersinia in children - implementation

- ◆ All central hospital laboratories invited
- ◆ Serogroup from 2 laboratories -> study nurse
- ◆ Study nurse calls attending physician, who
 - obtains verbal consent from family
 - obtains contact information on patients
- ◆ Study nurse
 - mails survey questionnaire to patient/guardian
 - identifies healthy child clinic by address of patient

Yersinia in children - logistics

- ◆ Delays
 - from sample date to study nurse: 10 d - 3 w
 - study nurse --> physician --> study nurse 1-4 d
 - mail questionnaire --> return: 1-2 w
 - sample date --> return questionnaire: 2 - 4 w
 - 'total recall delay' 40 - 60 days
- ◆ Study nurse telephone contacts
 - minimum eight per patient+controls cluster
- ◆ Questionnaires carefully filled

Current status of Yersinia case-control study

- ◆ Information from laboratories on 28 patients
 - 1 did not consent (private physician)
 - 3 were too old
- ◆ For 24 recruited patients
 - 2 miss controls from the same healthy child clinic
 - 7 have only 1 control
 - total 37 controls
- ◆ Natl. register: notifications from 36 patients

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