

ROBERT KOCH INSTITUT



PAE



OUTBREAK INVESTIGATION

Module

(OIM)

From data analysis to communication of findings

**Organized by the Postgraduate Training for Applied Epidemiology (PAE)
in cooperation with EPIET/EUPHEM**

CASE STUDY

Outbreak of gastroenteritis after a high school dinner in Copenhagen, Denmark, November 2006

Adapted to the needs of the EPIET Outbreak Module

**December 7-11, 2015
Berlin, Germany**

Objectives

At the end of the case study, participants should be able to:

- Conduct an investigation to identify the source of an outbreak
- Explain the contributions of epidemiological and microbiological investigations used in foodborne outbreaks
- Conduct a questionnaire survey, including questionnaire design using EpiData Manager and online tools Carrying out data cleaning and descriptive, univariable and stratified analyses using STATA
- Critically evaluate the results from statistical and microbiological analyses and identify food vehicles most likely associated with becoming ill
- Understand the difficulties in food trace back in the globalised world

This case study was designed under an ECDC service contract for the development of training material (2010). The data were slightly modified for training purposes.

Source :

This case study is based on an investigation conducted by
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Software required for this exercise:

- STATA version 12, 13 or 14
- EpiData Manager/ EpiData EntryClient

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The first version of this case study was designed under an ECDC service contract for the development of training material (2010).

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Info for the entire case study:

First, try to find the results yourselves.

If you need help you can find the solutions in the separate **Help document** to this case study.

This icon identifies tasks for which help is provided:



1-Introduction

On November 14th 2006 the director of a high school in Greater Copenhagen, Denmark, contacted the regional public health authorities to inform them about an outbreak of diarrhoea and vomiting among participants from a school dinner party held on the 11th of November 2006. Almost all students and teachers of the school (750 people) attended the party. The first people fell ill the same night and by the November 14th the school had received reports of diarrhoeal illness from around 200 – 300 students and teachers, many of whom also reported vomiting.

In Session 1 we will discuss what you would do after receiving this information.

Exercise 2 – Questionnaire design, Data entry, data validation and export for analysis

The epidemiologists in the outbreak team decided to perform a retrospective cohort study in order to identify the food item that was the vehicle of the outbreak. The cohort was defined as students and teachers, who had attended the party at the high school on 11 November 2006.

Information about the survey and a link to the questionnaire was circulated to students and teachers via the school's intranet with the request that everyone who attended the school party on 11 November 2006 should fill in the questionnaire. Practically all students and teachers check the intranet on a daily basis, because it is the school's main communication channel for information about courses, homework assignments, cancellation of lessons etc. The information about the investigation was also displayed on the screen in the main hall of the school. The school's intranet was also accessible for ill students or teachers from home.

Investigators used a questionnaire similar to the one provided in the next page (see table 1). For the purpose of the case study a reduced number of variables are included in the questionnaire compared to those that were included in reality.

Using EpiData you will discuss the following:

- a. Creating data entry forms from scratch.
- b. Validating duplicate data and finding possible case-id problems in data
- c. Importing and exporting data
- d. Proper documentation and versioning of files when working in a group.

Table 1: Sample questionnaire

Questionnaire on illness after the school dinner on November 11th 2006

1. ID _____

2. Personal information:

2.1. Sex: _____

2.2. Age: _____

3. Which group do you belong to:

3.1. student ☐ teacher ☐

3.2. If student, which class do you go to: _____

4. Were you ill after the school dinner party on Saturday, November 11th 2006?

Yes ☐ No ☐ (If NO, continue with question 7).

5. If YES, which symptoms did you have? (Please set a cross in every line).

	Yes	No
Diarrhoea (looser stools than normal at least 3 times in 24 hours)	<input type="checkbox"/>	<input type="checkbox"/>
Bloody diarrhoea	<input type="checkbox"/>	<input type="checkbox"/>
Vomiting	<input type="checkbox"/>	<input type="checkbox"/>
Abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>
Nausea	<input type="checkbox"/>	<input type="checkbox"/>
Fever (more than or equal 38°C)	<input type="checkbox"/>	<input type="checkbox"/>
Headache	<input type="checkbox"/>	<input type="checkbox"/>
Joint pain	<input type="checkbox"/>	<input type="checkbox"/>

6. If you had diarrhoea or vomiting, when did it start?

6.1. Date

Saturday, 11 November	<input type="checkbox"/>	Tuesday, 14 November	<input type="checkbox"/>
Sunday, 12 November	<input type="checkbox"/>	Wednesday, 15 November	<input type="checkbox"/>
Monday, 13 November	<input type="checkbox"/>	Thursday, 16 November	<input type="checkbox"/>

6.2. Time

00:00-05:59	<input type="checkbox"/>	12:00-17:59	<input type="checkbox"/>
06:00-11:59	<input type="checkbox"/>	18:00-23:59	<input type="checkbox"/>

7. Consumption of food at the school dinner.

Did you eat during the school dinner on November 11th 2006? Yes ☐ No ☐

(If NO, continue with question 9).

8. What did you eat? (Please set a cross in every line).

	No	Ate less than 1 portion	Ate whole portion	Ate more than 1 portion	I don't know
Tuna mousse	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Shrimps (served with tuna mousse)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Green salad (served with tuna mousse)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Roasted veal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Pasta salad with pesto	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Rocket salad with beans and tomato	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cold red pepper sauce	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bread rolls	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

9. What did you drink? (Please set a cross in every line).

	No	1 glass	2 glasses	3 glasses or more
Champagne	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Draught beer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Red wine	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
White wine	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Thanks for participating in the study!

Exercise 3-A – Data management and .do files

As you can already foresee, Stata requires a fair amount of programming. In this session we will learn how to organise our data, how to save the commands used for a particular analysis and how to save the results in specific files known as do-files and log-files. We will also see how to include the creation of log-files by typing commands in the do-file.

The extension of Stata files containing data is **.dta**, for files containing the commands is **.do**, and for files containing the results is **.log**.

Task 3.1 – Get familiar with your data

Open the Stata software and decide on your working directory by typing in the Stata command window the change directory command (**cd**) and the route for the folder you choose in quotation.

Example: Type, e.g. `cd "C:\DATA\OM\session3 "` to specify the directory.


Type **cd** to know in which directory you are. Alternatively you can have a look at the bottom right of the screen and check what your working directory is.

Change your working directory to the folder session3. Open in Stata the dataset **copenhagen3.dta** by typing the following command:

```
use copenhagen3.dta, clear
```


Familiarize yourself with the information displayed in the different windows (command, variables and results) and explore the menus.



If you want to see your dataset you can either click on  or type **browse** in the command-window.

For a quick overview of the data you can use the following commands: **browse**, **describe**, **codebook**, **list**, **count**.



You can start saving your commands on a do file. Open a do file by clicking icon  and a new window will open with an appearance similar to a text-editor where you can type, run and save your commands.

Remark: You have been sent several .ado-files. To integrate them into Stata, you can type

```
adopath
```

in Stata and check, where Stata looks for .ado-files. These paths can be different on different computers. Put the files in one of the folders given there, for example into C:\ado. If the folder does not already exist, you can create it.

Task 3.2 – Create a plan of analysis

Think of the different analysis steps you want to carry out in order to find the source of the outbreak!

Exercise 3-B – Data check and recoding with Stata

Task 3.3 – Check the dataset "copenhagen3.dta"

List any inconsistencies or outliers you have found in the data and think about what consequences this could have on your analysis.

Use do-files.

Describe your dataset, check frequency tables for outliers or unusual values, means, medians, modes, quartiles, etc.

- Familiarize yourself with the **tabulate** command for the categorical variables of the dataset
- Familiarize yourself with the **summarize** command for the continuous variables of the dataset

Save the commands in a do-file named **data_checking.do**.



Optional Task 3.4 – Log-files

Use log-files. Save the output you generated in task 3.3 in a log-file named **Copenhagen_checking.doc**



Task 3.5 – Recode the data after the checking procedure

a) Create a case definition.

In the study, the following case definition was used: a case is a person from the cohort, who presented with diarrhoea or vomiting within 48 hours of the meal. So anyone who presented with diarrhoea or vomiting from 6pm on November 11th to 5:59pm on November 13th was included as a case. Anyone with symptoms outside this time window is defined as a control as this person probably didn't become sick at the party.

For the analysis sake, we exclude any people from the cohort who didn't eat at the dinner because we specifically hypothesise a *food item* to be the vehicle of the outbreak. Excluding persons reduces the sample size and therefore the power slightly, but the investigators considered that this would increase specificity.

b) Recode inconsistent age.

In the data checking procedure, you have found that one person has an inconsistent age. Looking at the questionnaire again revealed the true age was 18, not 8.

c) Recode sex.

For the data analysis, you should recode the sex variable from a string type to a numeric variable.

Save the commands in a do-file named **recoding.do**.



Exercise 3-C – Descriptive analysis with Stata

Task 3.6 – Dataset description and tabulation

Use the dataset **copenhagen3_recode.dta** in folder session3 and use the appropriate commands in order to fill out tables 2 – 5 on the next page.

- a) Describe signs and symptoms of cases
- b) Determine the median incubation period
- c) Describe the cohort in terms of person
- d) Calculate the overall attack rates as well as the attack rates stratified by person characteristic

Reminder: an attack rate is calculated by dividing the number of cases who ate a specific food by the total number of people who ate the food

Save the commands in a do-file named **descriptive.do**.



Optional Task 3.7 – Export your output to excel

Copy your most favourite table to excel



Table 2. Description of symptoms among cases

Symptoms	#	Total replies	% of Total
Diarrhoea			
Bloody diarrhoea			
Vomiting			
Abdominal pain			
Nausea			
Fever			
Headache			
Joint pain			

Table 3. Descriptive epidemiology: Incubation period

Incubation period	in hours
Mean	
Median	
Range	

Table 4. Descriptive epidemiology: cohort by age

Age	in years
Mean	
Median	
Range	

Table 5. Descriptive characteristics and attack rates of cohort by group, class and sex

Characteristics	Number	% of Total	Number of cases	Attack rate (in %)
Group	Student			
(n=)	Teacher			
Class	1			
(n=)	2			
	3			
Sex	Male			
(n=)	Female			
Total				

Exercise 4 – Describing time in Stata

In this session we will construct several types of epidemic curves using different software and aggregate data with Excel and Stata.

Task 4.1 – Epidemic curves – in Stata

Create the epidemic curve for onset of illness among cases:

- In Stata using **copenhagen4.dta**

When creating the epidemic curve, what intervals would you ideally use on the x-axis?



Optional Task 4.2 – Varying the time intervals

Try to generate another time variable with 6 hour intervals



Optional Task 4.3 - Epidemic curves - in Stata

Create an epidemic curve for the teachers using the "epicurve" command.



Optional Task 4.4 - Epidemic Curves – in Excel

Create the epidemic curve for onset of illness among cases:

- In Excel using data exported from Stata ("Modified bar chart")
- Create a stacked histogram showing the cases in intervals with gender in different colours in Excel



Exercise 5 –Univariable analysis

To identify (the) potential vehicle(s) in this outbreak, the investigators proceeded with an analytical study.

Task 5 – Study designs and appropriate univariable analyses

Using Table 6 and **copenhagen5.dta** in folder session5, calculate appropriate measures of association and 95%CI for the consumption of the various food items, using the following different study designs.

- a) Retrospective cohort
- b) Case-Control: discuss, if it is appropriate using this dataset
- c) Optional: Case-cohort study with the same number of controls as cases!

Save all commands in a do-file named **univariable.do**.

Table 6: Univariable analyses for various food and drink items, using different study designs

Cohort study		Case control study
Food item	RR (95% CI)	OR (95% CI)
Shrimps		
Veal		
Pasta		
Sauce		
Champagne		



Exercise 6 –Different statistical tests in Stata

In this session you will decide about the appropriate statistical tests to use and to interpret the test results.

Task 5 – Decide about the appropriate tests to use for comparison

Perform the following comparisons in Stata. What kind of information is compared, what test should be used?

- a) Is there a relationship between the gender of the persons and being a case?
- b) Is there a difference in the class the students go to between cases and non-cases?
- c) Is there a difference between age in males and females?
- d) Is there a dose response relationship between the food items and being sick? Look at the food items that seem most suspicious to you!



Exercise 7 - Establishing the aetiology of the outbreak

Symptomatic party attendees were asked to submit stool samples via their general practitioners to the local clinical microbiology laboratory where they were cultured for standard enteric bacteria. The result of the laboratory analysis from the initial eight patients came back as negative. However, the local laboratory was unable to analyse for the presence of norovirus, so this analysis was not performed.

Our initial working hypothesis was that the outbreak had a viral or toxic aetiology. Norovirus is generally acknowledged as the most frequent cause of foodborne outbreaks in industrialised countries and the fact that there seemed to be a rapid onset of symptoms (mainly vomiting) following exposure suggested that norovirus might be the causative agent in the outbreak.

Task 7.1 –

- a. What are the standard enteric bacteria that most diagnostic microbiology labs will screen for?
- b. Briefly review what you know about the epidemiology and diagnosis of norovirus.



Task 7.2 - Reviewing results of previous sessions, establish if the Kaplan Criteria were met.

You decide to use the four so-called Kaplan Criteria, to investigate if norovirus may be the aetiological agent of the outbreak

Application of the Kaplan Criteria is very useful because norovirus outbreaks occur frequently and diagnostics are often not available. Especially for larger outbreaks, a norovirus aetiology may be established using the Kaplan Criteria only.

The four criteria are:

1. A median illness duration of 12 to 60 hours.
2. A median incubation period of 24 to 48 hours (and all btw 15-80 hours).
3. Vomiting present in more than 50% of symptomatic people.
4. No bacterial agent found in stool samples.

When all four criteria are met, it may be concluded that the outbreak was caused by norovirus.

Nevertheless, it is important to keep in mind that about 30% of norovirus outbreaks do not meet these criteria, so if the criteria are not met, it does not necessarily mean that outbreak was not caused by norovirus.



At the local clinical microbiology laboratory, stool samples from 20 further patients were examined for: *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia enterocolitica*, *Clostridium difficile* and *Bacillus cereus*. A total of three samples were positive for *Salmonella*, the first culture results became available on 22 November 2006..

At the same time, stool samples from all five kitchen workers who had prepared the dinner were tested at the national reference laboratories at the SSI. They were all negative for enteric pathogens, including norovirus

Task 7.3a – How do you interpret these results?

The finding of *Salmonella* spp. is surprising because one would expect a median incubation period of at least 24 hours in a *Salmonella* outbreak. A look at the epidemic curve tells us that the median incubation period in this outbreak was <18 hours. It would also be unusual to have such a low proportion of positive stool samples (3 of 20), if *Salmonella* was the only pathogen causing the outbreak.

Task 7.3 b - Given these results what might be the next steps?

The outbreak investigation team decided to have all stool samples sent to the Danish national reference laboratories at SSI where they were additionally tested for diarrhoeagenic *Escherichia coli* and norovirus.

In total, stool samples from 48 cases were examined at SSI. The results were as follows:

- Samples from 30 patients were tested by PCR for norovirus genogroup I and II. They were all negative.
- Samples of all 48 cases were examined for enteric bacteria. Of these, 18 cases were positive for enterotoxigenic *Escherichia coli* (ETEC). Identification of ETEC was done by a PCR method and this test was not available at the local laboratory that did the initial tests.
- A total of four cases were positive for *Salmonella*, all were *S. Anatum*. One of these cases was also among those positive for ETEC. No other bacterial agents were found in the stool samples.

Task 7.4 - Briefly assist each other reviewing what ETEC is and what the pathogenic mechanism consists of. What are the next steps?



The fact that this was an outbreak where several aetiological agents were identified, may point to a contamination from an environmental source. It also makes it less likely that kitchen staff excreting bacteria could have contaminated the food (and we already know that kitchen staff claimed not to have had symptoms, and that their stools tested negative).

As a next step laboratory analysis of the foods and ingredients was performed. The left-over foods from the party included veal with cold red pepper sauce, pasta salad with pesto, mixed salad containing rocket salad, beans and tomato. Samples of these foods were cultured for pathogenic enteric bacteria. A test for norovirus in such foods could not be performed at the time of the outbreak; only oysters, berries and water samples had been successfully analysed for the presence of norovirus

All foods were found to be negative for pathogenic bacteria, except for the pasta salad with pesto. In leftovers of the pasta salad with pesto *Salmonella* Anatum was found. Furthermore, the pasta salad with pesto was found to be heavily contaminated with generic *E. coli*. As much as 10^5 bacteria/g were found. However, it was not possible to detect any ETEC in the food.

Task 7.5 – Discuss the significance of this finding.

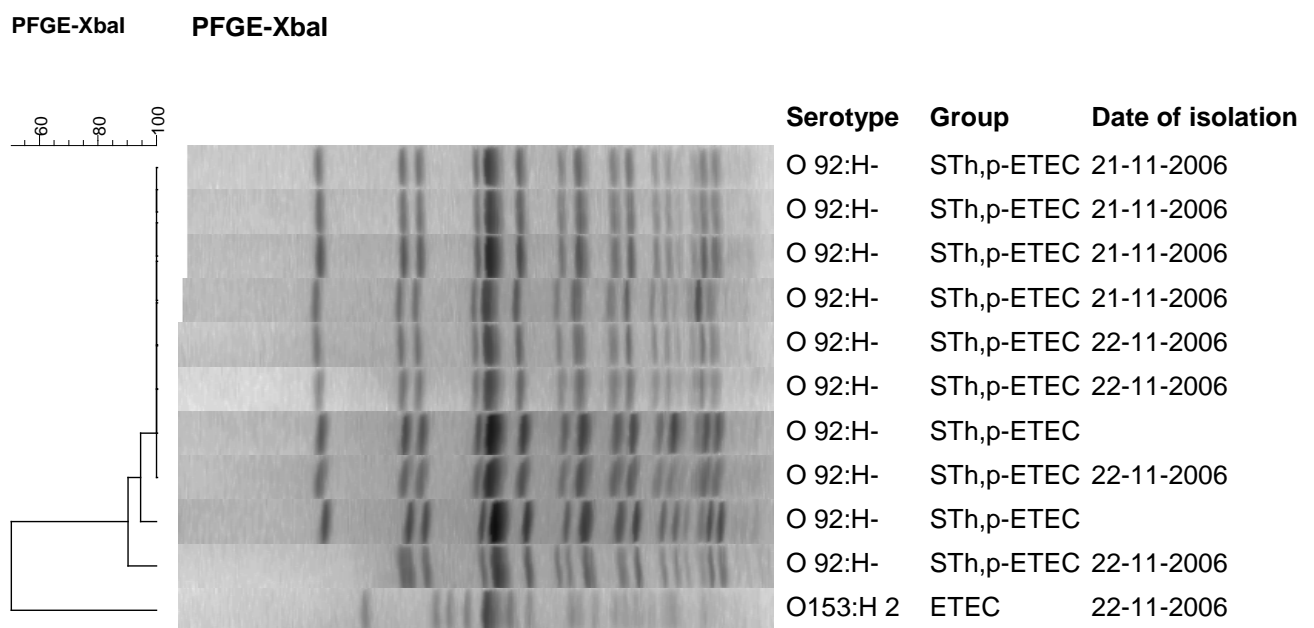


Task 7.6 - PFGE typing of outbreak strains

The *Salmonella* strains and the ETEC strains were firstly serotyped . As noted above, the four *Salmonella* strains from patients were all serotype Anatum. Of the 18 ETEC isolates, 17 isolates were serotype O92:H- and one isolate was serotype O153:H2.

Next, strains were subtyped by PFGE using the restriction enzyme, *Xba*I.

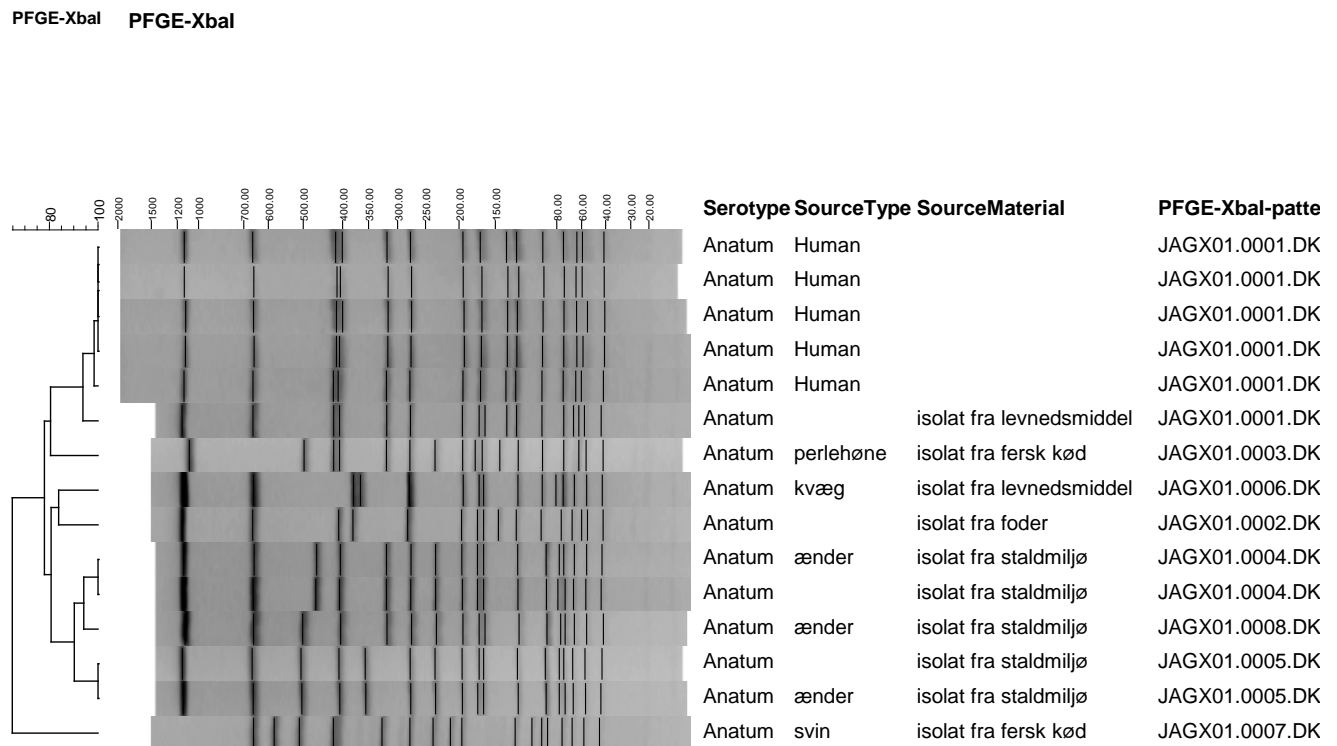
Figure 1: PFGE-typing of the ETEC strains performed at the SSI during the outbreak period.



Questions:

- Explain (briefly) to the person sitting next to you, the principle of PFGE typing.
- What do we see in the picture?
- How many different PFGE profiles of ETEC were there?
- Explain the dendrogram on the left.

Figure 2: PFGE-Typing of the the salmonella strains performed at the SSI during the outbreak period



Danish course: “isolate fra”=isolate from; “levnedsmiddel”=food; “fersk kød”=meat; “foder”=feed; “staldmiljø”=stable(cowshed,pigsty); “perlehøne”=guinea fowl; “kvæg”=cattle; “ænder”=ducks; “svin”=pigs.

Questions:

- The *Salmonella* picture looks slightly different from the ETEC one. Why might that be?
- How many different PFGE profiles do you see among the cases of the outbreak?
- Did the pesto isolate match?
- There are also other isolates on the gel, why and do they match?
- How do you interpret the dendrogram?



Finally:

A comparison was made to previous PFGE profiles present in the Bionumerics database in Denmark. Had the outbreak occurred today, an international comparison would also have been performed. ECDC is developing a new system for molecular surveillance. It is currently being implemented for four pilot organisms: *Salmonella*, STEC, *Listeria monocytogenes* and *Mycobacterium tuberculosis*.

Exercise 8 – Analytical epidemiology – Stratified analysis

You can see that eating pasta and eating veal as well as drinking champagne are associated with the highest risk of becoming ill. There are, however, many other food items that are associated with an increased risk (even if not statistically significant). At this stage we cannot conclude anything, but need to check for effect modification and confounding.

This should be done by stratification.

Task 8.1 – Stratified analysis

Use the dataset **copenhagen6.dta** in folder session8

a) Think about which variables you might want to include when checking for effect modification or confounding

b)-c) refer to this:

Start by looking if eating veal is an effect modifier or a confounder of the association between eating pasta and being a case, look at the 2X2 tables.

b) Try and phrase the question that you are trying to answer with this analysis

c) Use the **cs** command

d) To summarize your results, use **csinter** command

e) Then design the appropriate stratification tables to fill in tables 8 and 9 on the next page

f) Found any possible confounding? If so, check if all the criteria for a confounding are met!

g) Interpret the results

Save all commands in a do-file named **stratified.do**.



Table 8: Stratification by pasta

Risk factor	RR for risk factor , stratified by eating <u>pasta</u>		M-H Test for homogeneity (p-value)	Crude RR	Adjusted (M-H) RR	% Change between crude and adjusted OR	Effect modifier or confounder?
	Yes	No					
veal							
sex							
group							
champagne							
sauce							
shrimps							

Table 9: Stratification by veal

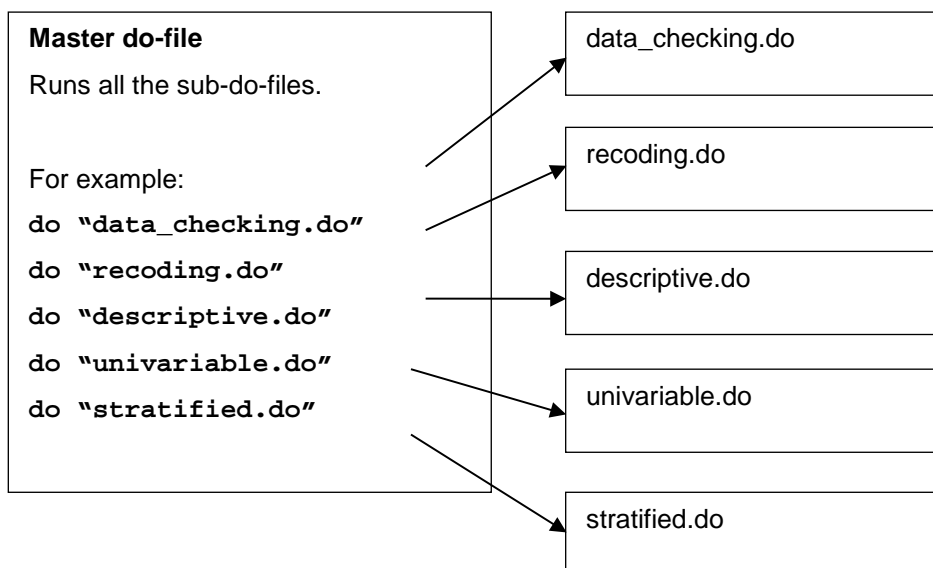
Risk factor	RR for risk factor , stratified by eating <u>veal</u>		M-H Test for homogeneity (p-value)	Crude RR	Adjusted (M-H) RR	% Change between crude and adjusted OR	Effect modifier or confounder?
	Yes	No					
pasta							
sex							
group							
champagne							
sauce							
shrimps							

Optional Task 8.2 – Integrate the analysis steps in one master .do-file

You have now performed a set of important analysis steps and have created a number of .do-files. They can all be integrated in one master .do-file in which you can integrate the entire analysis you performed so far. Below you find what a master .do-file can look like. Try it out with the existing .do-files you created!

For this, create a new directory with the original dataset used in session 3 (before data checking) and all the .do-files you created so far. In this directory, also create this master .do-file as described below.

Also remember, next time you plan an analysis with STATA, this step can also be done before you start typing commands for the different analysis steps. Such a “prospective master .do-file” can help organising your thoughts before you start the analysis!



Exercise 9: Communication

In this session you will finalize an outbreak report. For this you need to consider what you know about the outbreak in Copenhagen.

You will have filled the template provided to you before the module with the available information and findings and now need to focus on the discussion of the results and the conclusions and recommendations.

Concluding Remarks

In summary, it is fair to say that there was both epidemiological and microbiological evidence that the pasta salad with pesto was the most likely vehicle of transmission in this outbreak. Further work focused on how the pasta salad with pesto could have become contaminated and on lessons learned from this outbreak.

The regional food authority examined the premises of the kitchen and interviewed the kitchen staff. This is what these investigations revealed:

All but one food item had been prepared on the day of the party. The pasta salad had been made one day before and the pesto for that salad had been prepared from fresh ingredients without heat treatment another day before that and stored in a refrigerator. Because the amount of pesto was very large, it had to be mixed with a professional kitchen mixing machine for quite a long time. During that process the temperature of the ingredients became warm and this may have caused bacteria to multiply. Also, if such a large volume of warm food is placed in a refrigerator in one big container, it will take hours before the central parts reach refrigeration temperatures. The correct practice of dividing large portions into smaller ones is not always followed in busy kitchens.

The pesto may be said to have been a fine example of globalisation: It had been prepared from five ingredients (parmesan cheese, olive oil, fresh basil, garlic and pine nuts), which originated from five different countries. Samples of these ingredients were collected for microbiological investigation. All were negative for *E. coli* (<10 cfu per gram). However, except for the pine nuts, all samples were from different batches than the one used for the preparation of the pesto in question.

The investigators decided to collect more information on the different ingredients in order to determine the ingredient most likely to have been contaminated. Questions considered were: Has the ingredient previously been identified as the source of an outbreak? Is there any step in the cultivation or production process of the ingredient that is prone to faecal contamination? Sources of information were food safety experts, the Internet and literature searches.

- Pine nuts are harvested by burying the cones in the ground, because that makes the cones open up. This practice is a possible source of contamination, but the available sample from the same batch as used for preparation of the pesto tested negative.
- Parmesan cheese – there were no outbreaks of ETEC related to hard cheese described in the literature although salmonella contamination had occurred.
- But there were reports of outbreaks of ETEC or *Salmonella* associated with contaminated basil. One example (although it occurred a year after the present outbreak) was an outbreak of *Salmonella* Senftenberg in the UK and other countries, where basil imported from Israel was involved (Pezzoli et al, 2008, Foodborne Pat Dis 5:661-8). Trace-back investigations showed that the basil in the present outbreak was also imported from Israel.
- The remaining ingredients garlic and olive oil were not considered possible sources.

The Statens Serum Institut contacted the Ministry of Health in Israel, who in turn initiated an inspection of the production company that was declared as the origin of the basil in the importation papers. However, as this producer claimed not to have grown basil in the last four years, neither the exact origin of the basil, nor the environmental conditions during production and handling could be clarified.

ECDC has made a toolbox, which may be of help for foodborne or waterborne outbreaks that cross borders. It's called the "Toolkit for investigation and response to Food and Waterborne Disease Outbreaks with an EU dimension". It can be found here: ecdc.europa.eu/en/healthtopics/food_and_waterborne_disease/toolkit/

The EU has a system for notification between member states about foods that may be harmful to human health, the RASFF (rapid alert system for food and feed). Information about this and other international communications systems can be found in Tool 8 in this toolkit.

Outbreak learning points to consider:

- It is important to be aware that ETEC, usually known as the cause of travellers' diarrhoea, can also be acquired domestically by foodborne transmission.
- At the same time, ETEC will very often not be diagnosed, because it requires specialised tests which are not part of the routine diagnostic packages for domestic diarrhoea or are not at all available at primary level microbiological laboratories.
- As in many other foodborne outbreaks, a violation of time-temperature-regulations had likely occurred in this outbreak, too. Inspection of the kitchen where the dinner had been prepared and detailed interviews with the food handlers allowed identification of this problem.
- Introduction of microbiological quality standards for imported fresh produce should be considered.
- Collaboration between countries is important when imported food is involved in outbreaks. Different networks exist at WHO and EU levels for notification of threats and trace-back of food products, including the ECDC EPIS system (cases), the EU commission EWRS (cases) and RASFF (food) systems and the WHO Infosan system (food)

The outbreak investigation was later published as: Pakalniskiene J, Falkenhorst G, Lisby M, Madsen SB, Olsen KE, Nielsen EM, Mygh A, Boel J, Mølbak K.: A foodborne outbreak of enterotoxigenic *E. coli* and *Salmonella* Anatum infection after a high-school dinner in Denmark, November 2006. *Epidemiology and Infection*, 2009, 137(3):396-401.