EpiSouth

Network for the Control of Public Health Threats
in the Mediterranean Region and South East Europe

Role of the laboratory in outbreak investigations

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Learning objectives

- Understand the roles of laboratory before and during an outbreak

- Understand problems that may arise in the collaboration between PH microbiologists and epidemiologists during outbreaks and identify possible solutions

Laboratories and outbreaks

Before the outbreak

- Outbreak detection

  Laboratory detects the outbreak

During the outbreak

- Outbreak response and management

  Epidemiologist captures an increased incidence

In between outbreaks

- Trend monitoring
- Intervention evaluation
- Monitoring progress towards a control objective

1. Role of laboratory BEFORE the outbreak

On-going routine surveillance to provide an early warning about emerging groups of cases potentially with a common source

Laboratory can identify clusters/outbreaks of:

- ✓ Infections with an unusual pathogen
- ✓ Specific subtype of a pathogen/new serotypes
- ✓ Multidrug-resistant pathogens

2. Role of laboratory DURING the outbreak

Epidemiologist captures an increased incidence

a) Provision of general microbiological advice
b) Identify the agent causing the outbreak
c) Identify common exposures with seemingly unrelated cases
d) Confirm cases in an outbreak
e) Distinguish different outbreaks
f) Confirm an outbreak
g) Investigate vaccine-related outbreaks
h) Indicate possible sources

a) Provision of general microbiological advice

- Range of plausible organisms and toxins to help focus the epidemiological components of the investigation
- Appropriate clinical specimens to collect and quantity of specimens

b) Identify the agent causing the outbreak

Correctly identifying the agent may allow:

- Confirms the clinical diagnosis to target interventions
- Provides additional details on the pathogen (e.g. subtypes of a pathogen – Influenza H1N1, *Yersinia enterocolitica* serotype O:3)
- Allows for a more specific case definition that include laboratory criteria

b.1. Confirm diagnosis to target interventions

Influenza or legionellosis?

Antivirals or antibiotics

Strain of meningitis

Type of vaccine to use

Pneumococcus type?

Relation to current vaccine

Drug resistance

Antimicrobial to use?

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- Allows for a more specific case definition that include laboratory criteria

b.2. Identifying the pathogen causing the outbreak: typing of pathogens

- Variety of processes that describe detailed characteristics of microorganisms of the same species
- Allows further subdivision into different organism types or subtypes
- Organisms that are indistinguishable by typing are said to have epidemiological relatedness, and therefore to have come from a recent common ancestor and common source.

b.2. Identifying the pathogen causing the outbreak: typing of pathogens

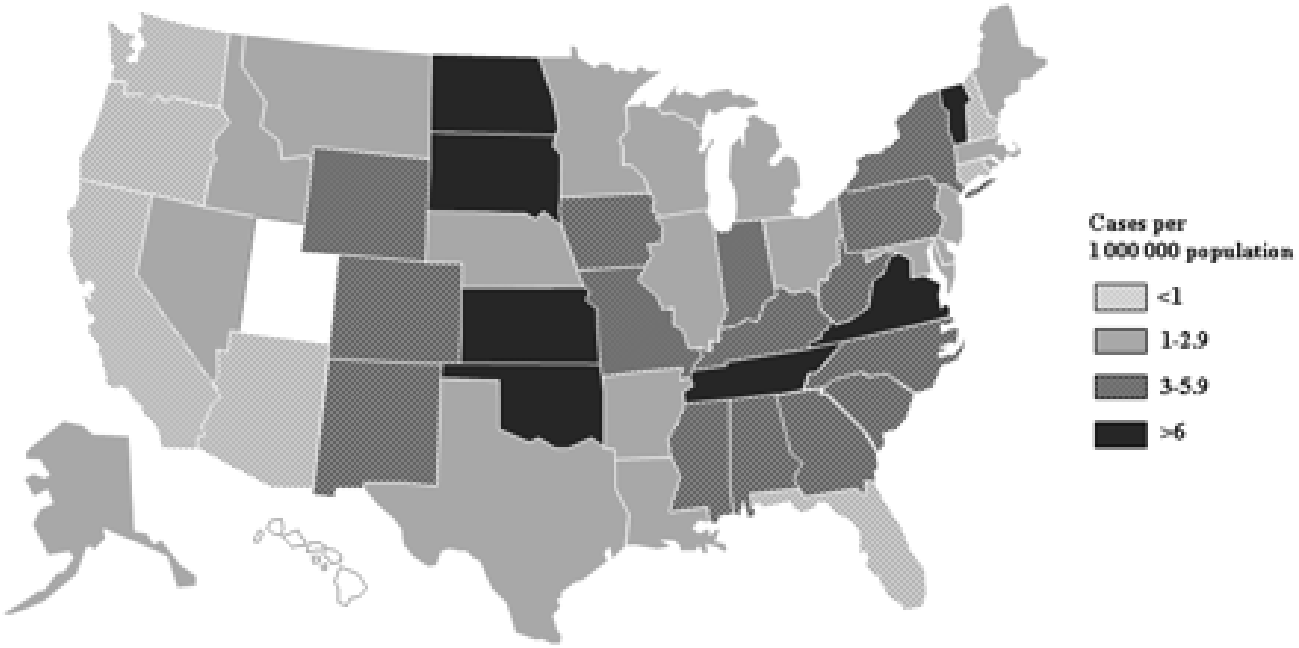
- Phenotypic (based on function and visible traits of the organism)
  - ✓ Serotyping
  - ✓ Biotyping
  - ✓ Phagetyping
  - ✓ Drug resistance

e.g. Vibrio cholerae serogroup O:1; biotype El Tor; Ciprofloxacin resistant

- Genotypic (analysis of nucleic acids within the organism)

Real life outbreak #1 – *Salmonella* (1)

In summer 2006 a U.S nationwide great increase in *Salmonella* infections



Real life outbreak #1 – *Salmonella* (2)

2011	*Salmonella* serotype	Number of cases
1	Enteritidis	1,424
2	Typhimurium**	981
3	Newport	959
4	Javiana	753
5	S. I 4,[5],12:i:-***	314
6	Muenchen	201
7	Heidelberg	169
8	Montevideo	150
9	Infantis	130
10	S. I 13,23:b:-	119
11	Oranienburg	114
12	Saintpaul	113
13	Braenderup	110
14	Bareilly	108
15	Agona	73
16	Mississippi	69
17	Thompson	67
18	Typhi	63
19	Berta	52
20	Anatum	50

FoodNet 2011 Surveillance Report, CDC

- About 40.000 cases of *Salmonella* infections reported/year and 7000 confirmed in U.S
- More than 2 400 Salmonella serotypes have been described and reported

Serotyping of isolated outbreak strains identified *Salmonella* serotype *tennessee*

b) Identify the agent causing the outbreak

Correctly identifying the agent may allow:

- Confirms the clinical diagnosis to target interventions
- Provides additional details on the pathogen (e.g. subtypes of a pathogen – Influenza H1N1, *Yersinia enterocolitica* serotype O:3)
- Allows for a more specific case definition that include laboratory criteria

Real life outbreak #1 – *Salmonella* (3)

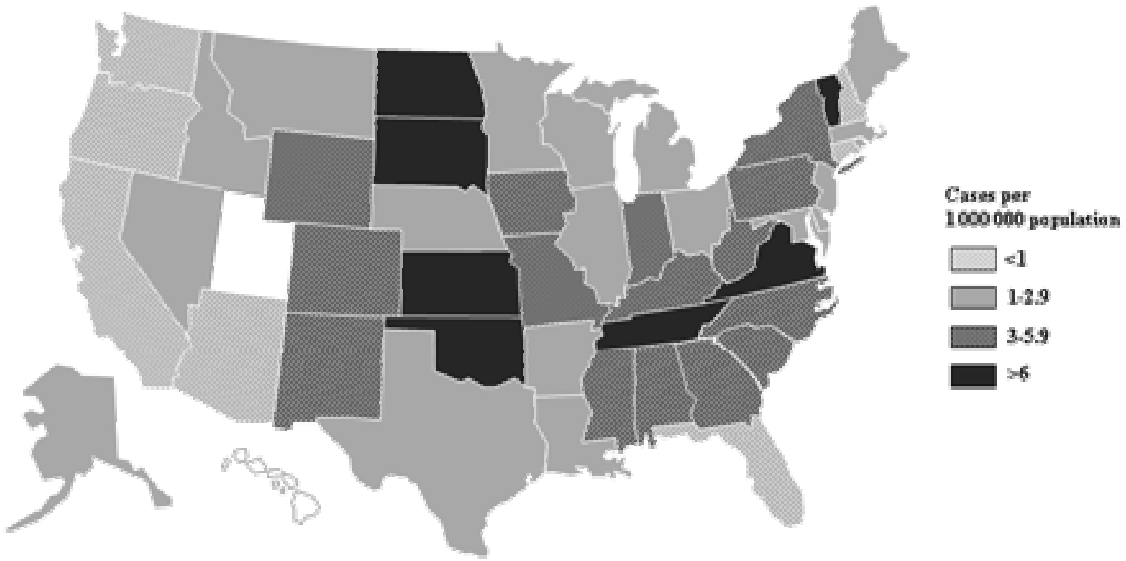
Case definition:
infection with *S.tennessee* in a person ≥18 years old with a history of diarrhea and illness onset from 1 August 2006

Identification of organisms common to different cases, increases the specificity of the case definition

c) Identify common exposures with seemingly unrelated cases

Are seemingly unrelated cases (e.g. geographically widespread) related to a common exposure?

Real life outbreak #1 – *Salmonella* (4)



More than 1000 cases in 48 states

Epidemiologic data (case-control study) suggested peanut butter as the possible source. (Peanut butter mOR, 12.1 [95% CI, 3.6–63.3]) Sheth et al. CID 2011

d) Confirm cases in an outbreak

Is case A truly a case in my OB or not?

Subtyping of cases excluded persons with other serotypes of *Salmonella*

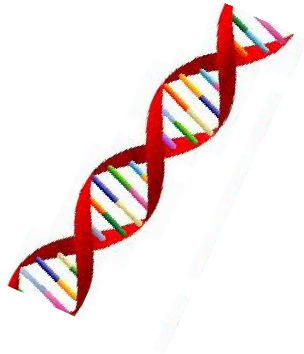
But sometimes phenotyping is not enough to confirm cases in an OB….
We need to further narrow down the case definition and exclude non-related cases!!

b.2. Identifying the pathogen causing the outbreak: typing of pathogens

- Phenotypic (based on function and visible traits of the organism)
  - ✓ Serotyping
  - ✓ Biotyping
  - ✓ Phagetyping
  - ✓ Drug resistance

- Genotypic (analysis of nucleic acids within the organism)

Genotyping of the pathogen



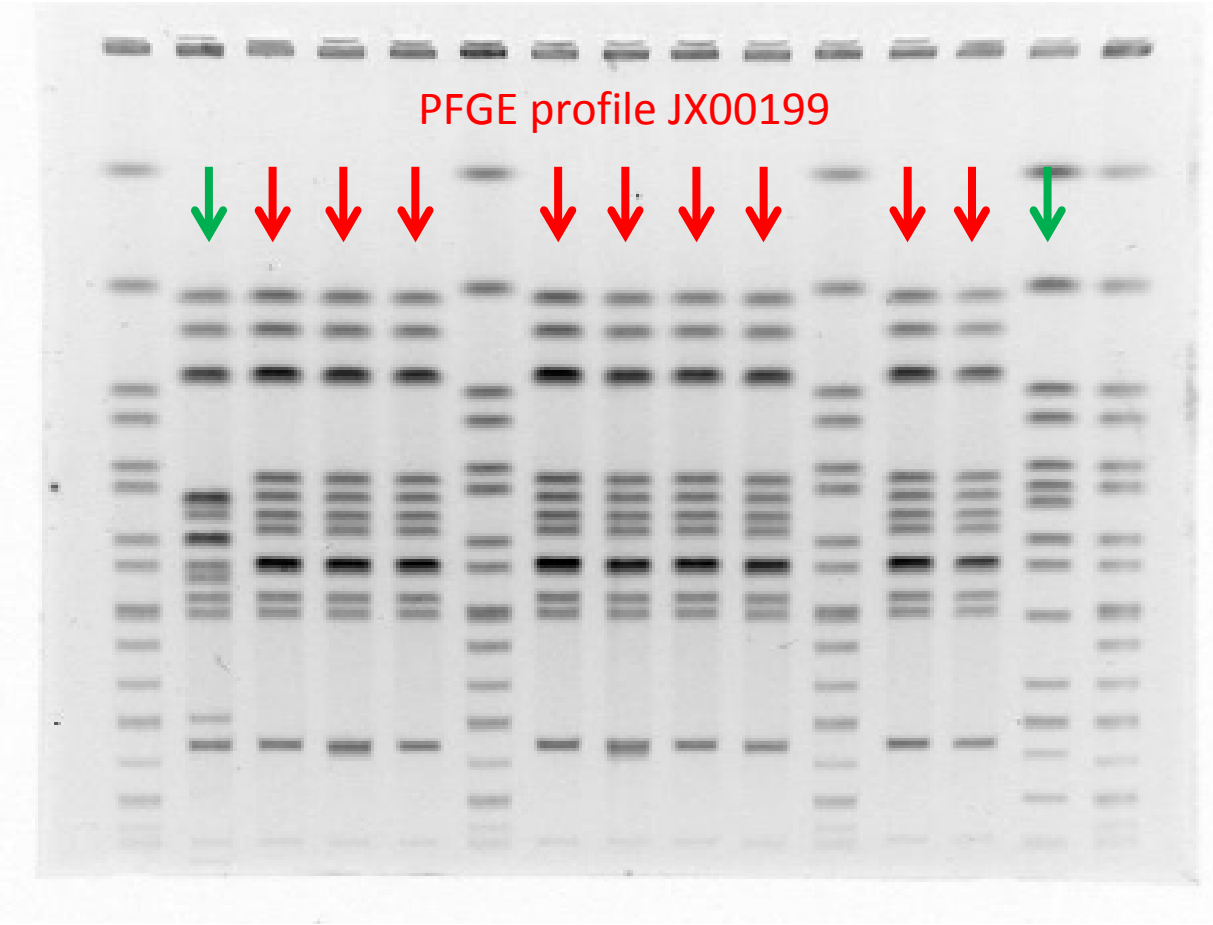
Is the use of genetic variation in pathogens in the study of disease

Goal → provide laboratory evidence that *epidemiologically related* isolates collected during an outbreak are also *genetically related* and thus represent the same strain

Typing by Pulsed Field Gel Electrophoresis (PFGE)

- Provides a genetic fingerprint of the bacteria so we can further narrow down persons associated with an OB
- Can be applied to any bacteria
- Ideally, PFGE patterns of isolates representing the OB strain would be indistinguishable from each other and distinctly different from those of epidemiologically unrelated strains

Real life outbreak #1 – *Salmonella* (7)

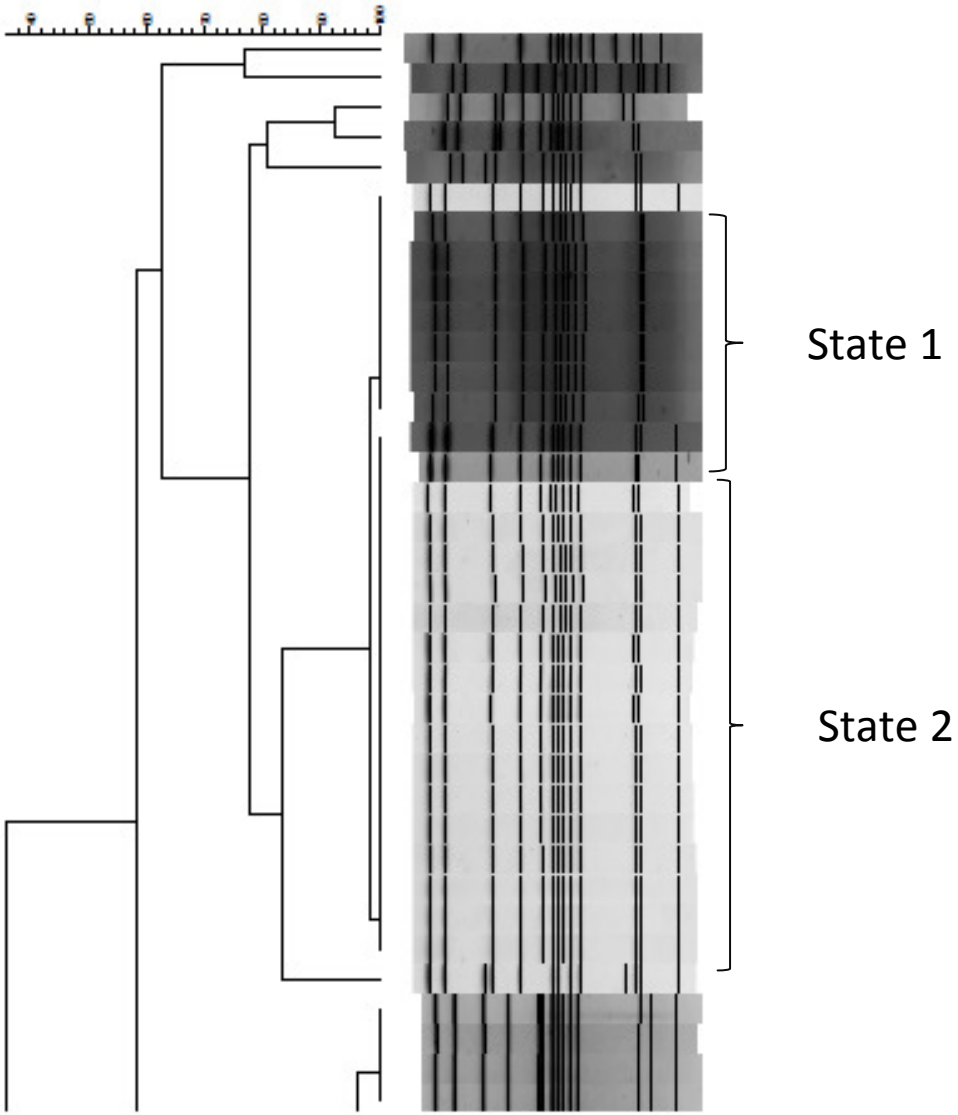


PFGE testing on *Salmonella tennessee* OB isolate cases, 9 are matches

PFGE allows to further narrow cases in an OB helping to include or exclude persons

PulseNet U.S.: a database of PFGE profiles

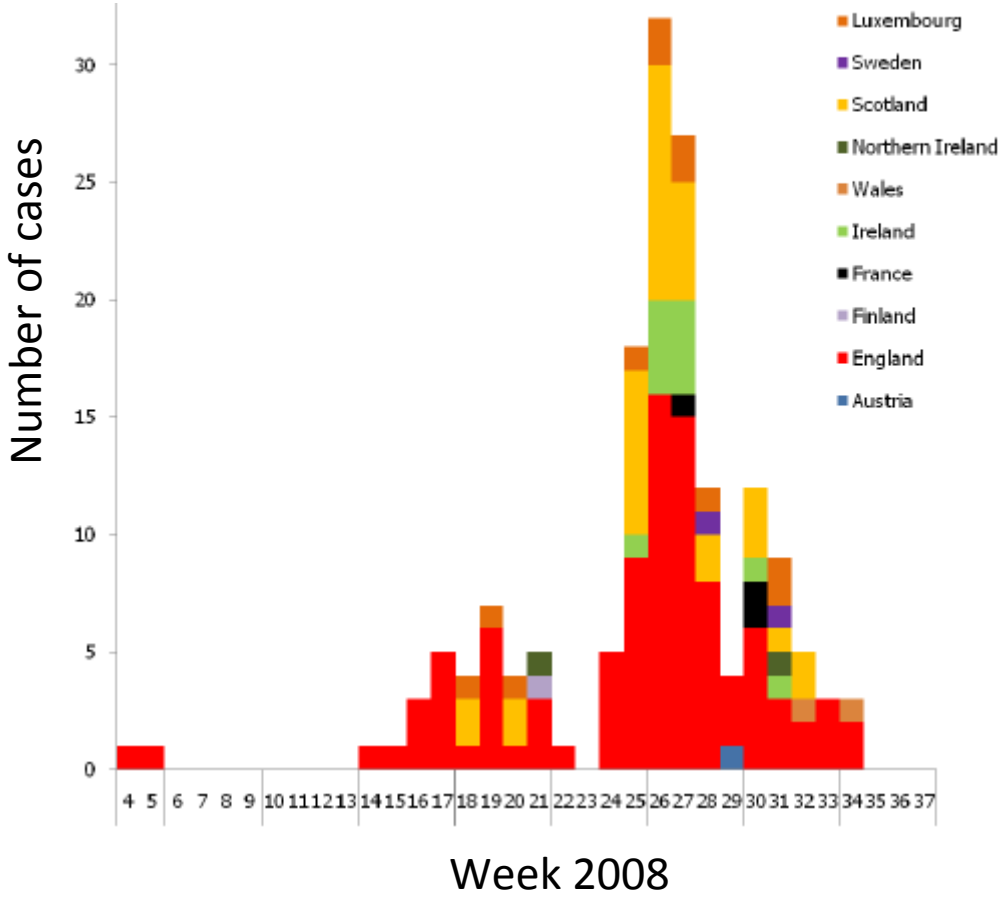
- American database representing nearly over one half a million isolates of bacteria from food, the environment, and human foodborne illness since 1996.
- It connects foodborne illness cases together, using DNA fingerprinting of the bacteria to detect and define outbreaks



Detection of outbreaks in laboratories in Europe

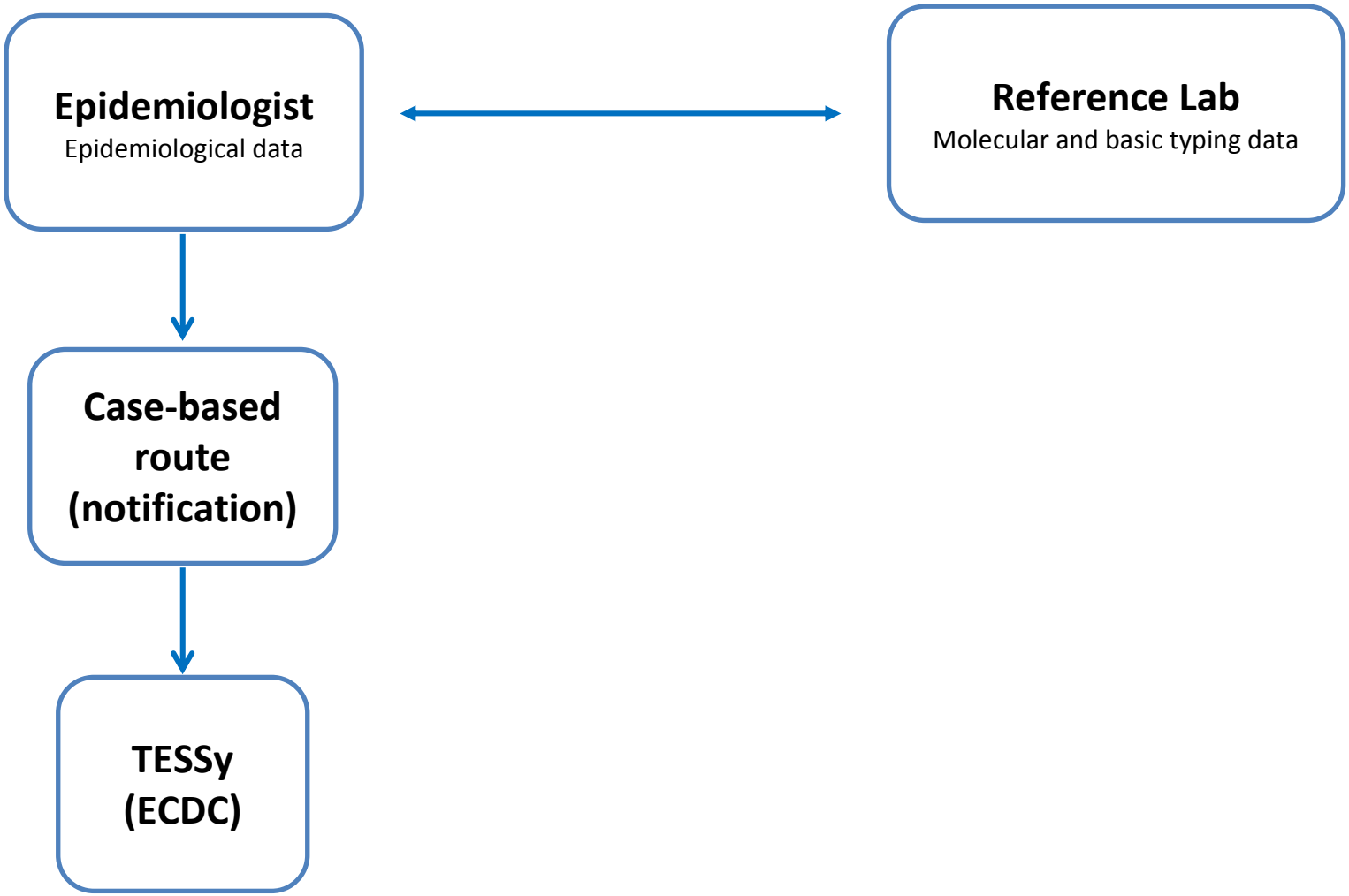
Multinational outbreak verified by PFGE

Salmonella Agona, N=163 cases, EU, Feb-Sep 2008

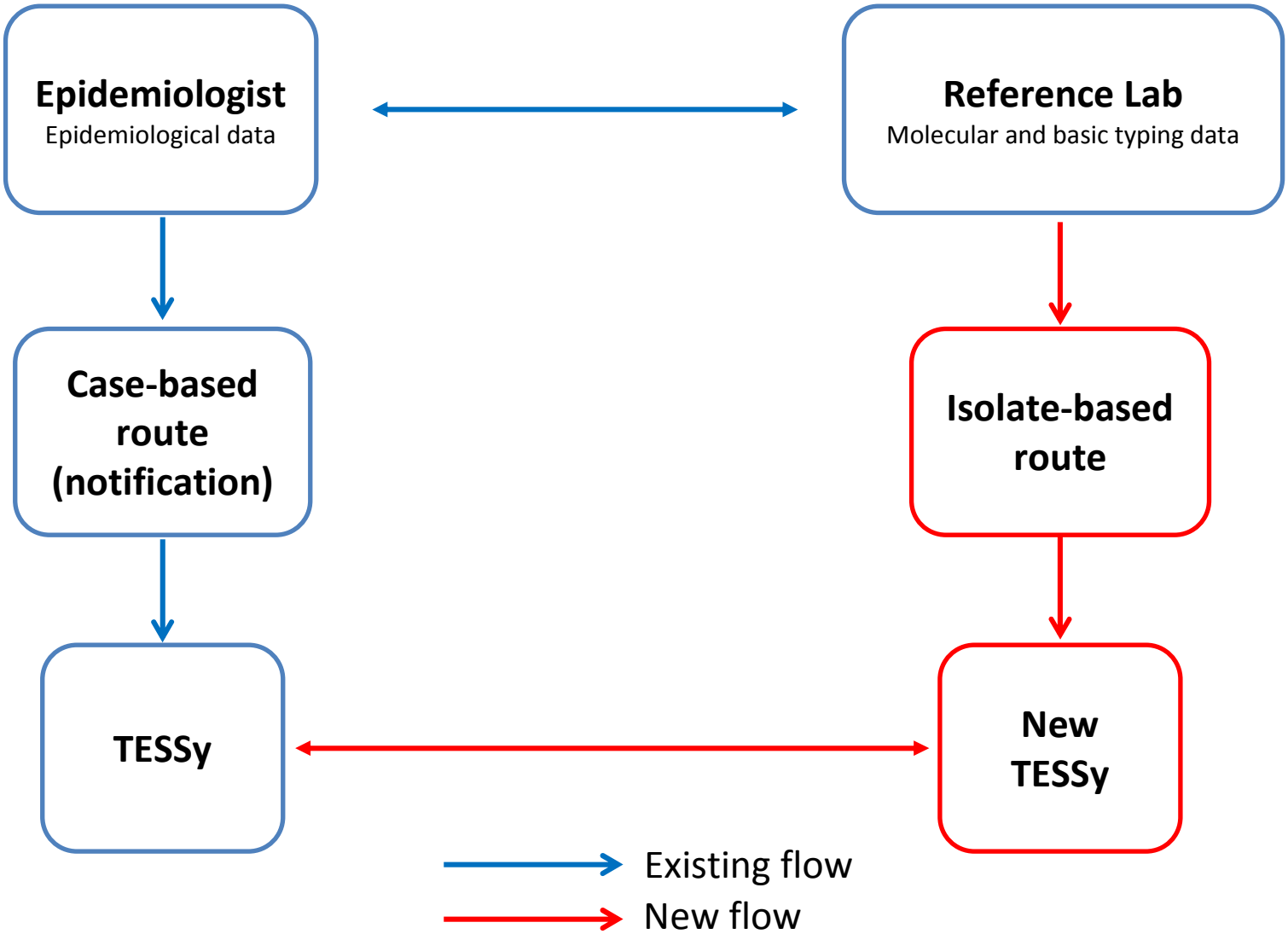


2008: PFGE images shared by email

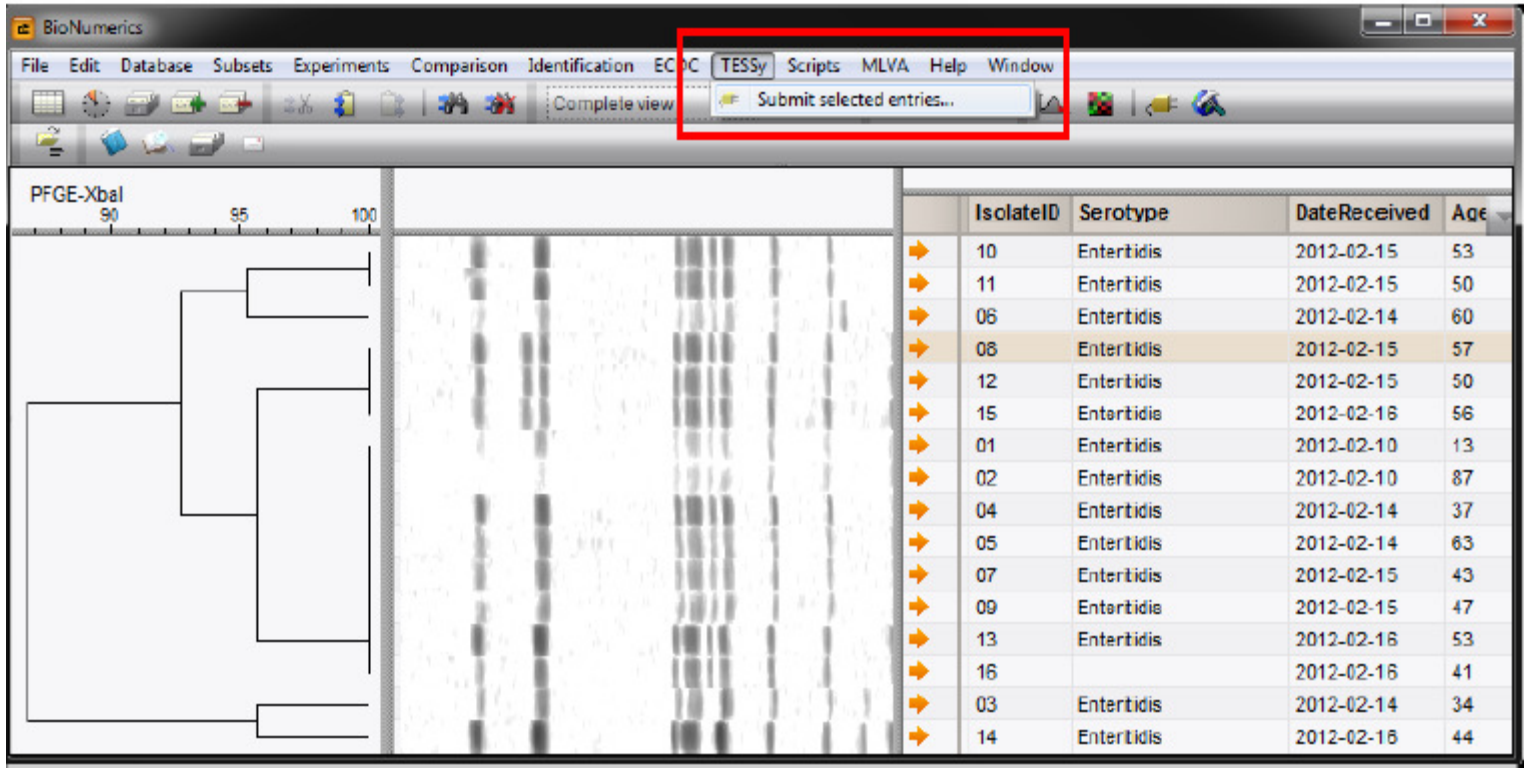
Existing flow to detect of international outbreaks in Europe



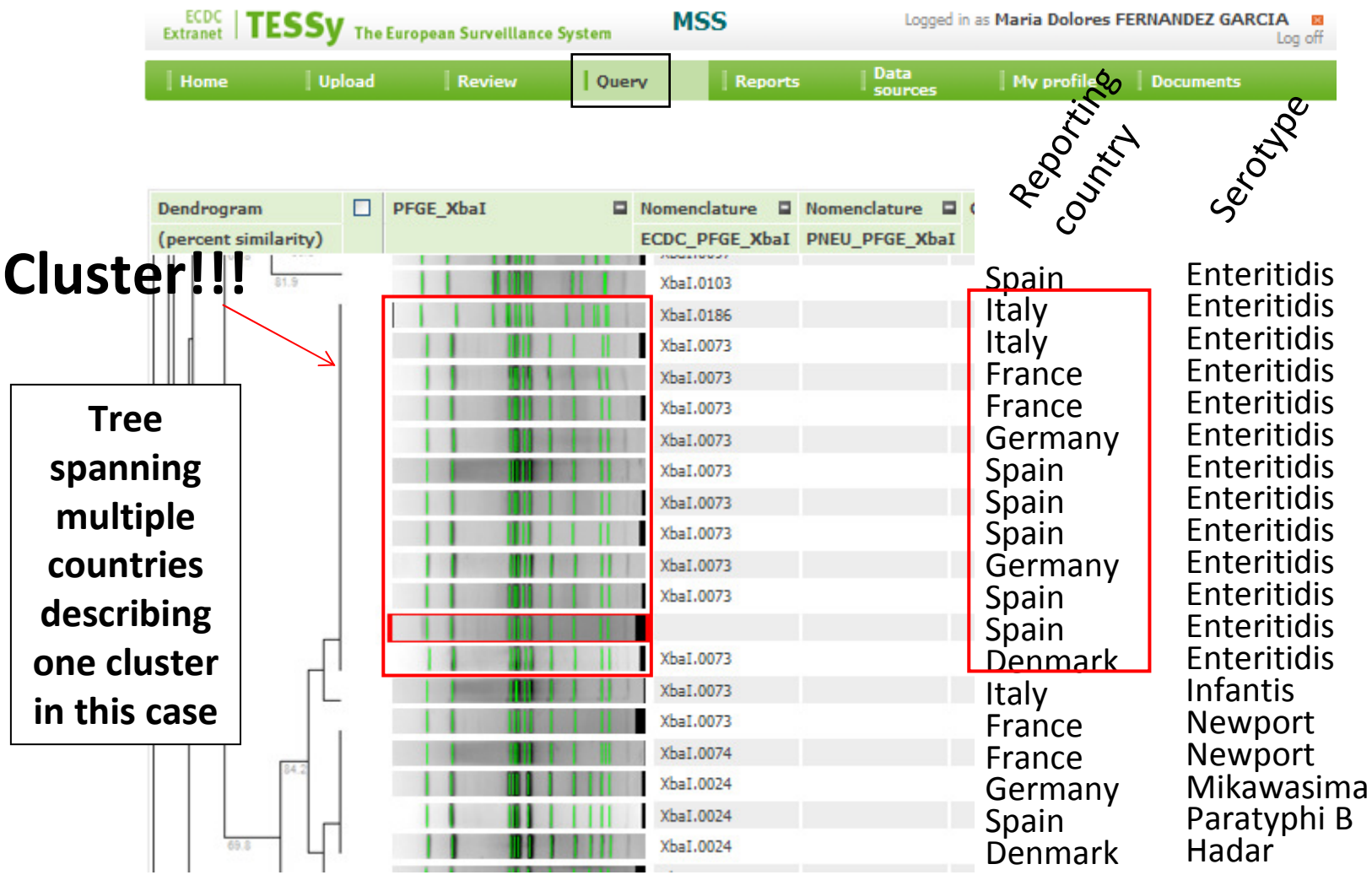
Molecular Typing Surveillance for rapid detection of international outbreaks by laboratories (started by ECDC 2012)



First countries upload molecular data from own system to the New TESSy



Then countries can review related international clusters/outbreaks



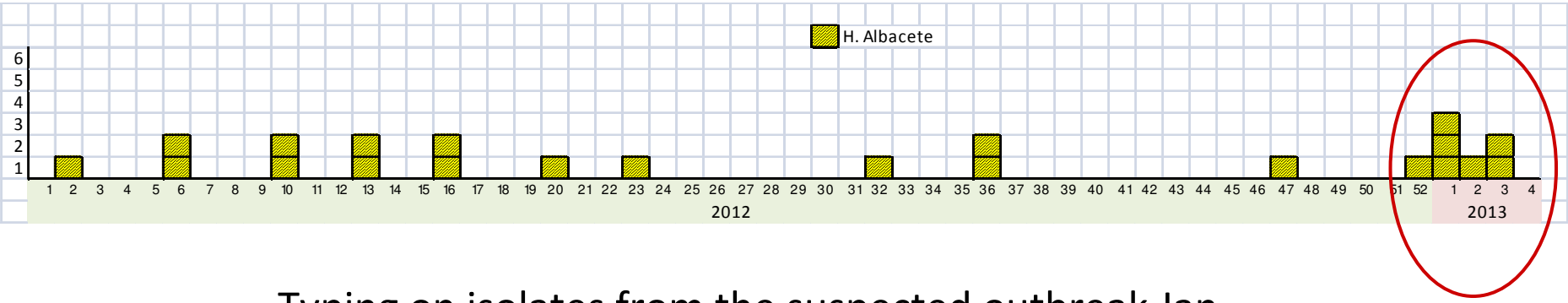
e) Confirm the outbreak

Is an outbreak really an outbreak?

Are different isolates the same strain?

Real life example #5 - the increase of *Y.enterocolitica* cases warranted the need for an OB investigation?

Increase of cases in Jan 2013 in a Spanish region

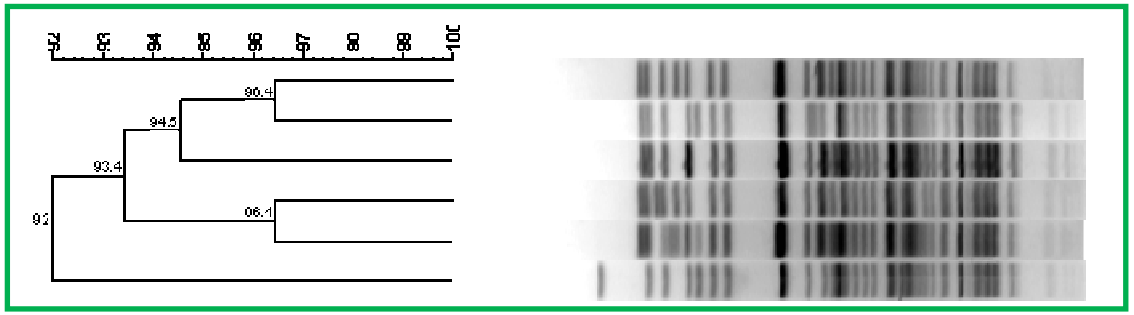


Typing on isolates from the suspected outbreak Jan 2013

Phenotyping

Key	Especie	Serotipo	Biotipo
213/13	Y.enterocolitica	O:3	4
4642/12	Y.enterocolitica	O:3	4
4643/12	Y.enterocolitica	O:3	4
211/13	Y.enterocolitica	O:3	4
212/13	Y.enterocolitica	O:3	4
4644/12	Y.enterocolitica	O:3	4

Genotyping: PFGE



No identical PFGE profile in isolates from Albacete → NOT AN OUTBREAK!!

f) Distinguish different outbreaks

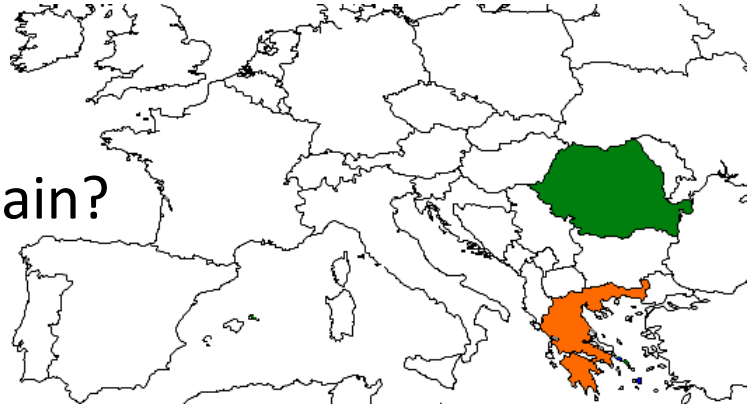
Are outbreak A and outbreak B related?

Help distinguish between apparently related outbreaks occurring in the same time frame

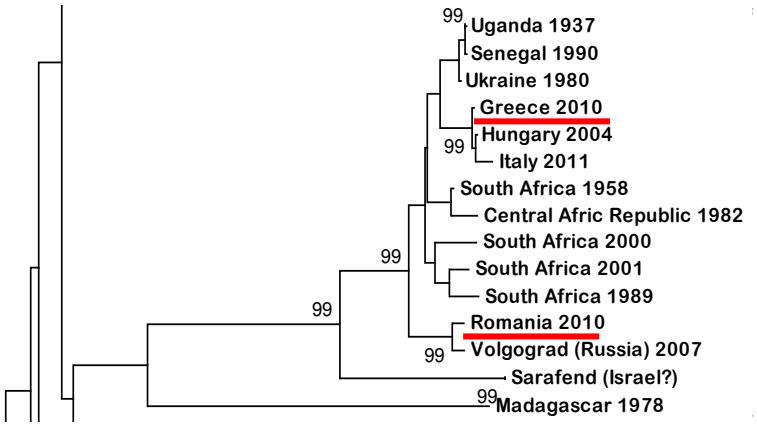
Real life outbreak #4 – West Nile Virus

Two outbreaks of WNV in Aug 2010

Were they caused by the same WNV strain?



Lineage 2



g) Investigate vaccine-related outbreaks (1)

Genetic characterizations of virus isolates establish the similarity or “match” between viruses and viruses used in a vaccine

e.g. Mumps outbreak

- Country X
- Introduced MMR vaccination into routine vaccination
- After some campaigns children developed fever/parotitis 2 weeks after vaccination
- Ministry of Health – Is this vaccine related?

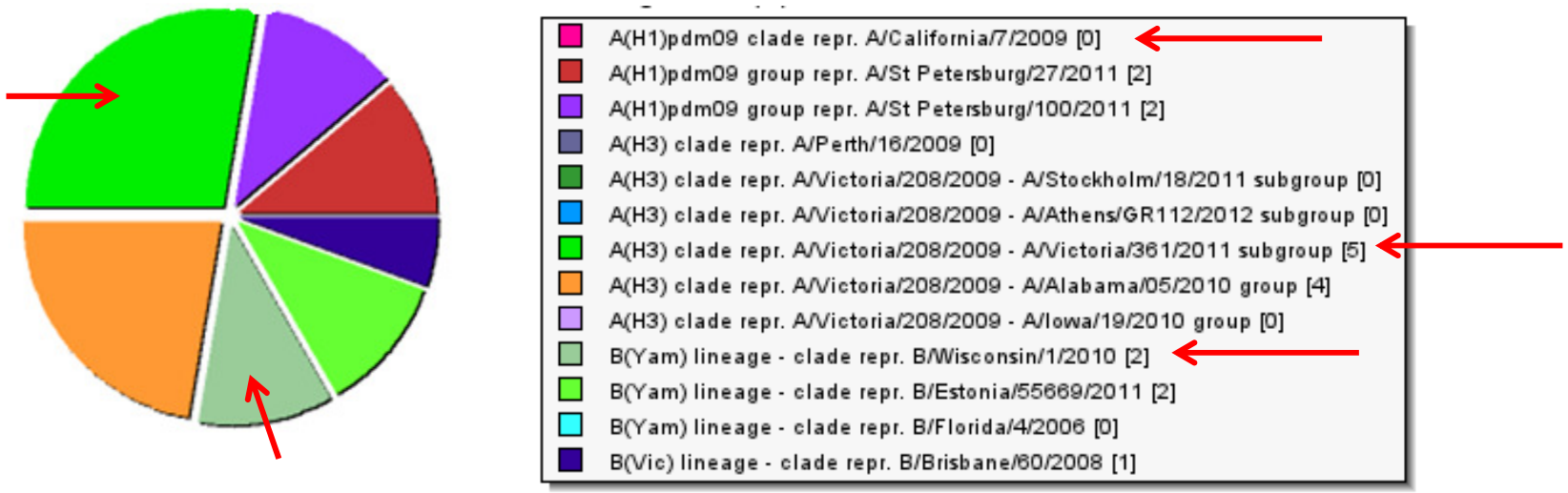
→ guide decision to stop vaccination campaign

g) Investigate vaccine-related outbreaks (2)

e.g. Seasonal flu:

Genetic characterizations of influenza virus isolates establish the similarity or “match” between flu viruses and flu vaccine

→ guide decision on the composition of vaccines for next season



← Viruses included in the seasonal influenza vaccine 2012/2013 of northen hemisphere

h) Indicate possible sources

What is the possible source of the organism causing the outbreak?

- Detection of organisms of the same type in potential sources

- In foodborne OBs: if a food item is available for testing, we can match the PFGE pattern of the bacteria in the food to the PFGE pattern on the stool of ill persons

Real life outbreak #6 – *Vibrio cholerae* (1)

- > 500 000 cases and >7000 deaths
- Cholera in Haiti in Oct 2010 for the first time in history
- Evidence points to the UN peacekeeping troops from Nepal as the source

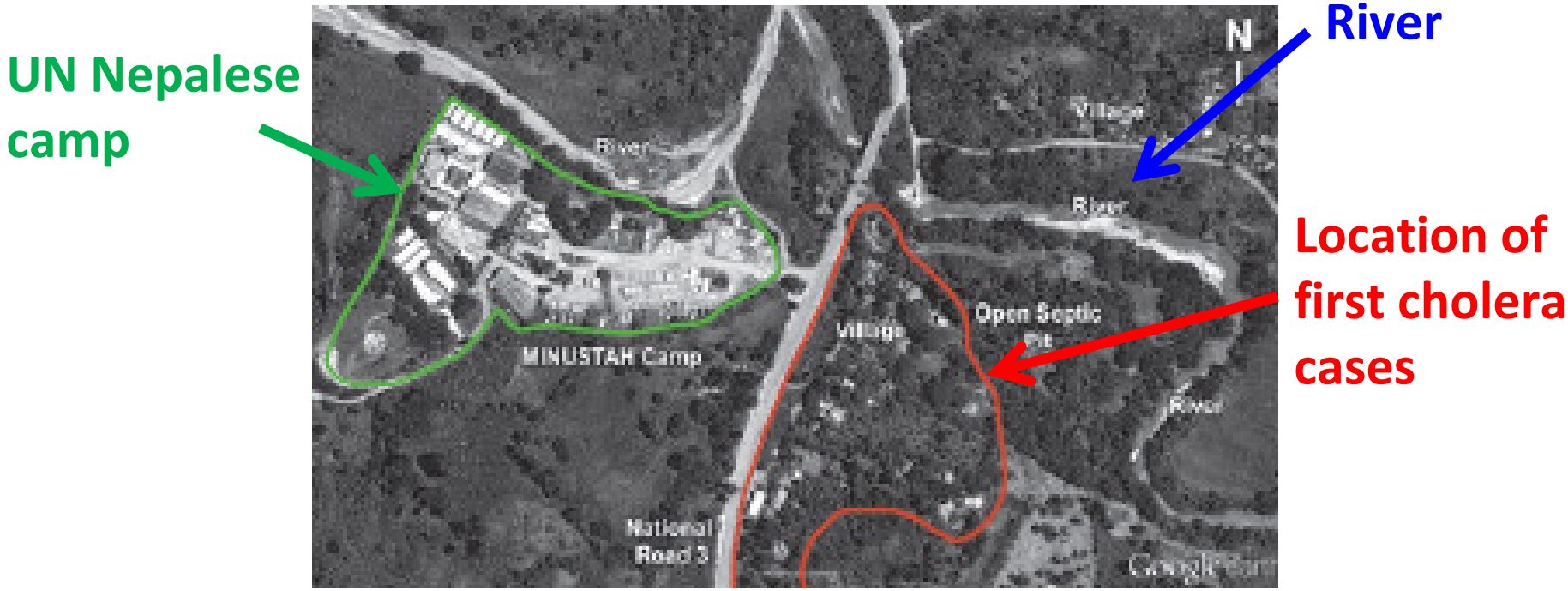
ORIGINAL ARTICLE BACTERIOLOGY

Nepalese origin of cholera epidemic in Haiti

Real life outbreak #6 – *Vibrio cholerae* (2)

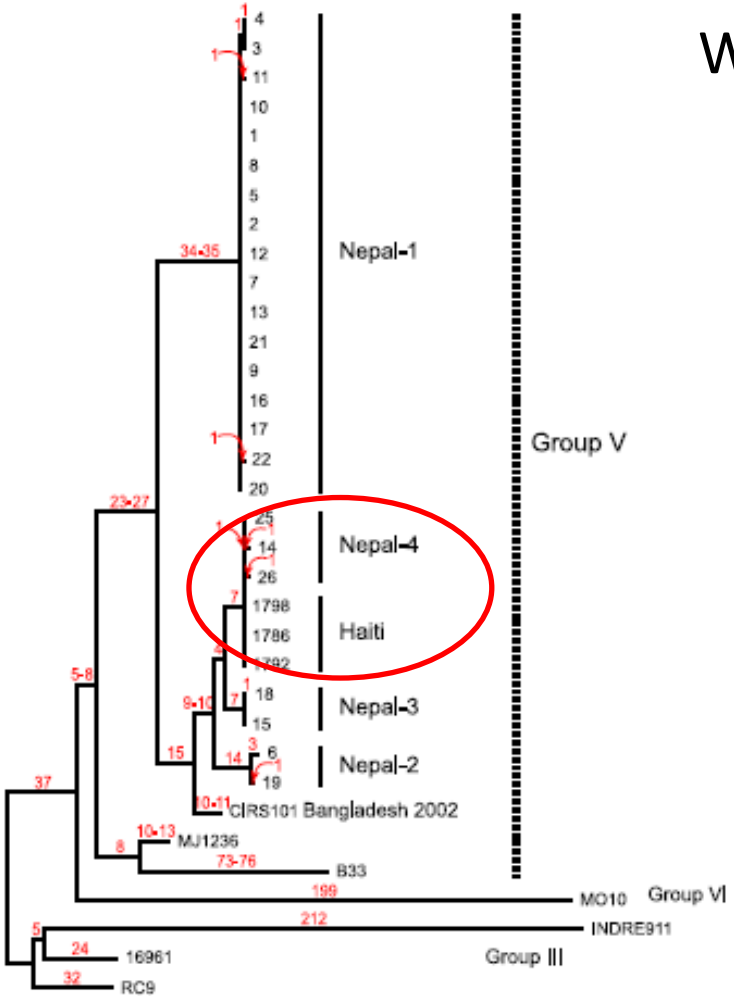
Epidemiological evidence

- UN troops came from Nepal where a cholera OB had just occurred
- A few days after they arrived, cases of cholera appeared in the village next to the UN Nepalese camp
- Pipes from the camp leaked faecal waste into the river



Real life outbreak #6 – *Vibrio cholerae* (3)

Molecular evidence



Hendriksen et al. mBio 2011

WGST → Whole-Genome Sequence Typing

- Compared complete genome sequences of isolates from the Haiti OB and *V.cholerae* isolates from Nepal collected in 2010
- One cluster contained 3 Nepalese isolates and 3 Haitian isolates that were identical
- PFGE patterns were consistent with an epidemiological link between the isolates from Nepal and Haiti

Moral of the stories…

Epidemiological and microbiological investigations are of vital importance during OBs

- Case questionnaires are usually done before typing results, so if multiple cases are citing the same source (restaurant, water source, etc) there may be something there
- After lab results indicate an OB may be occurring, epidemiologists can review questionnaires to get an idea of the source

The advent of typing has improved not just our ability to "catch" OBs, but to better define an OB and who's involved

Cooperation is necessary

Clinicians

Epidemiologists

Public Health labs

Public Health Authorities

Food Authorities

Others:

- Disease specialists
- Environmentalists
- Veterinarians
- Media people

But problems can arise…
1) organizational difficulties

Type of difficulty	Proposed recommendations
Samples sent with insufficient information on the patient/context	
Delays in reporting results	

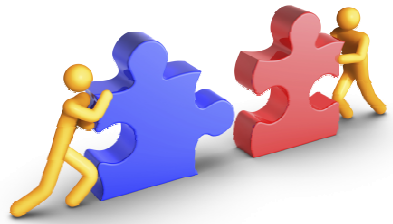
But problems can arise…
1) organizational difficulties

Type of difficulty	Proposed recommendations
Insufficient information on the patient/context	• Microbiologists must communicate how patient information guides their testing • Epidemiologists must learn how patient information helps guiding laboratory testing
Delays in reporting results	• Microbiologists need to understand why epidemiologists may require rapid results • Epidemiologists must enquire about the **time needed to obtain results** • Epidemiologists must become familiar with laboratory procedures

Your samples might not be the only ones in the lab

Technique	Theoretical timescale	Actual timescale
Direct visualization	Minutes/hours	Minutes/hours
Culture amplification	Days/weeks	Days/weeks
PCR amplification	Minutes/hours	Days/weeks
Serology	Hours	Weeks
Electron-microscopy	Minutes/hours	Not available!

Problems can arise…
2) technical difficulties



Type of difficulty	Proposed recommendations
Bad quality samples	
Samples not packed safely/ wrong transport medium/ labels not legible	

Samples from the field to the lab: Checklist

What samples should you take? And how?

- Blood, stools, swabs, water, food items, etc

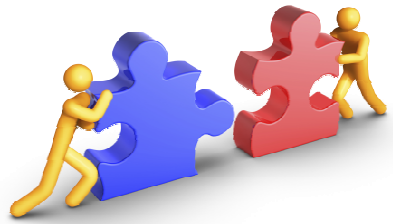
How should you ship the samples?

- How risk material?
- Transport medium?
- Cooling necessary?
- Leakage, in what container: “Box in a box in a box principle”
- Regulations?
- Reception

Ask the lab and inform that samples are arriving!

The result of any laboratory test is only as good as the sample received in the laboratory

Problems can arise…
2) technical difficulties



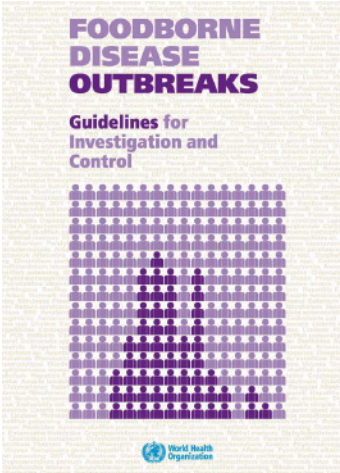
Type of difficulty	Proposed recommendations
Bad quality/quantity samples	• Disseminate guidance to collect appropriate samples to epidemiologists
Samples not packed safely/ wrong transport medium/ labels not legible	• Disseminate guidance for the packaging and transport of samples to epidemiologists

Problems can arise…
3) communication difficulties

Type of difficulty	Recommendations
Microbiologist not aware/not involved in OCT	
Delayed laboratory involvement	
Right person not spoken to (local/regional/national level)	
Lack of engagement of the lab to interpret results	
Arrival of samples is not announced in good time	
Pressure for unrealistic black or white answer/Difficulties in obtaining straight answers	
Final report not sent once the investigation is over	
Concern about data sharing and confidentiality: lab specialists have difficulties releasing their results	

Possible solutions: guide to effective laboratory involvement

- Consult the laboratory early about the alert!!
  - Identify a contact person
  - Invite a laboratory representative to join the OCT
  - Lab needs to schedule resources
- Establish and keep up lines of communication from the beginning to the end
- Build trust before the outbreak happens:
  - Meetings in peace time
  - Regular communication
- Prepare, plan, develop guidelines:
  - Adjust and communicate expectations
  - Clarify roles
  - Clear leadership: agree on authorship issues before the start of the project



- Support from strategic level
  - Administrative support;
  - Help with communication
- Efficient information sharing procedures
  - Sort ethics, data protection
  - Share data and information efficiently and openly; do not hide data and information
- Standard operating procedures, templates
  - Clarifying scaling up to “crisis mode”
- Identify common goals
- Understand that one is not only supporting the other, you work together for the same goals
- Understand that there are different perspectives
- Recognize different skills
- Respect different working cultures

Do you know your laboratory systems?
Do you know the level at which specimens from OBs are to be investigated?

- **Who is in charge of which disease?**
- **Who do you contact in which case?**
- Local labs
- Regional labs
- Hospital labs
- Reference labs
- International lab networks

Find out!!!

Remember you can always phone a friendly microbiologist(s)

Acknowledgements

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