



Agents and outbreaks I

Salmonella, Campylobacter, E. coli, Listeria

Presentation 3
BTSF FBO course

Enne de Boer

Food Safety Hazards

Food safety hazard – any factor present in food that has the potential to cause harm to the consumer, either by causing illness or injury

Food safety hazards may be

- biological (pathogenic bacteria, viruses, parasites, prions)
- chemical (mycotoxins, pesticides, veterinary drugs, environmental contaminants, processing contaminants, cleaning chemicals, etc.)
- physical (stone, piece of metal, etc.)

Foodborne illness – bacterial hazards

Bacterial food safety hazards include two categories according to the mechanism by which they cause illness

Infection – bacterial pathogens develop in the gut after ingestion of contaminated food. Examples: *Salmonella*, *Campylobacter*, pathogenic *E. coli*

Incubation time of at least 8-12 h before symptoms develop

Intoxication (poisoning) – bacterial pathogens grow in foods and produce toxins. Examples: *Bacillus cereus*, *Staphylococcus aureus*

Relative short incubation time because of preformed toxin in the food

Foodborne infections – bacteria

Aeromonas

Campylobacter

Cronobacter (E. sakazakii)

Listeria monocytogenes

Salmonella

Shigella

pathogenic *Escherichia coli*

Vibrio cholerae

Vibrio parahaemolyticus

Vibrio vulnificus

Yersinia enterocolitica

Yersinia pseudotuberculosis



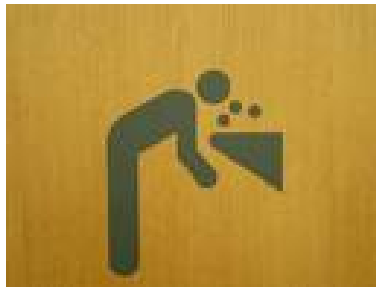
Foodborne intoxications – bacteria

Bacillus cereus

Clostridium perfringens

Clostridium botulinum

Staphylococcus aureus



Foodborne pathogens

Selection of foodborne pathogens of the European Union
Scientific Committee on Veterinary measures relating to public
health (2000)

- bacteria: *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, VTEC
- parasites: *Cryptosporidium*, *Echinococcus*, *Trichinella*
- viruses: mainly person to person transmission, directly or indirectly through food and water, but without animal reservoirs: norovirus, hepatitis A

Salmonella

Salmonella (non-typhoid) – characteristics (organism)

- widespread in wild and domestic animals
- more than 2600 serotypes
- transmission: raw foods of animal origin; surface water; cross-contamination; contact with animals



***Salmonella* in food (EU, 2011)**

Food	# samples	# positive	% positive
Broiler meat	25,611	1515	5.9
Pig meat	52,868	357	0.7
Table eggs	25,619	26	0.1

EFSA, (European Food Safety Authority), ECDC (European Centre for Disease Prevention and Control), 2013. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011; EFSA Journal 2013,11(4):3129, 250 pp.

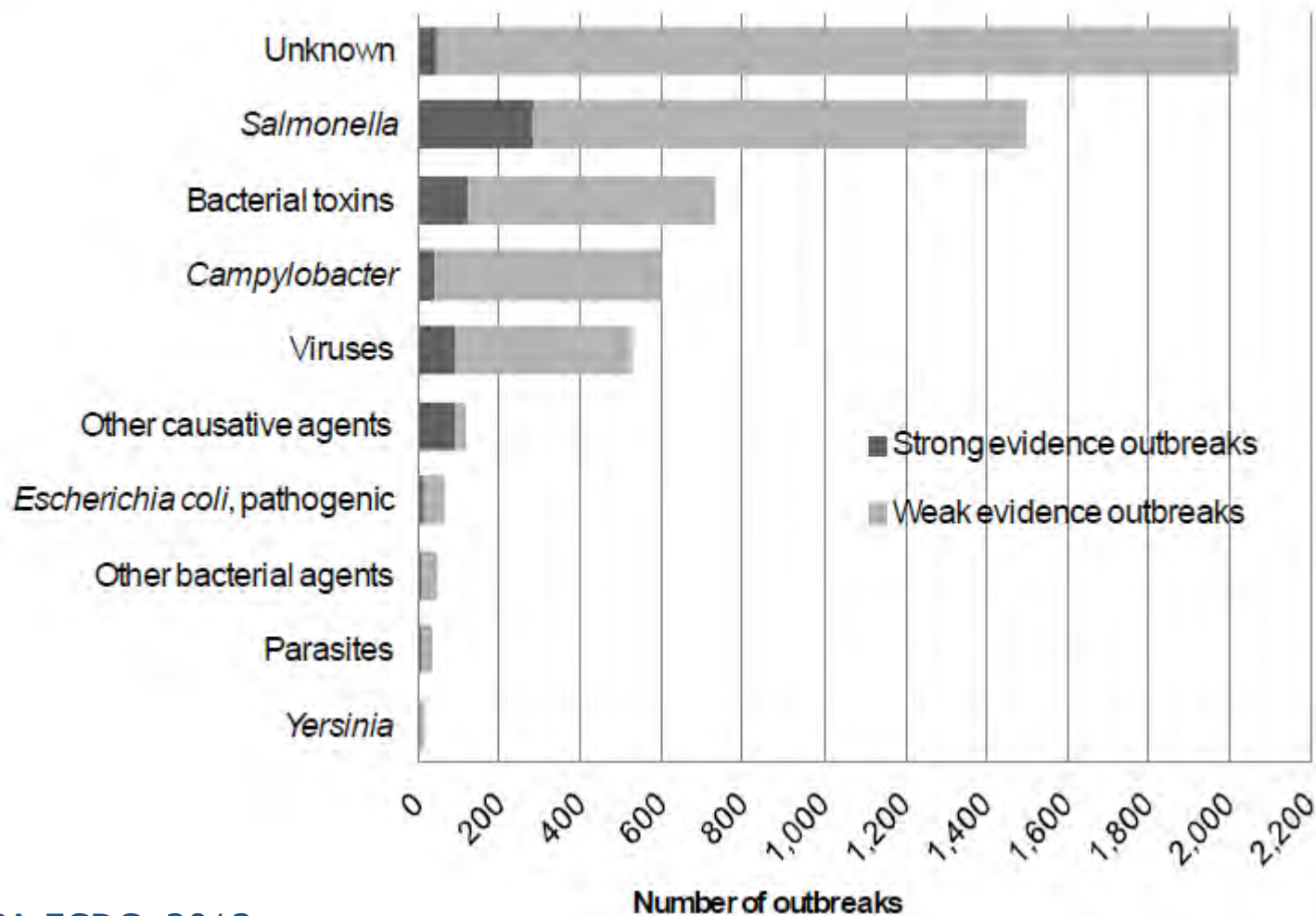
Salmonella

Salmonella infections – characteristics

- symptoms: diarrhoea, abdominal pain, fever, nausea, vomiting
- incubation time: 5 - 72 h
- period of illness: 3 - 7 days



Figure SU2. Distribution of food-borne outbreaks per causative agent in the EU, 2011



Distribution of the 10 most common Salmonella serovars in humans in the EU, 2011

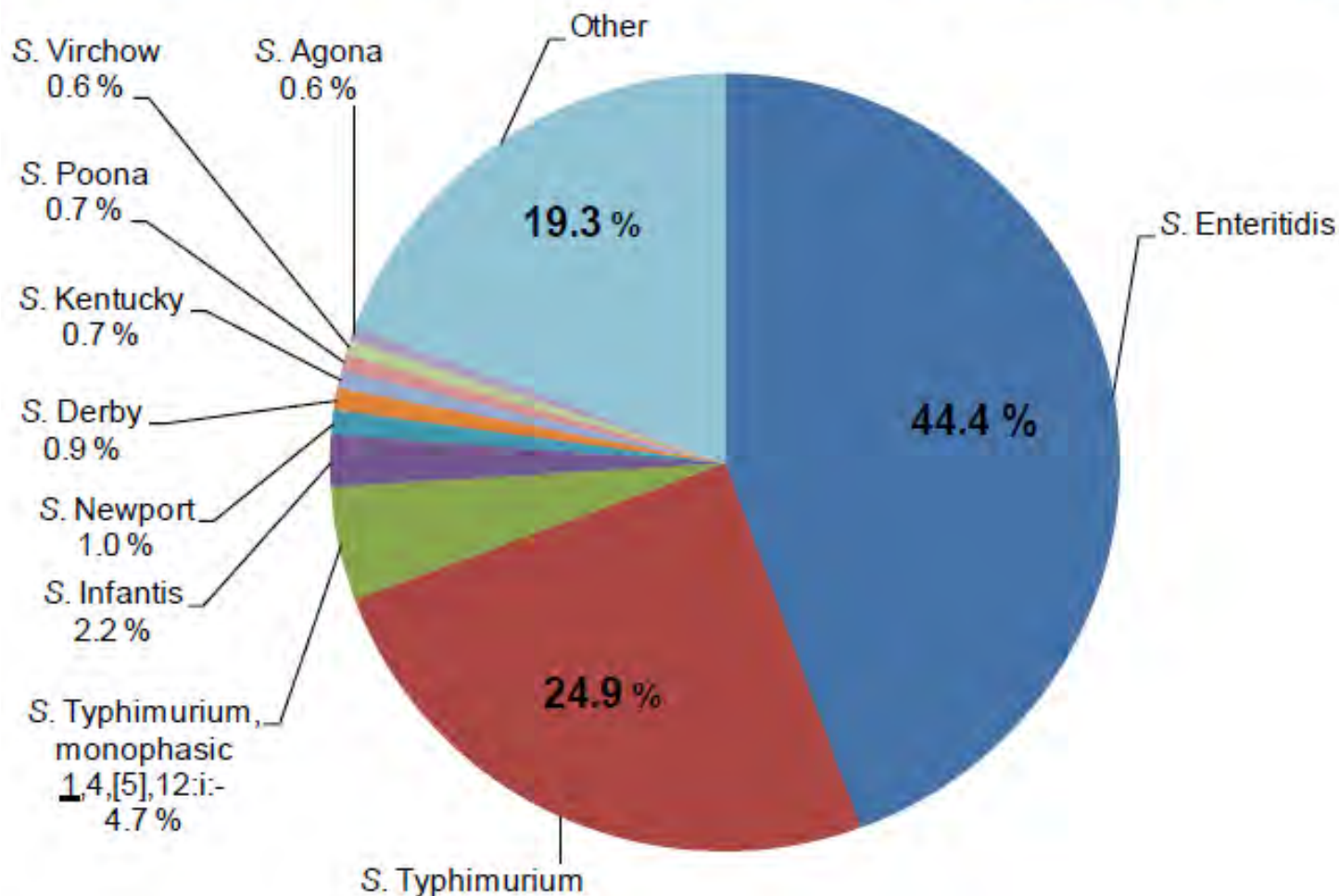


Figure SA1. Trend in reported confirmed cases of human salmonellosis in the EU, 2008–2011

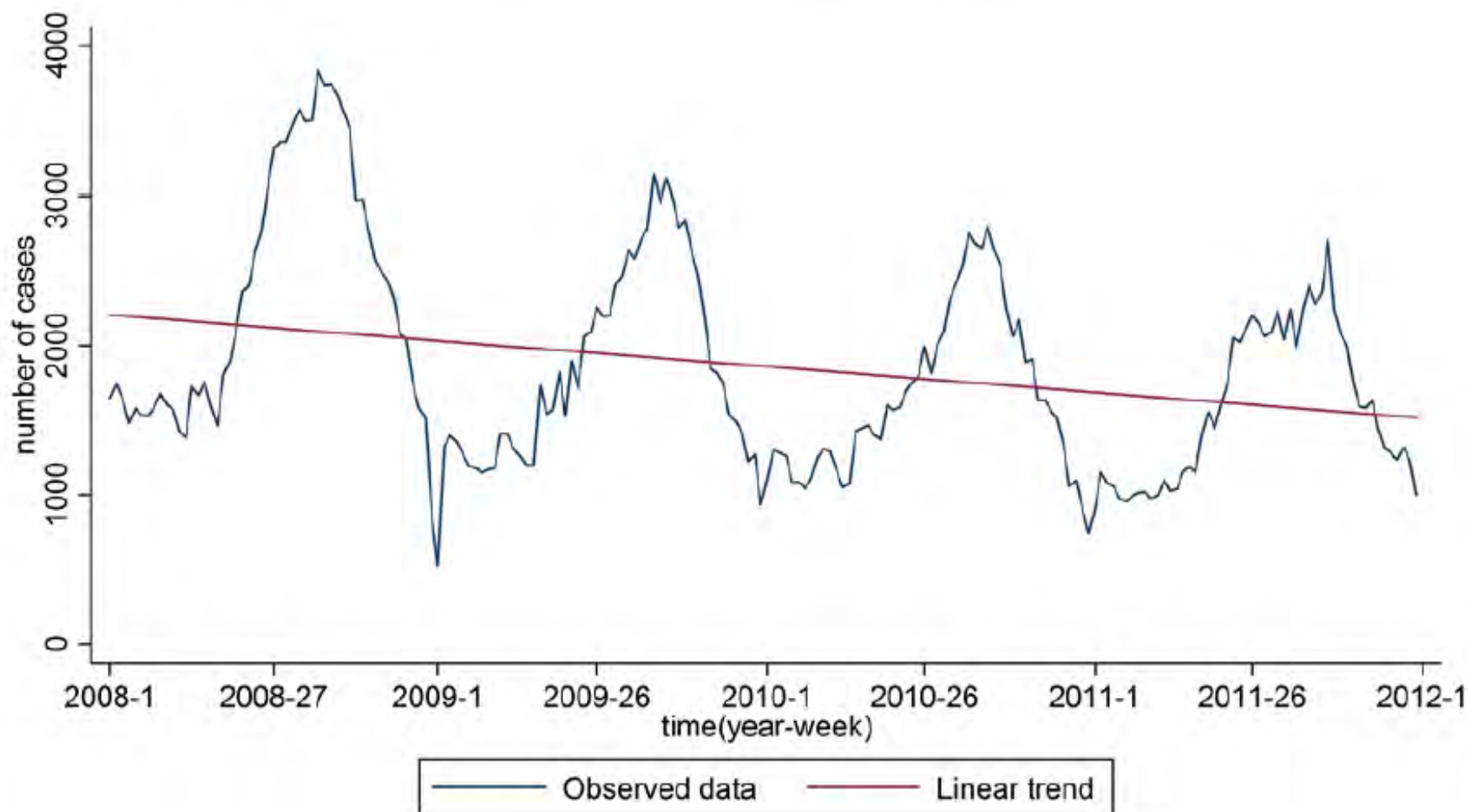




Figure SA4. Prevalence of *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Virchow* and *S. Hadar*-positive breeding flocks of *Gallus gallus* during production in the EU,¹ 2007–2011

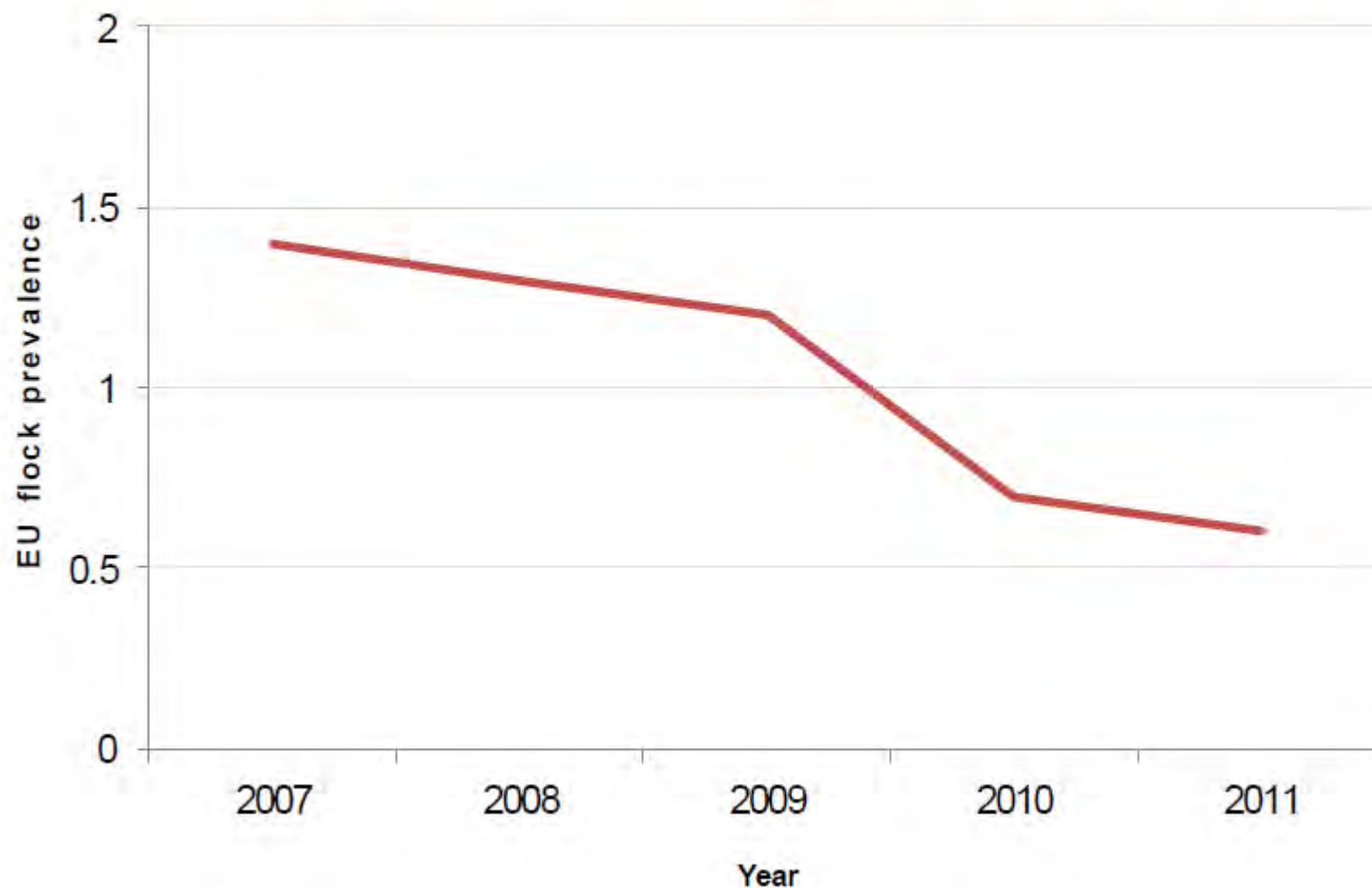




Figure SA8. Prevalence of *S. Enteritidis* and *S. Typhimurium*-positive laying hen flocks of *Gallus gallus* during the production period in the EU, 2008–2011

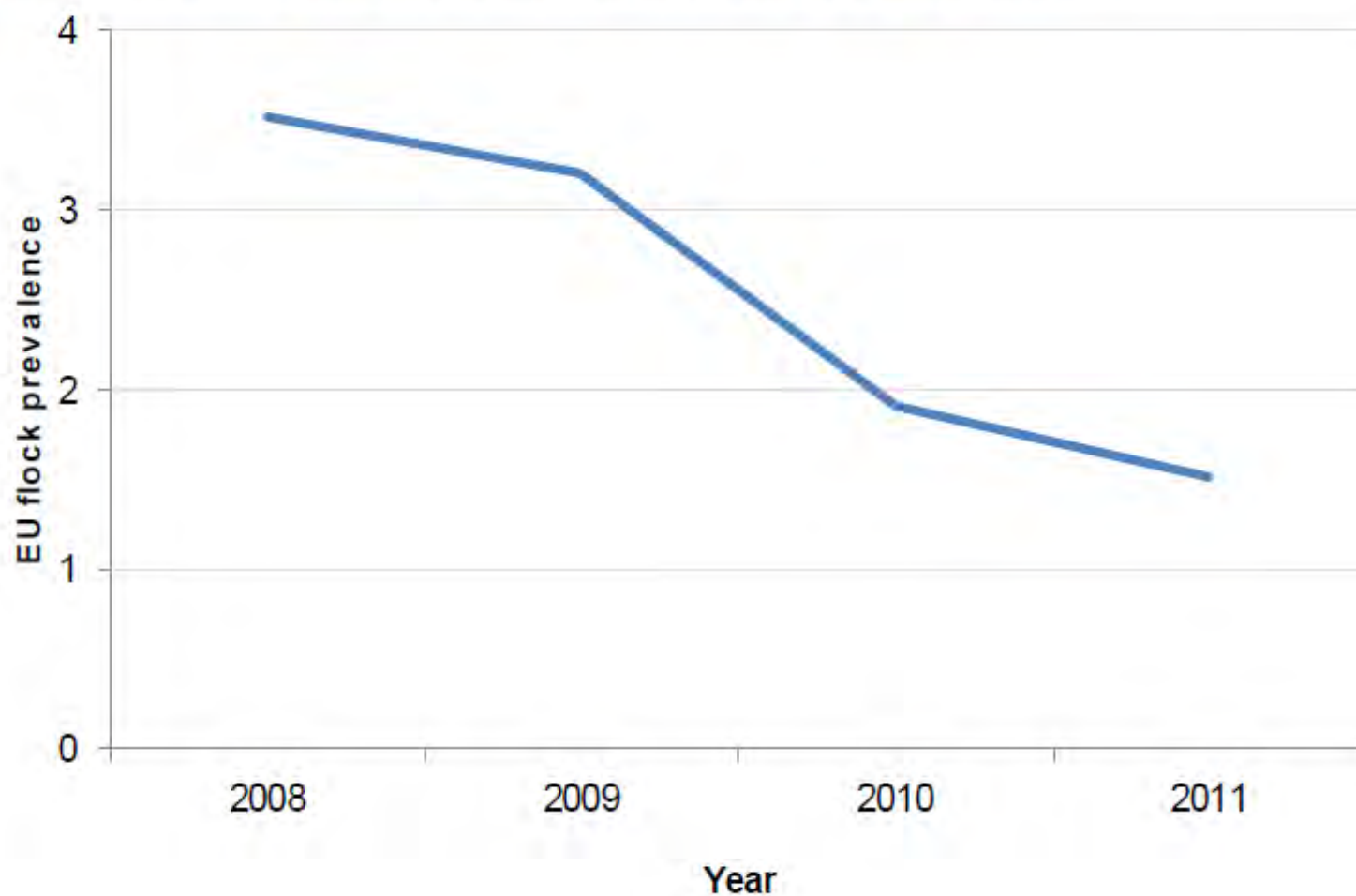


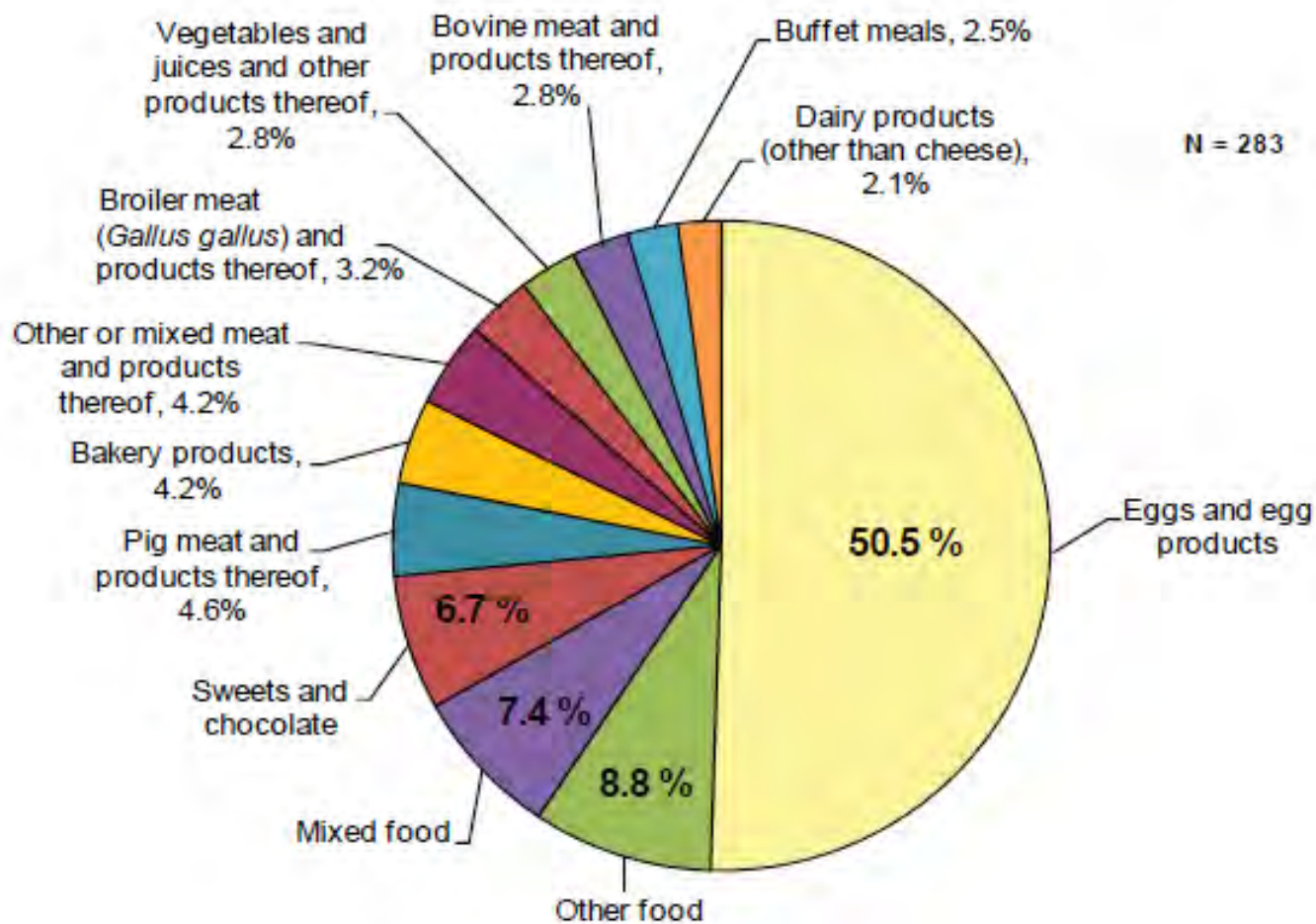


Figure SA15. Prevalence of *S. Enteritidis* and/or *S. Typhimurium*-positive broiler flocks of *Gallus gallus* before slaughter, 2011

EFSA, ECDC, 2013

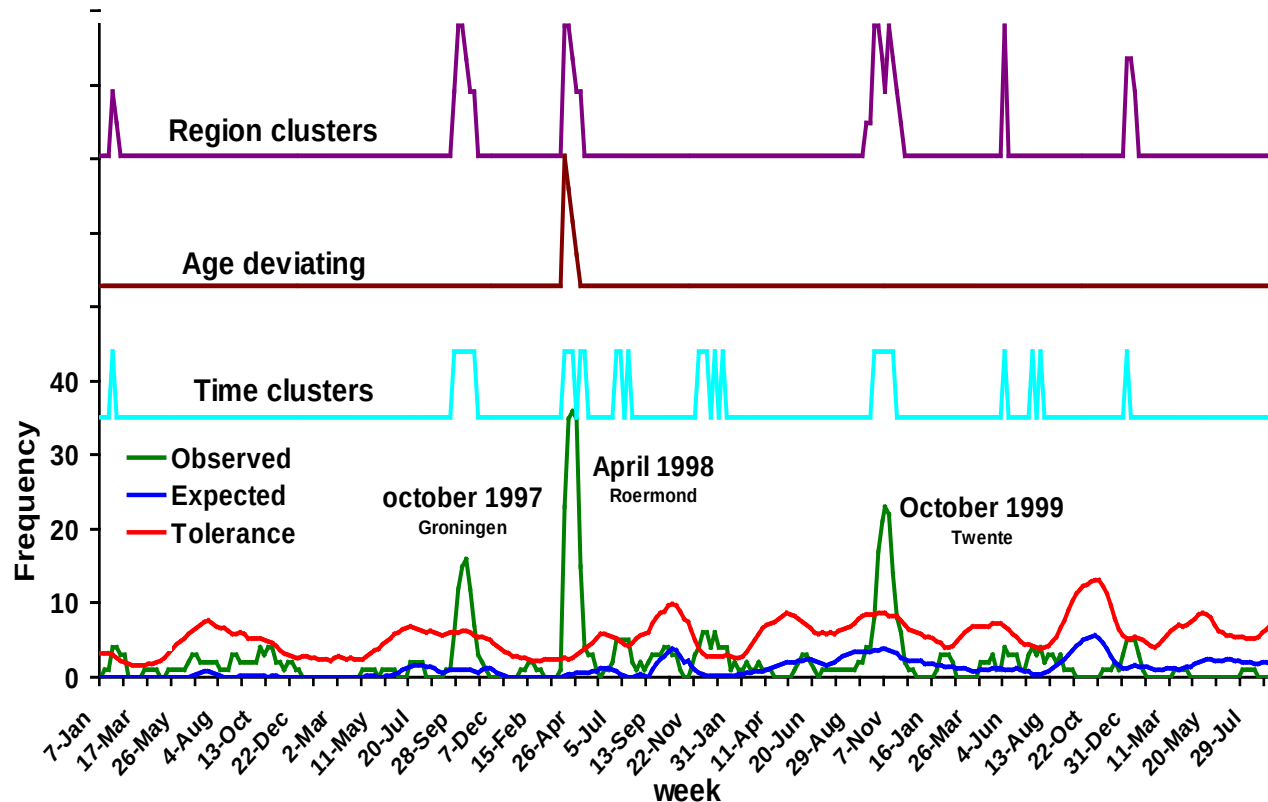


Food vehicles in *Salmonella* outbreaks, EU, 2011



Salmonella - outbreak investigation –

Early Warning System, outbreaks *S. Typhimurium* ft20



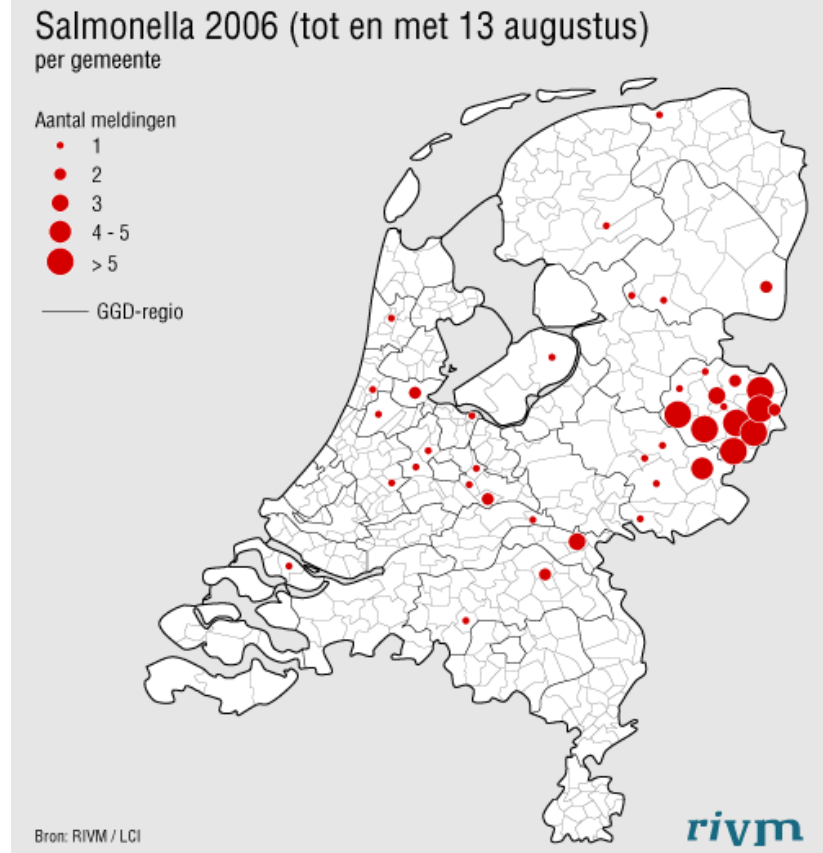
***Salmonella* - outbreak investigation – *Salmonella* in cheese -**

Example Netherlands

- ✓ From January 2006: an increase in *Salmonella* Typhimurium pt DT7
- ✓ A rare phage type, both in the Netherlands and in Europe (according to the international surveillance network Enternet)
- ✓ In 2006: > 200 laboratory-confirmed cases

***Salmonella* - outbreak investigation – - *Salmonella* in cheese -**

- STM DT7 patient isolates originate from one region
- STM DT7 in pork product from supermarket in the region and in cattle manure from farm X in the same region
- Alert to Municipal Public Health Service → questionnaire



***Salmonella* - outbreak investigation – *Salmonella* in cheese -**

➤ Questionnaire:

Farm X, a dairy farm with open access for the public, located in the epicentre of the outbreak; daily production of 500 non-pasteurized hard cheeses



➤ Case definition: Individual with gastroenteritis and a laboratory-confirmed STM DT7 infection with date of arrival of the isolate after 1 June 2006

➤ Case-control study (51 cases and 105 controls):

Cheese purchased from a farm, especially from dairy farm X and from a market stall, were associated with infection with STM DT7

***Salmonella* - outbreak investigation – *Salmonella* in cheese -**

Laboratory analysis of samples from farm X:



- March 2006: manure from a dairy cow with diarrhoea: positive
- April and July 2006: several 25-g samples of cheeses: negative
- August 2006: STM DT7 detected in the drain and from a cheese transport vehicle, but not from cheeses and cattle feed
- October 2006: from 8 types of cheese produced by farm X, 3 were positive; from an additional sampling of 10 cheeses 2 were positive

Enumeration using MPN-technique: 4.2 *Salmonella* / kg

***Salmonella* - outbreak investigation – *Salmonella* in cheese -**

Conclusions:

Cheese contaminated by STM DT7 caused salmonellosis affecting more than 200 persons. No new patients after destruction of contaminated cheeses and measures taken at the production farm

An intensive sampling scheme is especially important for suspicious foods with a high fat content and mild acidic pH; *Salmonella* in these matrices can easily pass the barrier of gastric acid in the stomach

- The integration of human, veterinary and food data into a common *Salmonella* database is crucial for early warning and source identification

***Salmonella* outbreaks – unexpected (?) vehicles**

Salmonella Thompson in smoked salmon (products)

September-October 2012; 950 confirmed cases; 200 hospitalized; 3 died

Netherlands and several other countries

Case definition: Persons in the Netherlands with *S. Thompson* cultured from any sample type since August 2012

source: Company A (NL), contaminated production line in Greece



***Salmonella* outbreaks – unexpected (?) vehicles**

Salmonella Bredeney in peanut butter

June – September 2012

USA, 41 persons ill in 20 states

61% of ill persons were children < 10 years

Case definition: person with confirmed *S. Bredeney* infection from 1 May 2012

Suspected: Creamy Salted Peanut Butter



***Salmonella* outbreaks – unexpected (?) vehicles**

Salmonella Braenderup in mangoes

September 2012

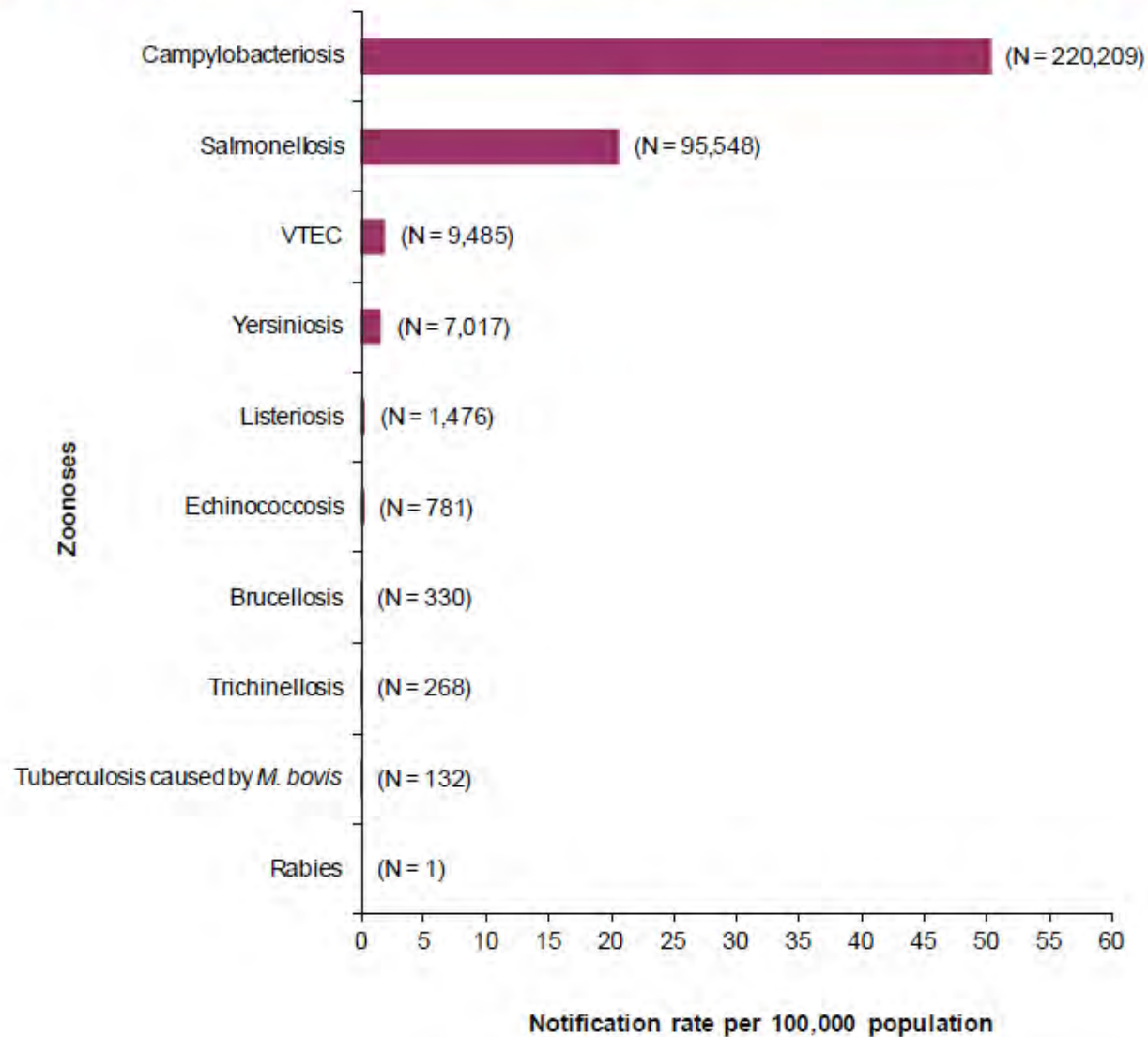
USA and Canada, 143 persons ill

Suspected: fresh-cut mangoes from Mexico





Figure SU1. Reported notification rates of zoonoses in confirmed human cases in the EU, 2011



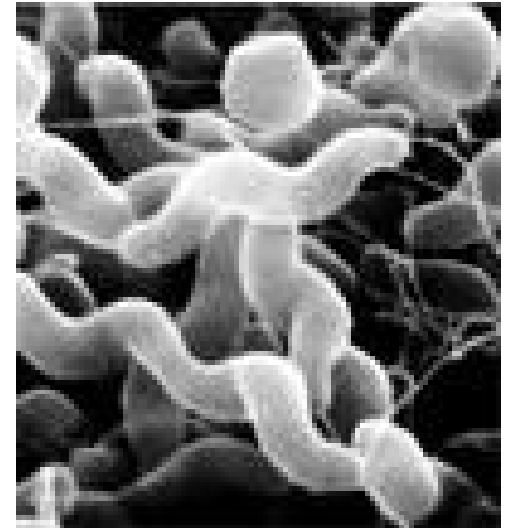
Campylobacter

Characteristics (organism)

thermotolerant species:

C. jejuni, *C. coli*, *C. upsaliensis*, *C. lari*

- Gram-negative spiral rods
- growth at 42°C, not at 25°C
- growth micro-aerobically (5% oxygen)
- heat-sensitive (killed > 48°C)
- no growth in foods
- present in gastrointestinal tract of animals/birds



Campylobacter

Campylobacter infections – characteristics

- symptoms of *Campylobacter* infection: diarrhoea, malaise, fever, abdominal pain; self-limiting; complications: arthritis, Guillain-Barré syndrome
- low infective dose (500 – 10.000 cells)
- incubation period: 1 - 7 days
- mostly sporadic infections

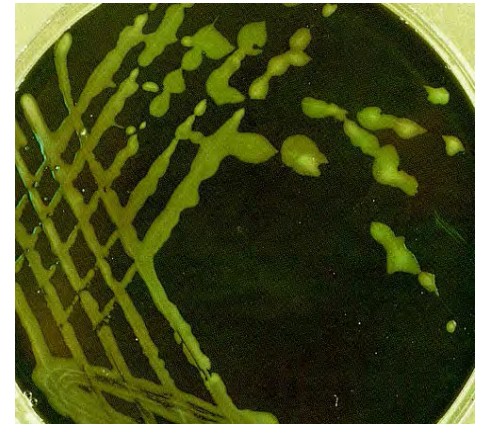
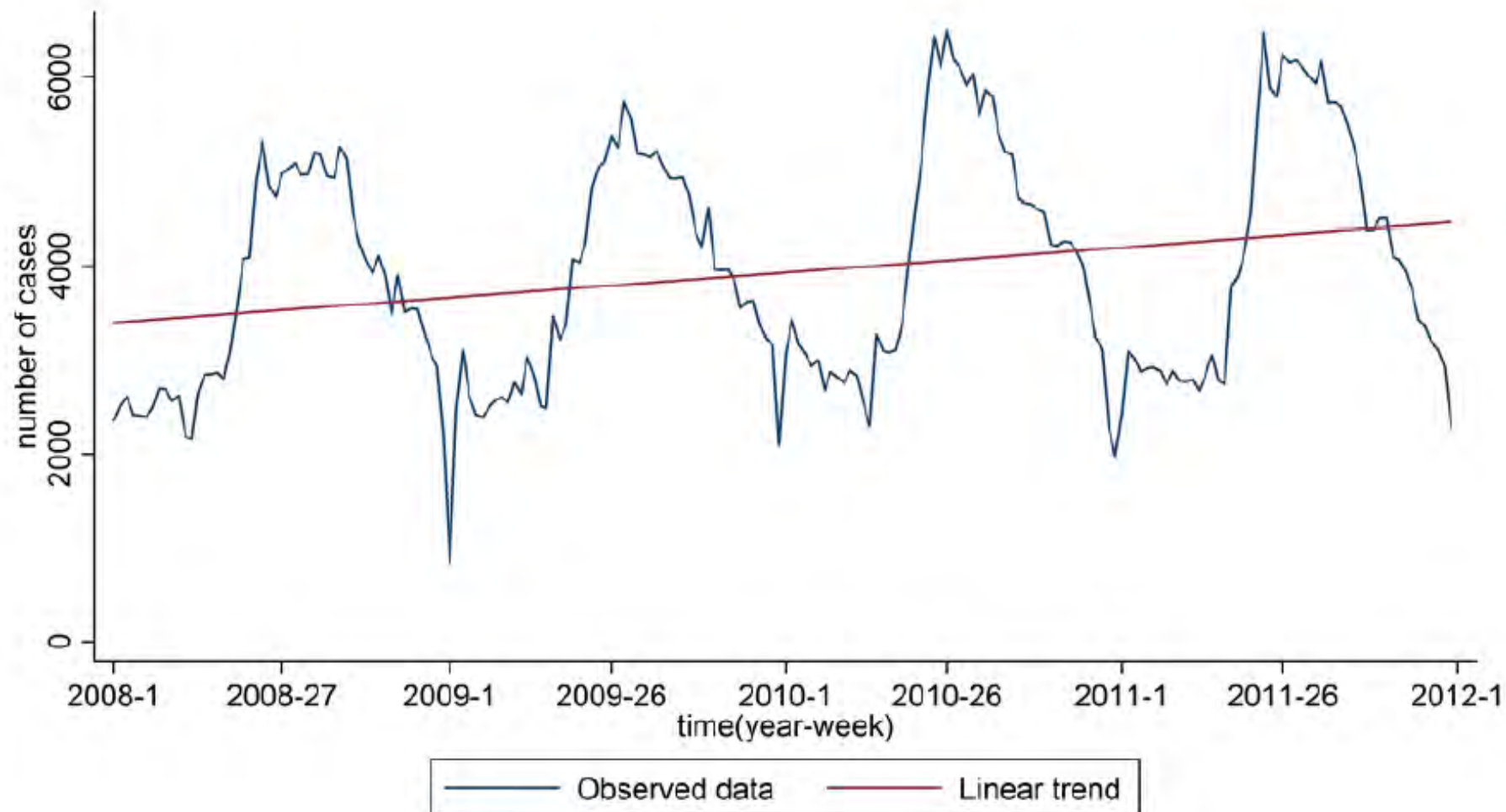




Figure CA1. Trend in reported confirmed cases of human campylobacteriosis in the EU, 2008-2011





**Prevalence (%) of *Campylobacter* spp.
contaminated broiler carcasses**

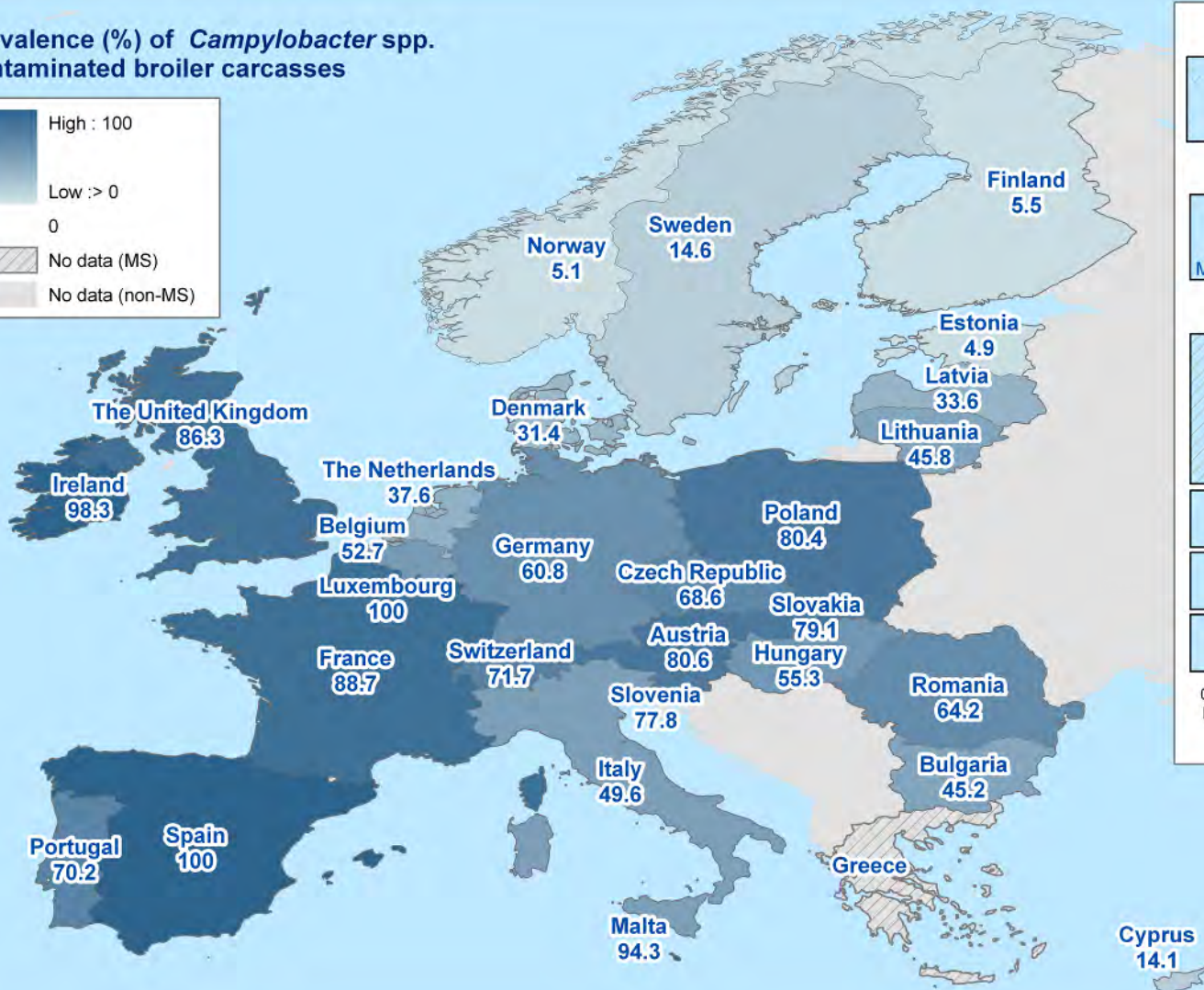
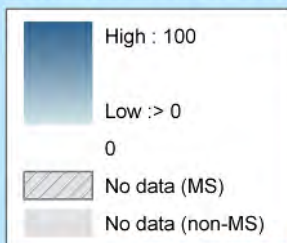
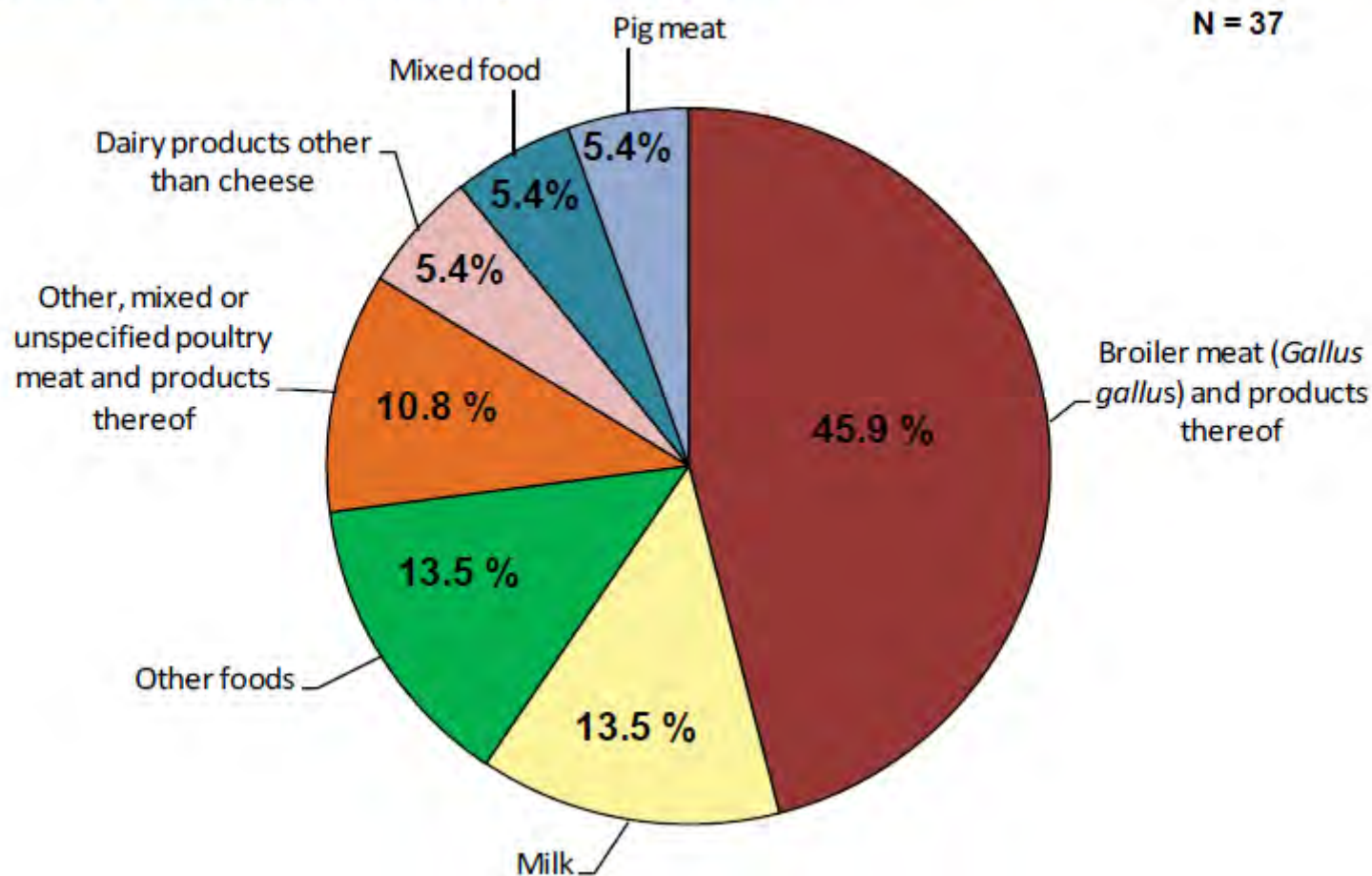




Figure OUT16. Distribution of food vehicles in strong evidence *Campylobacter* outbreaks (excluding strong evidence waterborne outbreaks) in the EU, 2011



***Campylobacter* - outbreak investigation – - *Campylobacter* in raw milk -**

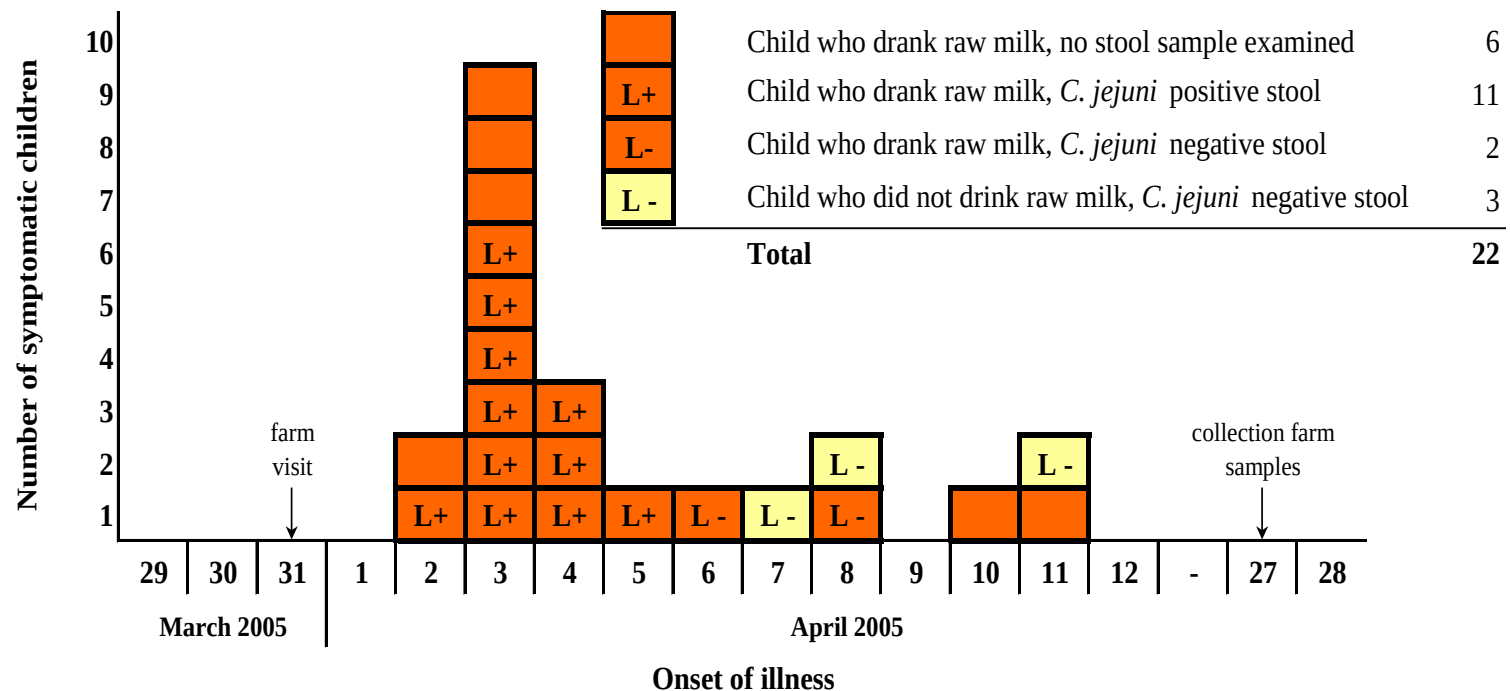
Example of outbreak in NL

Consultation of general practitioner by 9-year old boy with severe diarrhoea; isolation of *Campylobacter* from faeces

- General practitioner informs Municipal Health Service, as more children had developed diarrhoea; the children had been on a school trip to a dairy farm
- The Municipal Health Service notified the Food Safety Authority
- 22 / 34 school children ill
- symptoms: diarrhoea, fever, abdominal cramps, general feeling of illness

Campylobacter - outbreak investigation - - *Campylobacter* in raw milk -

The epidemic curve showing the number of symptomatic children by day of illness onset and laboratory result:



***Campylobacter* - outbreak investigation – - *Campylobacter* in raw milk -**

Farm investigation:

	Faeces dairy cattle (n=10)	Tank milk	Milk filter
# samples <i>Campylobacter</i> -positive	6	0	0

***Campylobacter* - outbreak investigation – - *Campylobacter* in raw milk -**

Characterisation of isolates:

	PFGE (<i>Sma</i>) pattern	flaA PCR-RFLP pattern	
Patients' isolates (n=11)	A	A	⇒ Common source
Dairy cattle faeces			
I <i>C. jejuni</i>	A	A	⇒ Infection source: dairy cattle
II <i>C. jejuni</i>	B	B	
III <i>C. jejuni</i>	C	C	
IV <i>Campylobacter</i> spp.	D	- ^a	
V <i>Campylobacter</i> spp	E	-	
VI <i>Campylobacter</i> spp	F	-	

^a PCR negative

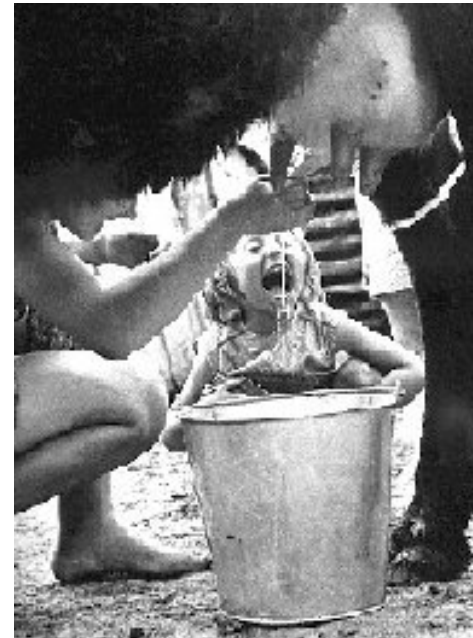
***Campylobacter* - outbreak investigation – - *Campylobacter* in raw milk -**

- Epidemiological investigation ➡ raw milk

86% of the cases could be explained by drinking raw milk

- Microbiological investigation ➡ raw milk

no discrimination between patients' isolates and isolates from dairy cattle by both PFGE and flaA PCR-RFLP



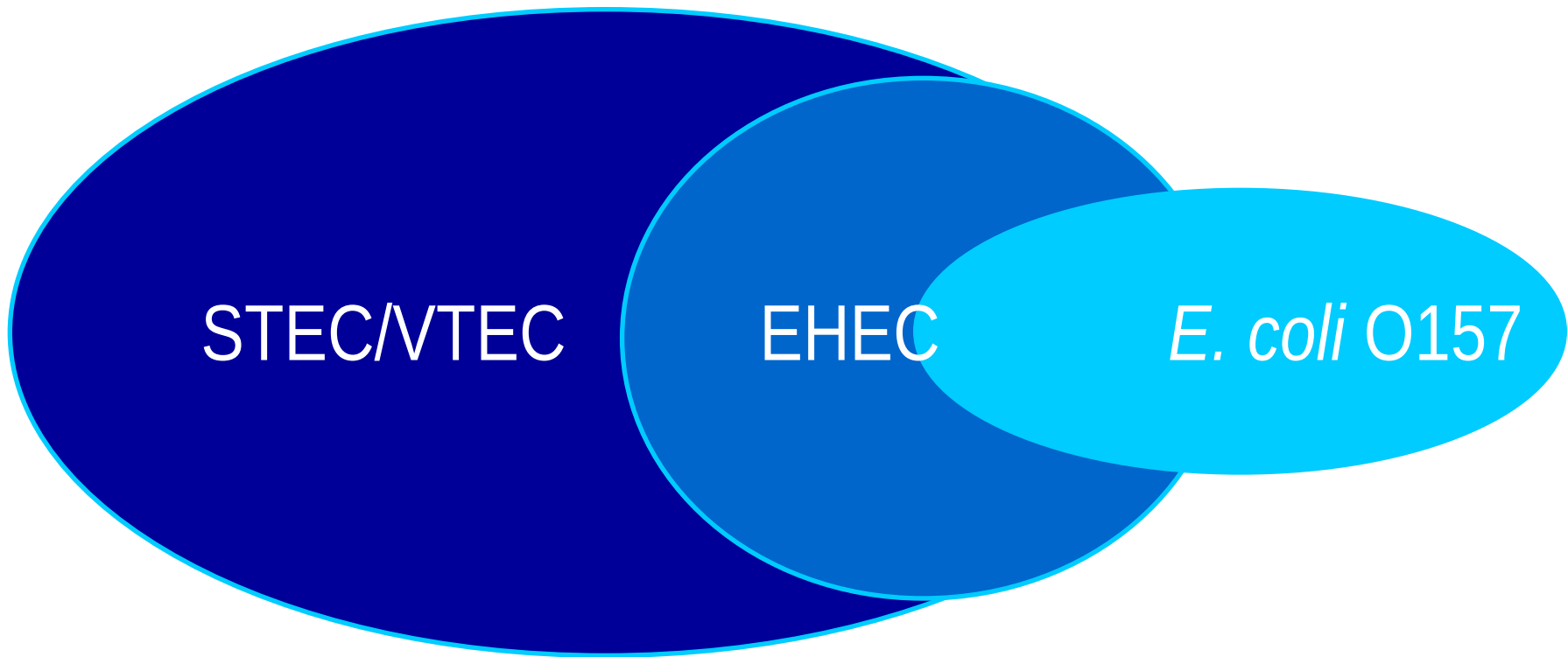
Escherichia coli

pathogenic *Escherichia coli*

- Enteropathogenic *E. coli* (EPEC) - infant diarrhoea
- Enteroinvasive *E. coli* (EIEC) - dysentery-like diarrhoea
- Enterotoxigenic *E. coli* (ETEC) - traveller's diarrhoea
- Enterohemorrhagic *E. coli* (EHEC) - bloody diarrhoea
- Enteroaggregative *E. coli* (EAggEC) – persistent diarrhoea
- Diffuse Adherent *E. coli* (DAEC) – diarrhoea
- Enteroaggregative hemorrhagic *E. coli* (EAHEC) – O104:H4



Shiga toxin-producing *Escherichia coli*



***E. coli* O157 - characteristics**

symptoms: 3-8 days after infection:

- diarrhoea, often after 1-3 d bloody
- 2-7% Haemolytic uremic syndrome (HUS), till 14 d after diarrhea (children up to 15%, also higher among elderly)
- 2-9% of the HUS patients die, children being more at risk (dehydration, sepsis); 25% chronic renal failure

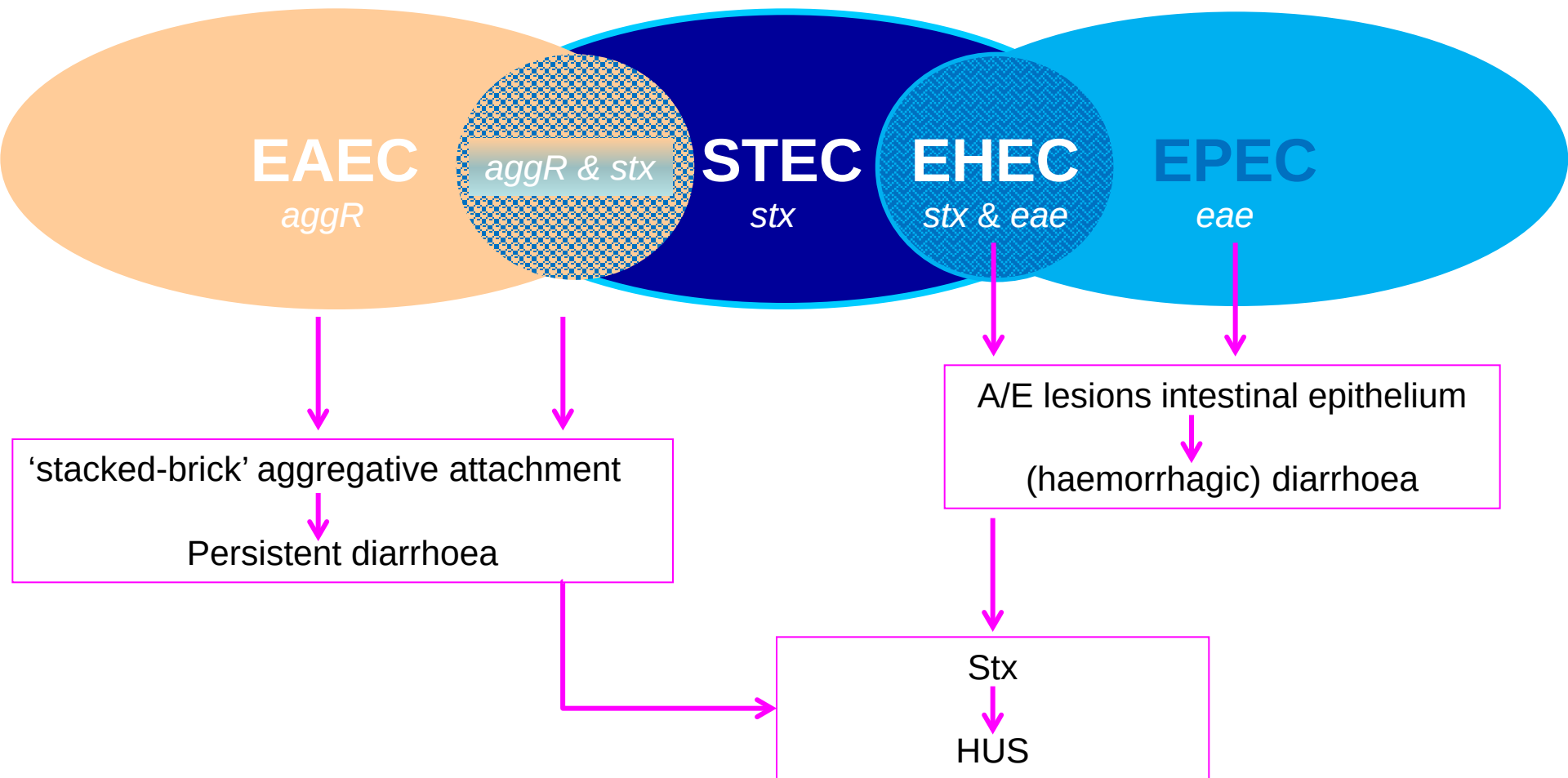
Age: < 5 years, > 60 years

Season: summer and (early) autumn

Infectious dose: ~ 10 - 200 bacteria

Transmission: food, contact with animals, contaminated water
person-to-person (faeces-oral)

STEC – EHEC – EAEC - EPEC



STEC / VTEC

- It is not possible to fully define human pathogenic VTEC or to identify factors for VTEC that absolutely predict the potential to cause human disease. There is no single or combination of marker(s) that defines a „pathogenic“ VTEC
- The detection of verocytotoxins alone, or of genes encoding for such verocytotoxins is not a sound scientific basis for assessing the disease risk to the consumer
- Strains positive for verocytotoxin 2 gene (*vtx2*)- and *eae* (intimin production)- or [*aaIC* (secreted protein of EAEC) plus *aggR* (plasmid-encoded regulator)] genes are associated with a higher risk of more severe illness than other virulence gene combinations. Other virulence gene combinations and/or serotypes may also be associated with severe disease in humans, including HUS

Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment (EFSA, 2013)

STEC / VTEC

Table 14: Proposed^(a) molecular approach for the categorisation of VTEC (*vtx* present)

Group	Genes ^(b)	Serogroups	Potential risk ^(c)	
			Diarrhoea	HUS/HC ^(d)
I	<i>eae</i> -positive or (<i>aaiC</i> and <i>aggR</i>)-positive	O157, O26, O103, O145, O111, O104	High	High
II	<i>eae</i> -positive or (<i>aaiC</i> and <i>aggR</i>)-positive	Any other	High	Unknown
III	<i>eae</i> -negative and (<i>aaiC</i> plus <i>aggR</i>)-negative	Any other	Unknown	Unknown

(a): As yet this proposed molecular approach must be regarded as provisional. This is because screening VTEC for the presence of *eae*, *aaiC* and *aggR* genes is not routinely undertaken by all laboratories reporting data to TESSy.

(b): Additional to the presence of *vtx* genes. *eae* = intimin-coding gene, *aaiC* = chromosomally-encoded gene encoding secreted protein of EAEC, *aggR* = plasmid-encoded regulator gene.

(c): Needs epidemiological studies for confirmation.

(d): HUS = haemolytic uraemic syndrome, HC = haemorrhagic colitis.

Selected STEC O157 outbreaks

<i>Year</i>	<i>Country</i>	<i>Cases</i>	<i>Source</i>	<i>Reference</i>
1982	USA (OR & MI)	47	Beef	Riley, 1982
1992	USA (multistate)	>500	Hamburger	CDC, 1993
1996	Japan	9451	Radish sprouts	Michino, 1999
2005	The Netherlands	21	Filet americain	Doorduyn, 2006
2006	USA (26 states)	183	Fresh spinach	Wendel, 2009
2007	The Netherlands Iceland	50	Lettuce	Friesema, 2008
2009	USA (multistate)	77	Cookie dough	Neil, 2012
2010	USA (multistate)	38	Raw milk hard cheese	CDC, 2010
2011	USA (multistate)	8	In-shell hazelnuts	CDC, 2011

Selected STEC non-O157 outbreaks – red meats

<i>Year</i>	<i>Serotype(s)</i>	<i>Country</i>	<i>Cases</i>	<i>Source</i>	<i>Reference</i>
1992	O111:NM	Italy	9	Ground beef	Caprioli, 1994
1995	O111:H-	Australia	161	“Mettwurst”	CDC, 1995
2000	O26:H11	Germany	6	“Seemerolle” cut of beef	Werber, 2002
2002	O148:H8	France	10	Mutton	Espié, 2006
2006	O103:H25	Norway	17	Lamb sausage	Sekse, 2009
2007	O26:H11	Denmark	20	Beef sausage	Ethelberg, 2009
2009	O123:H-	France	2	Ground beef burger	King, 2010
2010	O26	USA	3	Ground beef	FSIS, 2011

Selected STEC non-O157 outbreaks – other foods

<i>Year</i>	<i>Serotype(s)</i>	<i>Country</i>	<i>Cases</i>	<i>Source</i>	<i>Reference</i>
1994	O104:H21	USA (MT)	18	Milk	CDC, 1995
1999	O111:H8	USA (TX)	56	Salad bar	CDC, 2000
2000	O103	USA WA)	18	Punch	Brooks, 2005
2004	O111	USA (NY)	212	Apple cider	Bopp, 2008
2006	O121:H9	USA (UT)	73	Iceberg lettuce	Berger, 2006
2007	O145:H28 O26:H11	Belgium	14	Ice cream	De Schrijver, 2008
2011	O104:H4	Germany Other EU Non EU	3,842 125 12	Fenugreek sprouts	RKI, 2011



Figure VT1. Trend in reported confirmed cases VTEC infections in humans in the EU 2008–2011 (top) and 2008–2010 (bottom)

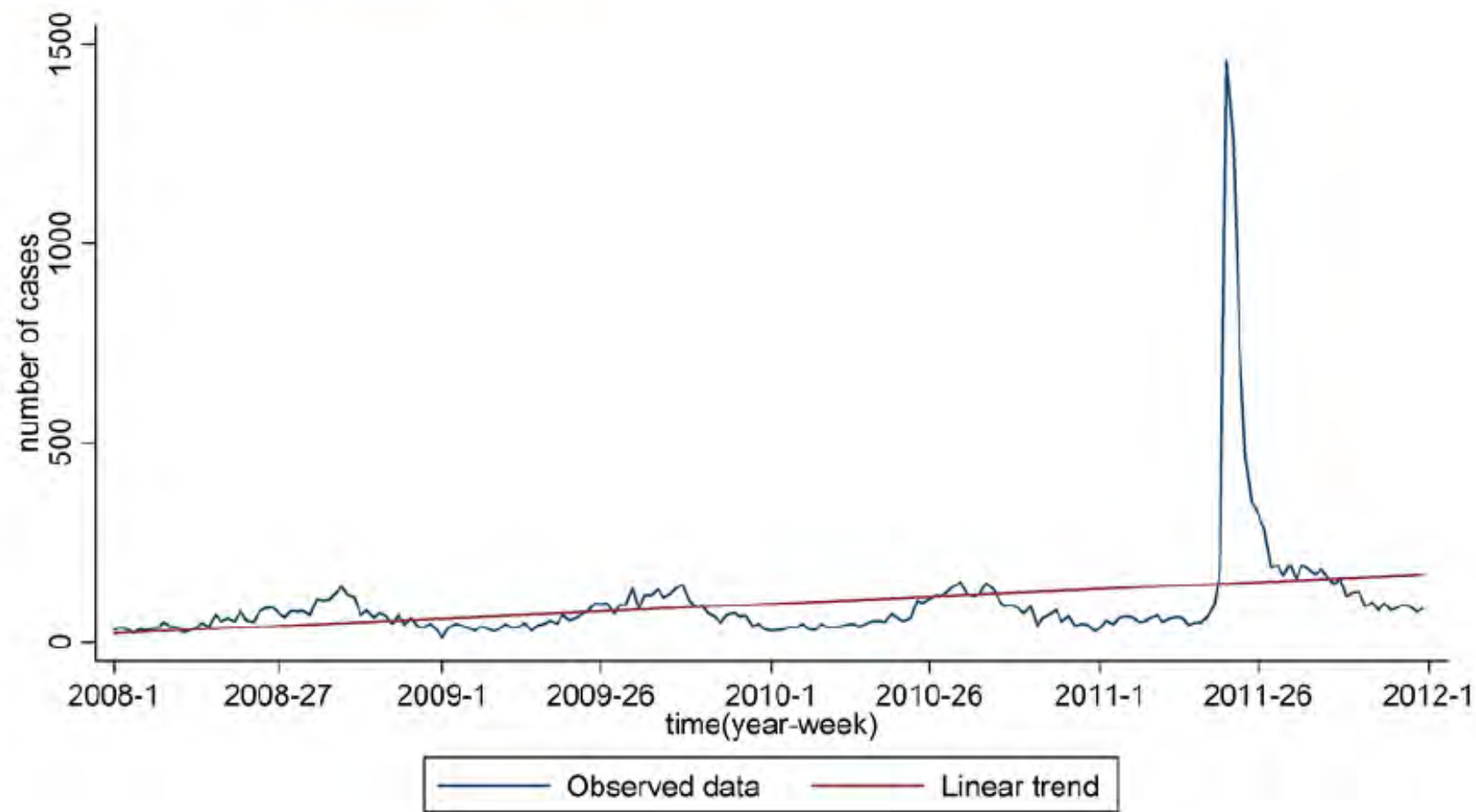
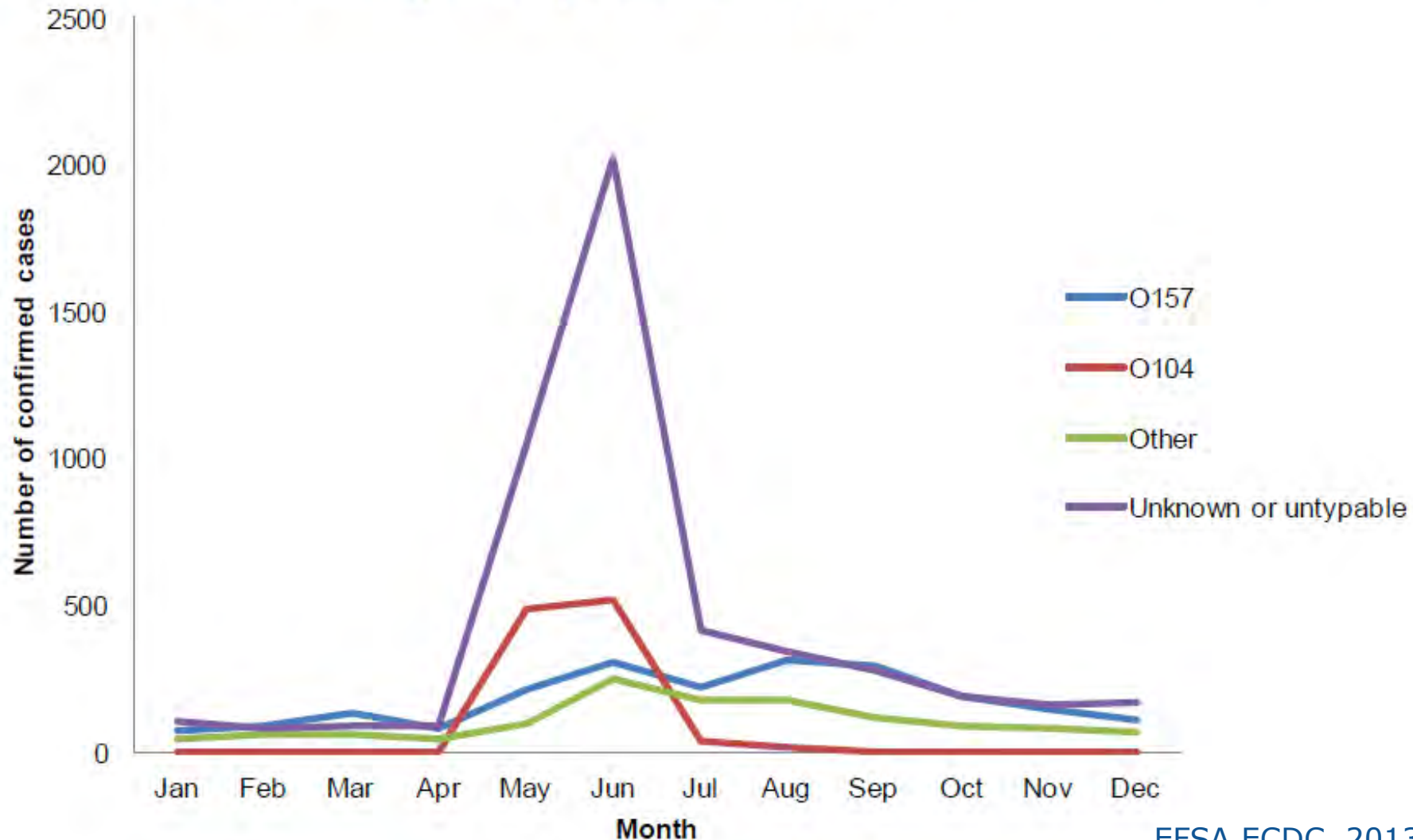




Figure VT3. Number of reported confirmed cases of VTEC infection in humans for serogroups O157, O104, other and unknown/untypeable, by month, in the EU, 2011



Outbreak investigation - EHEC O104:H4

21 May 2011 – Germany reports ongoing outbreak of STEC O104:H4

Cases with diarrhoea: 4000

Cases of HUS: 855

Deaths: 53



Characterisitics EHEC O104:H4

Virulence gene	<i>E. coli</i> Pathotype	present
AggR	EAggEC	+
ST-b	ETEC	-
LT-A	ETEC	-
ipaH	EIEC	-
hlyA	EHEC/STEC	-
eae	EPEC/EHEC	-
stx1	STEC/EHEC	-
stx2	STEC/EHEC	+
O104wzx	serotype O104	+
ESBL production		+

Natural reservoir for EAHEC O104:H4 is humans in contrast to STEC and EHEC, which have animal reservoirs

Outbreak investigation - EHEC O104:H4

Initial case control study indicates that the outbreak was associated with consumption of fresh salad vegetables, with a high proportion of adult women among the ill

Later, a detailed cohort study demonstrated an association with sprouted seeds



Outbreak investigation - EHEC O104:H4

Further epidemiological investigations revealed an association with consumption of sprouted seed mixtures, containing lentil and fenugreek sprouts

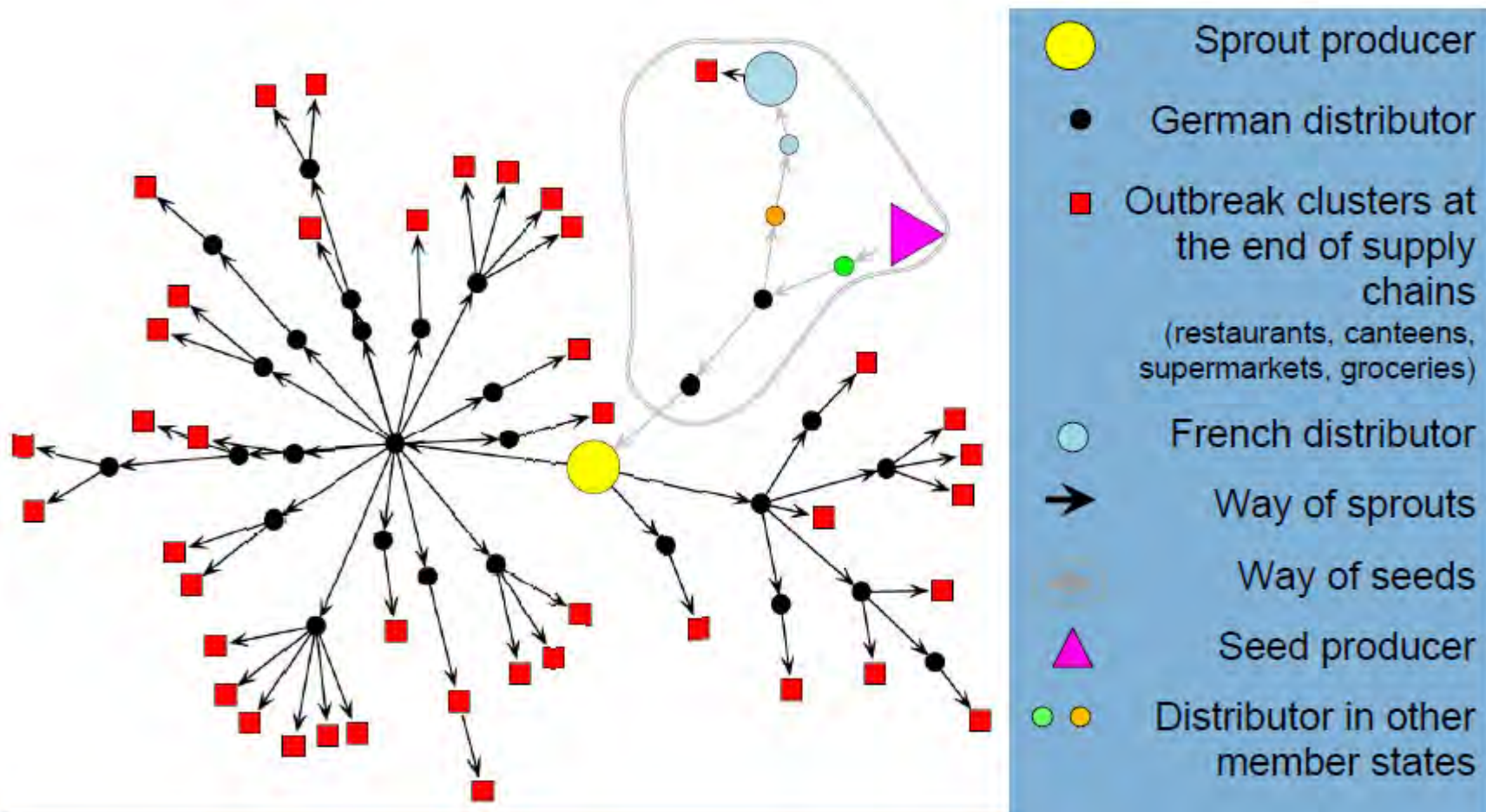
In a French outbreak of STEC O104:H4 (reported on 24 June 2011), without any link to the German outbreak, there was a strong association with the consumption of sprouted seeds (fenugreek, rocket, white mustard).

A specific lot of fenugreek seeds imported from Egypt was the most likely link between the German and French outbreaks



Outbreak investigation - EHEC O104:H4

Sprouts - results of the trace back



Outbreak investigation - EHEC O104:H4

None of the food samples, for which cross-contamination (from infected persons) could be excluded, was found positive for STEC O104:H4

Reported sample investigations (RASFF 11-658-add17), 30 August 2011: Total # samples: 10392



Outbreak investigation - EHEC O104:H4

Lessons learnt

- Lack of timely recognition and communication of increase in human cases with STEC infection and HUS
Serotyping of isolates from HUS patients should have been done earlier
- Timely recognition of a new pathogen with unexpected mix of virulence factors
- Detection methods were made available by EURL E. coli, but were not ready for use in some countries



Outbreak investigation - EHEC O104:H4

Lessons learnt

- Difficulty of identifying the causative food product
A special product and many patients did not remember the consumption of this product
- Traceability of the seeds was not easy
The safe production of sprouts seems to be difficult
- Sampling schemes for large lots of seeds were not available



Listeria monocytogenes

L. monocytogenes is widespread in the environment and consequently raw food products may harbour L.m. in low numbers and processed foods may be contaminated from the production environment



Findings of L.m. in foodstuffs are important in two main scenarios:

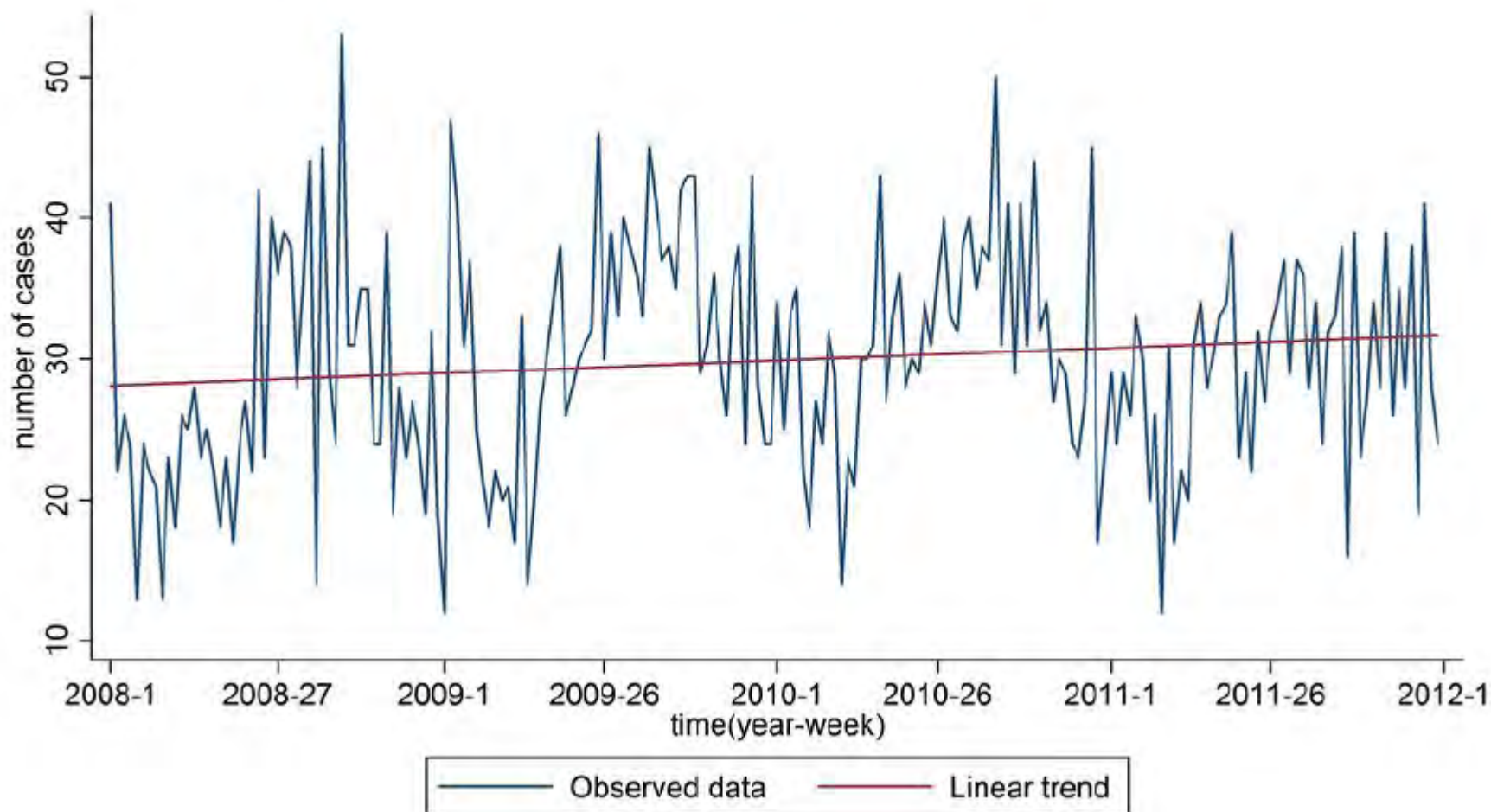
- Presence of L.m. in foods that are ready-to-eat (RTE) and able to support the growth of L.m.
- Consumption of foodstuff, which contains > 100 cfu/g of L.m. is regarded as a direct risk for human health

Listeria monocytogenes

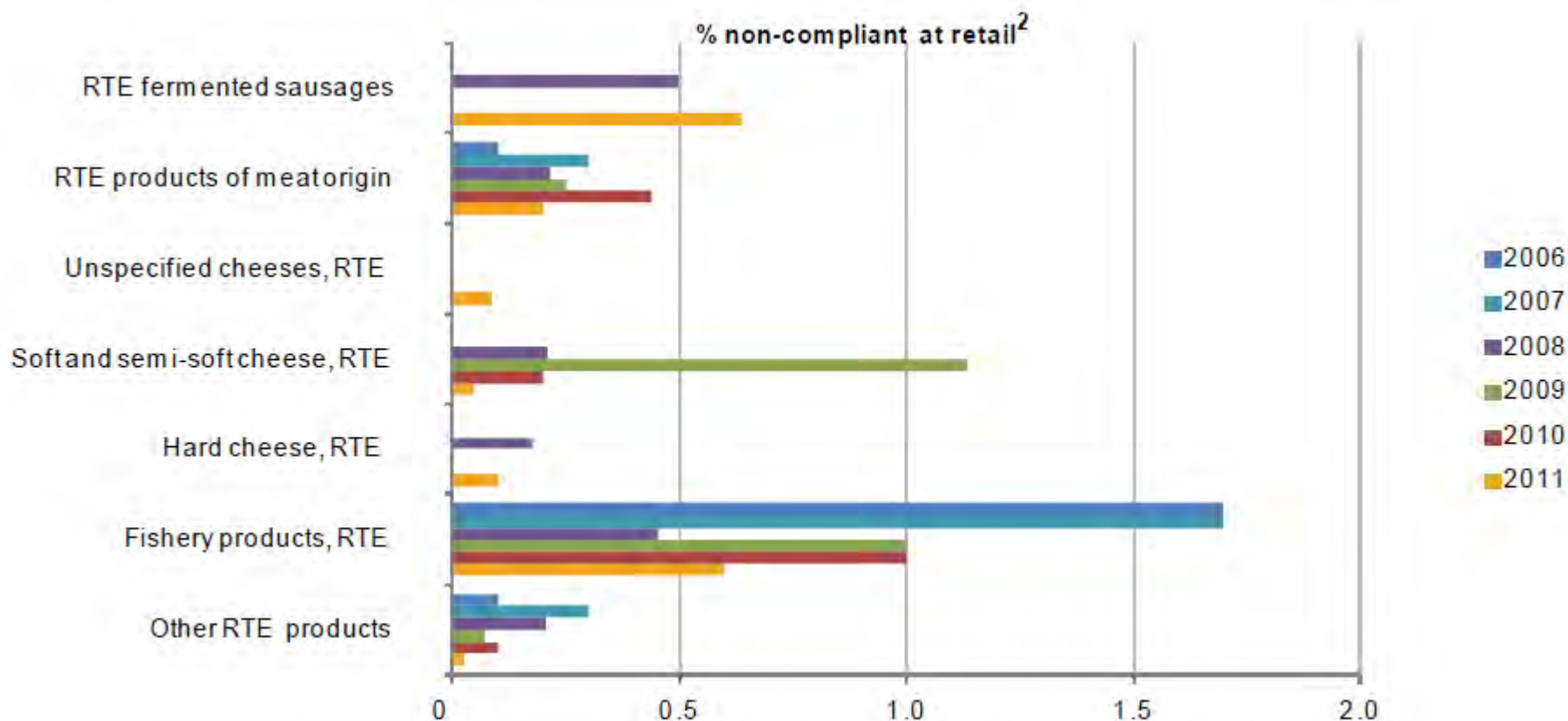
- Human disease cases are rare (3-4 cases per million inhabitants), but are important because of the severity of the disease
- symptoms: septicemia, meningitis, encephalitis, cervical infections in pregnant women may result in spontaneous abortion or stillbirth, gastro-enteritis
- Listeriosis is one of the most important causes of death from foodborne infection in industrialised countries
- Outbreaks are not easy to detect as patients are often not geographically clustered and the incubation period is long



Figure LI1. Trend in reported confirmed cases of human listeriosis in the EU, 2008-2011



Listeria monocytogens in foods at retail



***Listeria monocytogenes* - outbreak investigation**

Example from Belgium

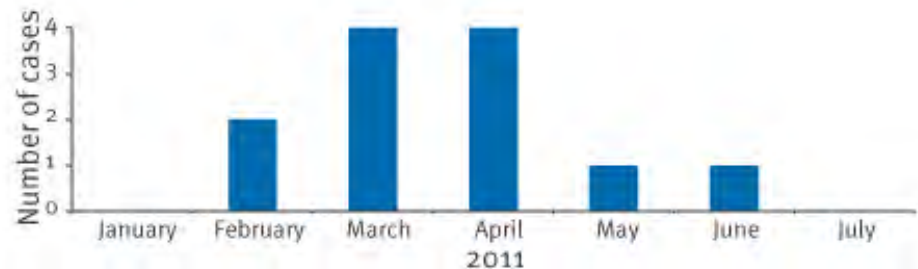
A cluster of time-linked cases and the identification of a clonal strain (*L. monocytogenes* 1/2a) suggest an outbreak of listeriosis in Belgium in 2011.

Suspect food: hard cheese made with pasteurized milk

Exchange of data between Belgium and France

FIGURE 2

Cases of listeriosis outbreak by month of diagnosis in the Belgian National Reference Centre for Listeria, Belgium, 2011 (n=12)



***Listeria monocytogenes* - outbreak investigation**

Example

Cantaloupes – United States, 28 States, August-October 2011

- 147 cases, 33 deaths, 1 miscarriage
- 88% of patients were aged >60 years
- Five different subtypes of *L. monocytogenes* have been associated with the outbreak
- Outbreak strains were isolated from whole and cut cantaloupe samples from patients' homes and a Cantaloupe Company
- Contamination through condensation from cooling systems



Source: CDC

Thank you for your attention

