



Business from technology

## **DETECTION OF SALMONELLA IN FOOD - EXPERIENCES AND INNOVATIONS**

**MoniQA 2nd international congress, Krakow**

***Emerging and persisting food hazards:***

***Analytical challenges and socio-economic impact***

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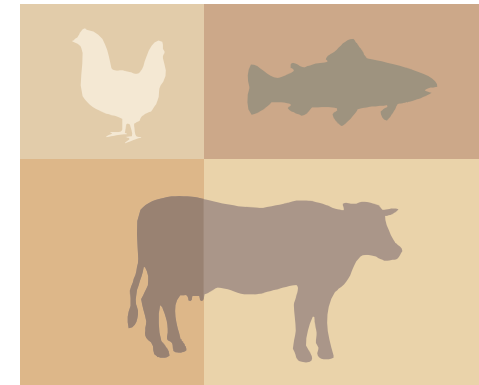
**9.6.2010**

## Background

- *Salmonella*, a genus within Enterobacteriaceae, an important human pathogen.
- Salmonellosis has been reported to be the most common food-borne bacterial disease in the world. In the United States it has been estimated that 1.4 million non-typhoidal *Salmonella* infections with 400 deaths occur annually.
- Especially in countries with poor sanitary conditions *Salmonella* causes annually several deaths. Although majority of the *Salmonella* cases are sporadic, outbreaks occur frequently.
- Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12-72 hours after infection. The illness usually lasts from 4 to 7 days.
- Although most people recover without treatment, severe infections may occur. Infants, elderly persons, and those with weakened immune systems are more likely than others to develop severe illness.

## Salmonella in foods

- *Salmonella* can be associated with many kinds of foods and water.
- Contamination of meat (cattle, pigs, poultry) may originate from animal salmonellosis, but most often it results from the contamination of meat with intestinal contents during evisceration of animals, washing, and transportation of carcasses.
- Likewise, vegetables and fruits may carry *Salmonella* if contaminated with fertilizers of faecal origin, or when washed with polluted water.



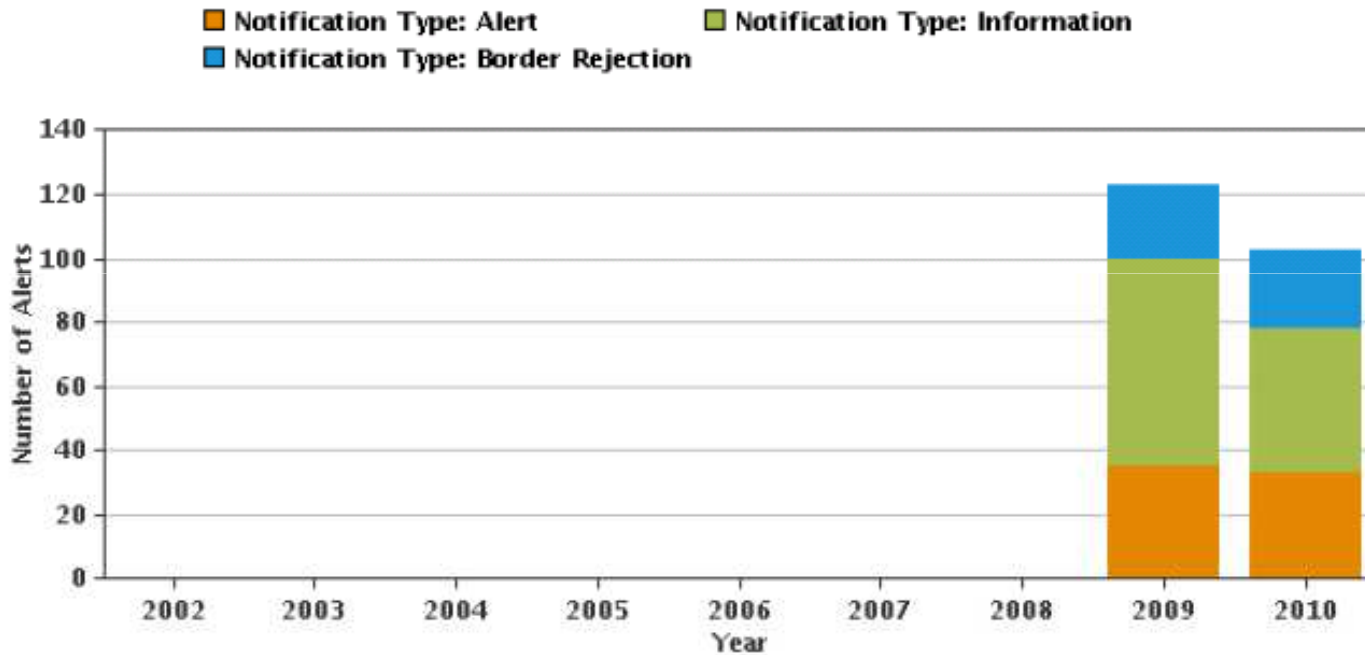
## Salmonella - EU

- EFSA reported in 2008 **131 468 cases** of zoonotic *Salmonella* infections in humans. There was a decrease for the fifth consecutive year (151 998 cases in 2007). *Salmonella* remained the second most frequent cause of food borne outbreaks. It is generally agreed, however, that the number of human cases are seriously underestimated and also underreported.
- *Salmonella* was found most frequently in raw chicken, turkey and pig meat. In EU the most important serovars in humans were *S. Enteritidis*, *S. Typhimurium* and *S. Infantis*.
- Together these three serovars accounted for up to 81% of the human *Salmonella* cases in the period 2005 to 2008, with *S. Enteritidis* being responsible about 60% of cases.
- EU outbreak data also estimated that eggs were the most important source of human salmonellosis in EU countries, and that the majority of *S. Enteritidis* cases was attributed to egg consumption (poultry reservoir).
- In EU 10-20% of human *S. Typhimurium* infections are guessed to be coming from the pig reservoir.



## Salmonella - RASFF alerts in 2010

RASFF alerts by year



For more information see: <http://www.fc24.eu>

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June 6, 2010, 12:25 PM

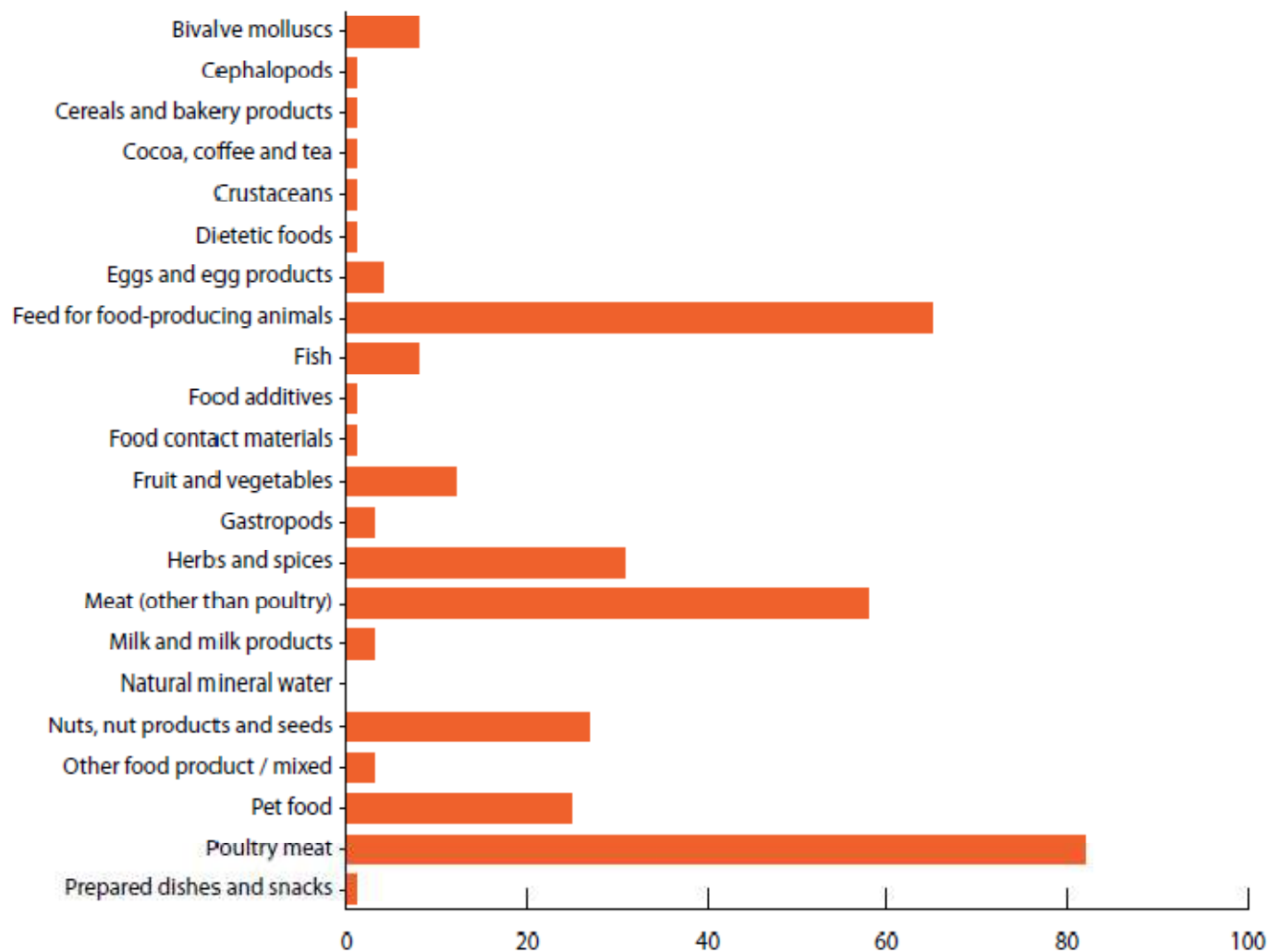
### Examples of alerts in May 2010:

Foodborne outbreak suspected (*Salmonella* Typhimurium) to be caused by dried sausages from France.

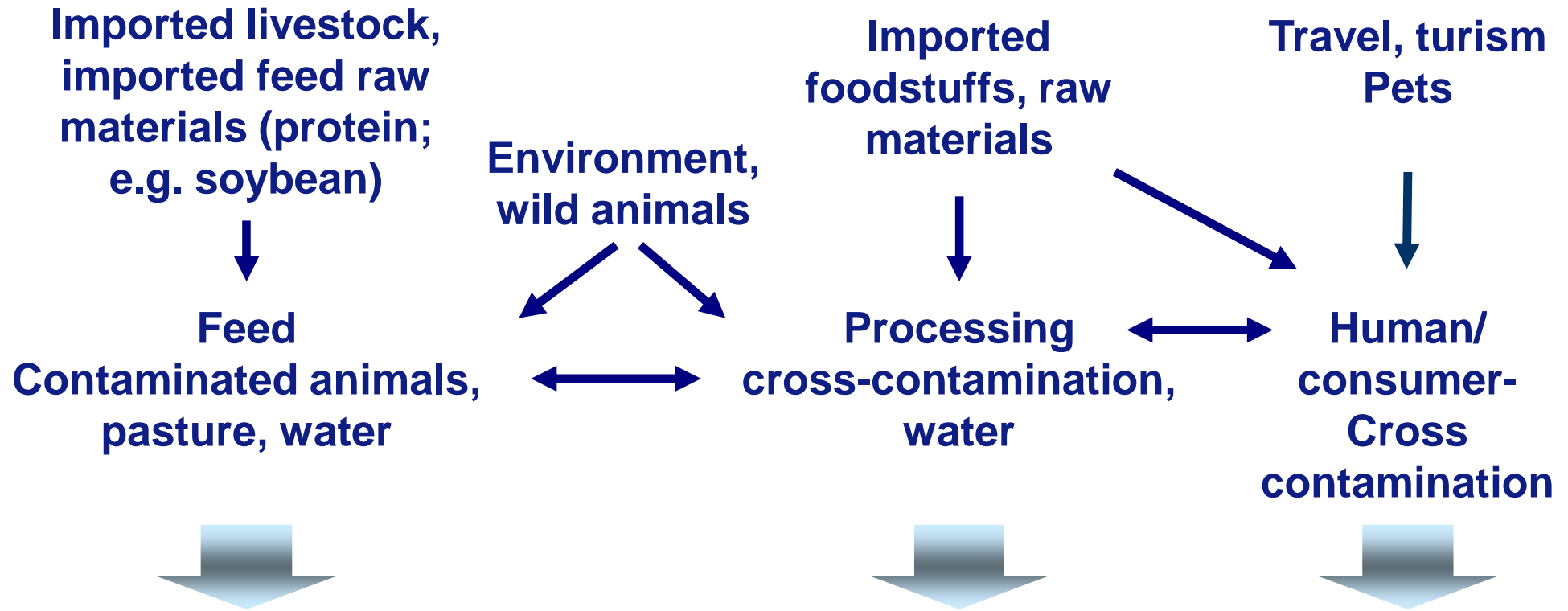
*Salmonella* in frozen chicken breasts from the Netherlands.

*Salmonella* in frozen kebab meat from Germany.

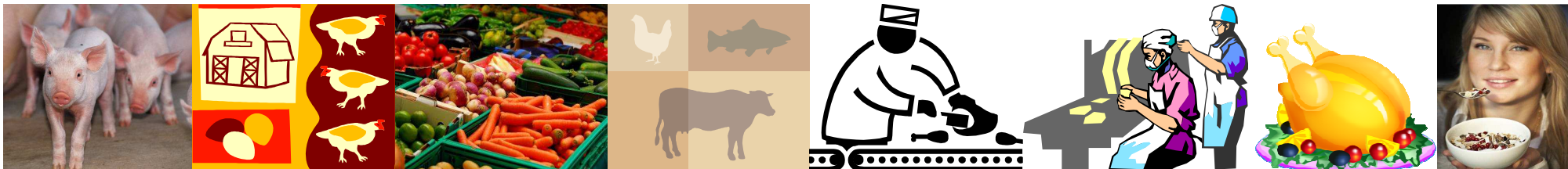
## Notifications of *Salmonella* in RASFF - 2008



## Potential routes of *Salmonella* in the food chain

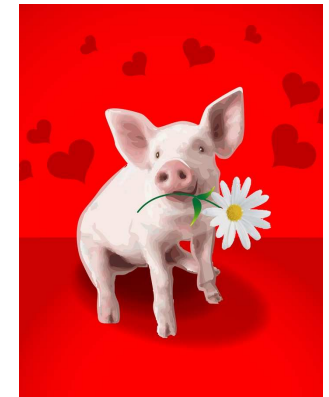


**Livestock**                      **Processing**                      **Human**



## Feed is an important source of *Salmonella*

- A recent study (Wierup and Häggblom, 2010) found that out of the 38 serovars which were isolated from vegetable feed ingredients (28) and from feed mills (10), 30 had also been isolated from human cases of salmonellosis diagnosed in Sweden 1997-2008.
- In addition, four (10 %) of the serovars isolated from feed were identical to the serovars found among the 10 most common serovars of human cases of salmonellosis in the EU (EFSA, 2009b).
- These data support the previous EFSA statement that all serovars of *Salmonella* are considered as potentially pathogenic to human. In addition, there are other external sources of *Salmonella* introduction (other than birds and rodents) e.g. water, staff, and wildlife) which have to be considered.
- Acid and heat treatment of feed has been reported to have benefits in elimination of *Salmonella*.
- Additional solutions to reduce the prevalence of *Salmonella* in the gut is, e.g. manipulate the gut flora through probiotics, prebiotics and antimicrobials.
- According to the EFSA opinion (2010) “To achieve control of *Salmonella* in slaughter pigs the two major sources should be controlled: ***Salmonella*-infected breeder pig herds, and *Salmonella*-contaminated feed**”.



## The feed borne outbreak of *Salmonella* Tennessee in Finland in the spring of 2009

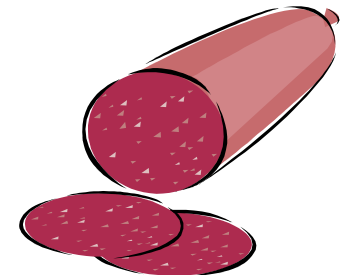
- *Salmonella* Tennessee epidemic caused by **contaminated feed broke up in pig and poultry farms** during the spring 2009. The epidemic was brought under control and no people were affected.
- The Rehuraisio feed mill is the largest in Finland and produce feed on several production lines for ruminants, pigs, poultry and fish.
- From all the pig and poultry farms, which had received feed from the Rehuraisio factory after 12.12.2008 samples were taken for *Salmonella* analysis. Faecal samples were collected from 800 farms (288 poultry farms and 546 pig farms) and feed samples from 600 farms.
- *S. Tennessee* was detected from 30 layer chicken farms and from faecal samples of 10 pig farms.
- According to the legislation Rehuraisio carry out own control of *Salmonella* in raw materials, the feed mill environment and in finished feed. According to Evira, Rehuraisio have reported their *Salmonella* findings in the feed mill environment during the control audits. Occasionally positive samples were detected in the production environment for the last 15 years. *Salmonella* were not detected in any samples of finished feed.
- During the outbreak *Salmonella* contamination was detected from environmental samples and cooler system in the production line.
- Major revision of the production line. Cleaning of the contaminated farms.
- Production line re-opened in Autumn 2009.



## Multistate outbreak of human *Salmonella* Montevideo (USA)

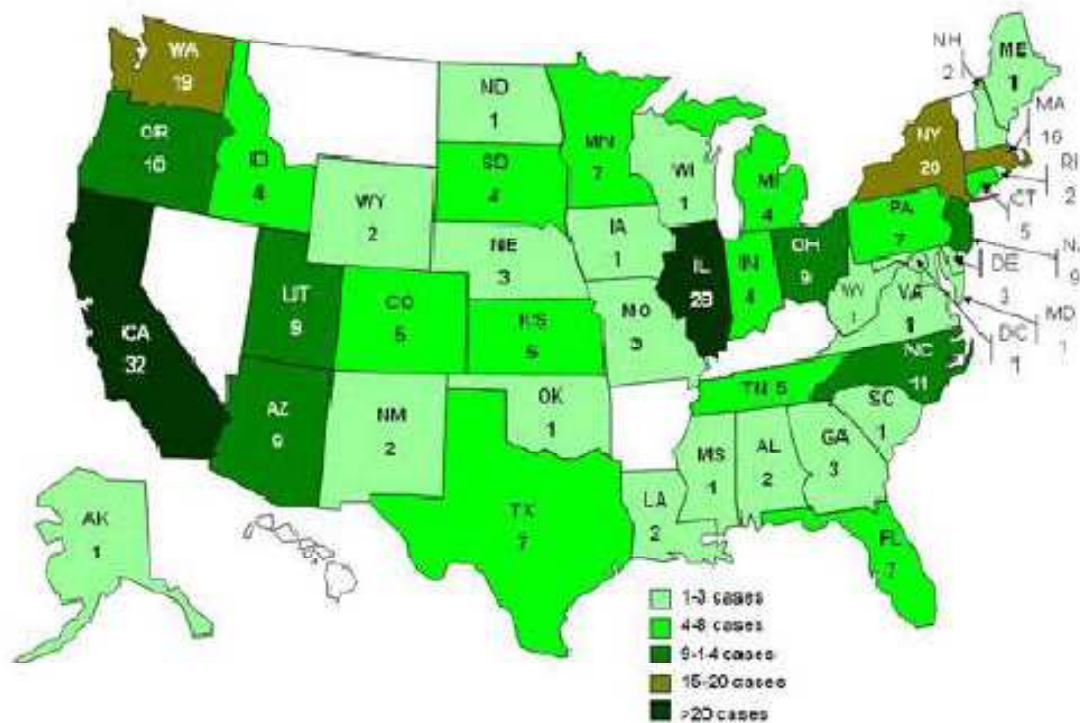
- Centers for Disease Control and Prevention (CDC) collaborated with public health officials in many states, the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS), and the U.S. Food and Drug Administration (FDA) to investigate a multistate outbreak of ***Salmonella* serotype Montevideo infections**.
- Testing found the outbreak strain of *Salmonella* Montevideo in samples of **black and red pepper intended for use in the production of Italian-style meats**.
- Since then, several recalls have been issued.
- E.g. **1.2 million pounds of Italian-style salami was recalled**.

Ref: CDC, 2010



## Investigation Announcement: Multistate Outbreak of Human *Salmonella* Montevideo Infections

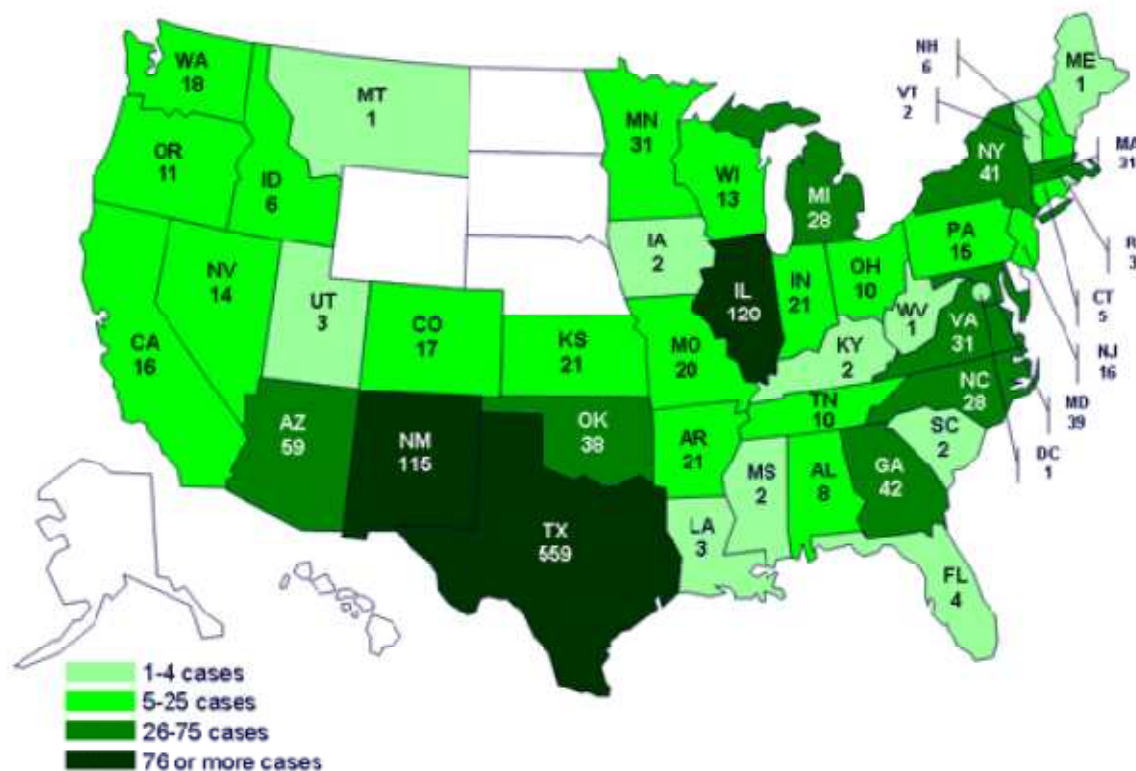
Persons Infected with the Outbreak Strain of *Salmonella* Montevideo, United States, by State, as of 04/28/10 at 9:00 pm EST (n=272)



- The latest confirmed case count is 272 persons in 44 states and DC, with April 14, 2010 as the most recent reported illness onset date.

- According to CDC the numbers of new cases have declined substantially since the peak in November 2009, but some of the recalled products have long shelf-lives and could cause illness if consumed.

## Cases infected with the outbreak strain of *Salmonella* Saintpaul, United States, by state, as of August 25, 2008 9pm EDT



• Jalapeño peppers were a major source of contamination and serrano peppers also were a source.

In addition, tomatoes were possibly a source, particularly early in the outbreak.

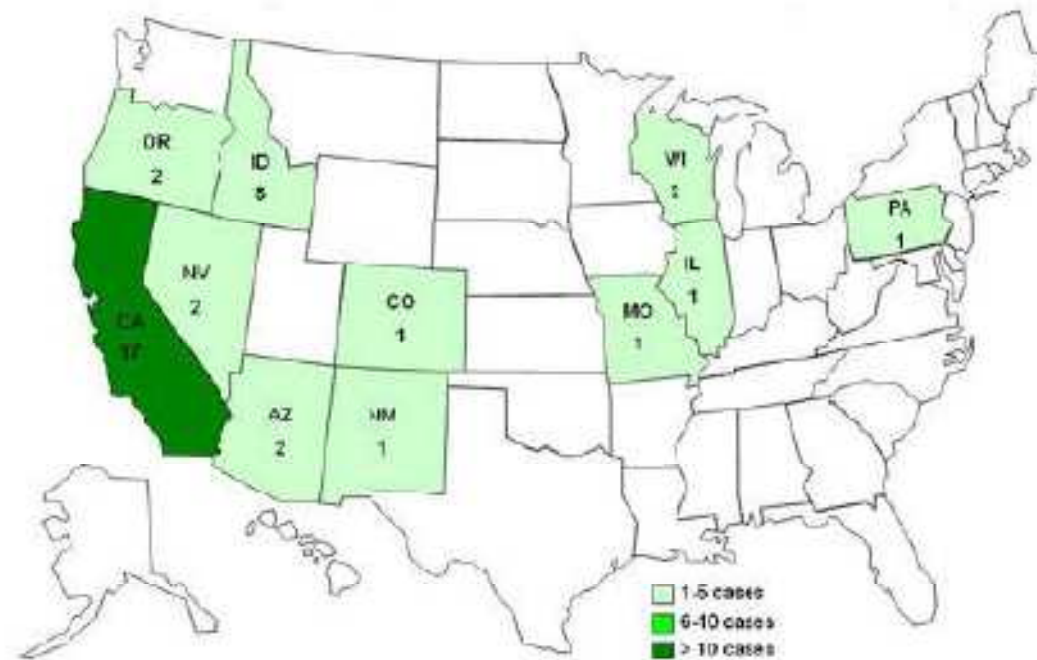
Jalapeño peppers were traced back to distributors in the United States that received produce grown and packed in Mexico.

\*1442 persons infected with *Salmonella* Saintpaul with the same genetic fingerprint.



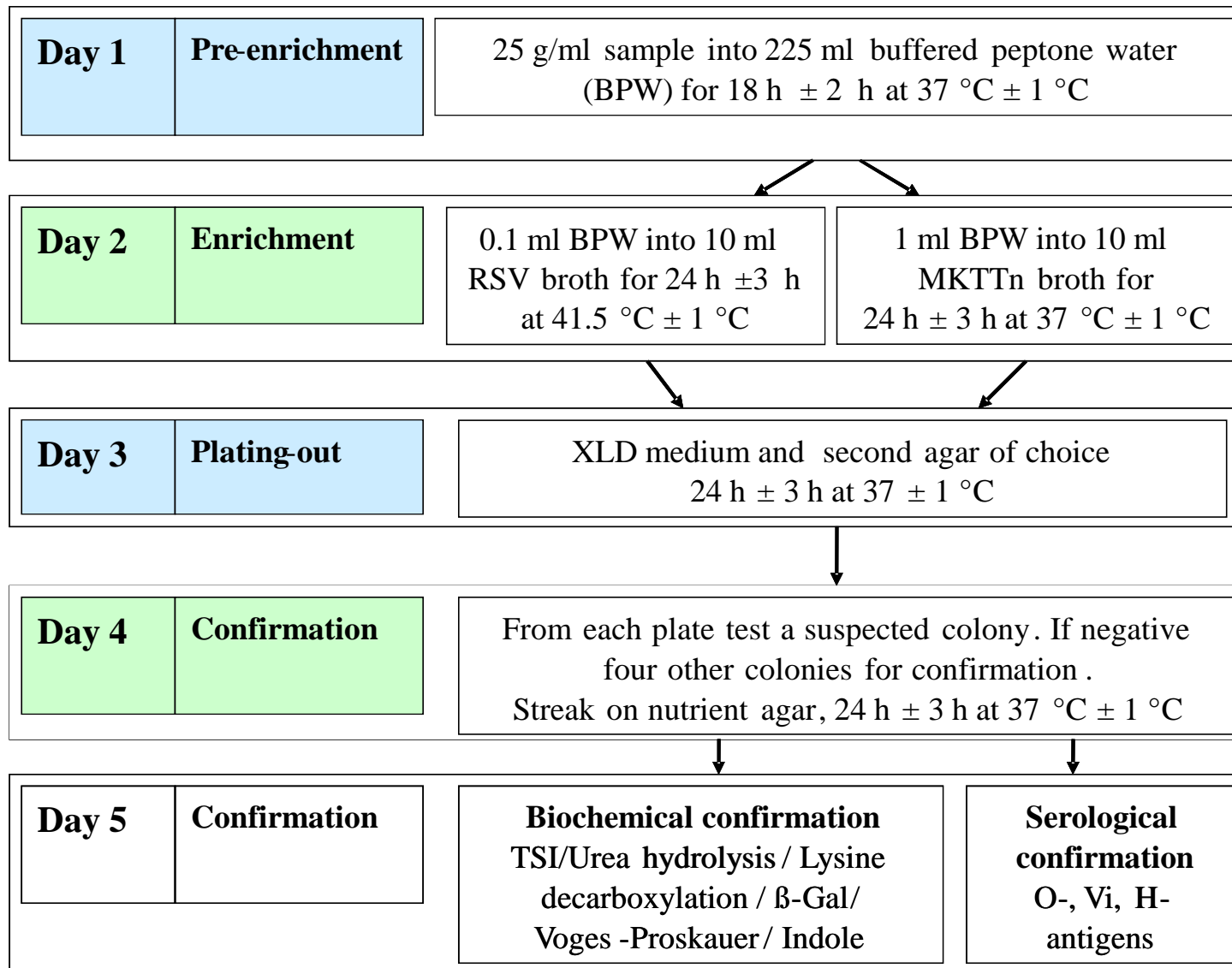
## Investigation Update: Multistate Outbreak of Human *Salmonella* Newport Infections Linked to Raw Alfalfa Sprouts

Persons Infected with the Outbreak Strain of *Salmonella* Newport, United States, by State, as of 06/02/10 at 11:00 pm EDT (n=35)



## Detection of *Salmonella* – traditional methods

- Critical steps in the detection of *Salmonella*, as in other microbiological analysis, are sampling, storage and handling of the samples.
- **ISO 7218:2007 standard** contains information about general requirements and guidance for microbiological examinations, whereas **ISO 6579:2002** (Microbiology of food and animal feeding stuffs-Horizontal method for the detection of *Salmonella* spp.) contains information about the detection of *Salmonella* spp. In addition ISO 6579:2002/Amd 1:2007 Annex D deals with the detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage.
- The presence of *Salmonella* in ready-to-eat foods is considered significant regardless of the level of contamination. Therefore isolation is carried out by enrichment culture of a defined weight or volume of food (normally 25 g).
- The traditional detection and isolation of *Salmonella* spp. from food and feed products utilises a multi-step protocol with non-selective pre-enrichment, followed by a selective enrichment step, isolation on selective agar media and a preliminary biochemical and serological confirmation.



Scheme of the *Salmonella* detection according to ISO 6579:2002 (Horizontal standard for the detection of *Salmonella*).

## Detection of *Salmonella*

- In several EU countries the use of rapid methods is approved as in-house control but not in the examination of samples belonging to national *Salmonella* control program. Several rapid and automated methods have been developed, validated and are on market for the detection of *Salmonella*.
- Several commercial PCR-based methods have been reported to have sensitivities equal to or higher than the culture-based methods, in addition, good specificity has been reported.
- PCR-based methods are useful in process control, primarily because of rapid indications of the presence or absence of *Salmonella*. Almost all rapid test protocols include a selective enrichment stage, and then apply concentration and/or rapid detection techniques to replace culture on selective agars and further confirmatory tests.
- Discrimination between live and dead cells, e.g. DNA chelating agents, in PCR.
- Conventional cultivation methods are needed for isolation of *Salmonella*. Particularly in epidemiological investigations, there is a need for typing, susceptibility testing. PCR-based methods need to be accompanied by suitable isolation methods that can find *Salmonella* also when the enrichment broth contains low levels.

Ref: Koyuncy et al. (2010)

## Examples of rapid methods for detection and identification of *Salmonella*.

Method	Manufacturer
<b><i>Immuno-enzymatic tests (ELISA-based)</i></b>	
Vidas <i>Salmonella</i> (SLM, easy SLM)	bioMérieux SA, France
Transia system	Biocontrol Systems, Sweden
Bioline Selecta, Bioline Optima	Bioline, Denmark
Rapidyme <i>Salmonella</i>	SA/NV Belgium
Tecra Unique <i>Salmonella</i> , Tecra Ultime <i>Salmonella</i>	3 M Microbiology, USA
Ridascreen <i>Salmonella</i>	R-Biopharm
Patharix, (RIMS) re-circulator immuno-magnetic-separation	Matrix MicroScience Ltd
<b><i>PCR-based methods</i></b>	
BAX <i>Salmonella</i> PCR (BAX System)	DuPont Qualicon, USA
iQ-Check <i>Salmonella</i>	Bio-Rad laboratories, France
TagMan® <i>Salmonella</i>	Applied Biosystems, USA
LightCycler <i>Salmonella</i> detection kit	Roche Diagnostics, Germany
foodproof <i>Salmonella</i> detection kit	Merck KGaA, Germany
Assurance CDS <i>Salmonella</i>	Biocontrol Systems
<b><i>Identification and immunological tests</i></b>	
VITEK® 2 Gram-negative card	bioMérieux SA, France
Oxoid <i>Salmonella</i> rapid test (ORST)	Oxoid, UK

## Summary

- As food trade is becoming more global and consumers prefer more fresh produce and uncooked ready-to-eat foods, the microbiological risks of foods have increased. The volume of global trade is increasing and food and feed stuffs are moving faster from one country to another. In addition, people are travelling more and further away than before.
- Hence, collaboration between various global networks/programmes needs to be reinforced. E.g. collaboration in harmonization of methods, training and organization of inter-laboratory comparison studies as well as for on-line reporting of new zoonotic cases needs to be active.
- The Rapid Alert System for Food and Feed (RASFF)



## Selected references

- Alakomi, H-L. & Saarela, M. *Salmonella*- importance and current status of detection and surveillance methods. Quality Assurance and Safety of Crops & Foods. 2009. Vol. 1. 3: 142-152.
- EFSA, 2006. Opinion of the scientific panel on biological hazards on "Risk assessment and mitigation options of *Salmonella* in pig production". The EFSA Journal, 341, 1-131.
- EFSA, 2008a. Opinion of the scientific panel on biological hazards on a request from the European Commission on a quantitative microbiological risk assessment on *Salmonella* in meat: Source attribution for human salmonellosis from meat. The EFSA Journal, 325, 1-23.
- EFSA, 2008b. Scientific opinion of the scientific panel on biological hazards on a request from the health and consumer protection, Directorate General, European Commission on microbiological risk assessment in feeding stuffs for food-producing animals. The EFSA Journal, 720.
- EFSA, 2009a. Analysis of the baseline survey on the prevalence of *Salmonella* in holdings with breeding pigs, in the EU, 2008, part A: *Salmonella* prevalence estimates. The EFSA Journal, 7, 93.
- EFSA, 2009b. The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. The EFSA Journal, 223.
- EFSA, 2009c. Joint opinion on antimicrobial resistance (AMR) focused on zoonotic infections. The EFSA Journal, 7.
- EFSA, 2010. The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. The EFSA Journal, 2010
- EFSA, 2010. Scientific Opinion on a Quantitative Microbiological Risk Assessment of *Salmonella* in slaughter and breeder pigs<sup>1</sup> EFSA Panel on Biological Hazards (BIOHAZ), EFSA Journal, 2010; 8(4):1547
- Koyuncy, S., Andersson, G. & Häggblom, P. 2010. Accuracy and sensitivity of commercial PCR-based methods for detection of *Salmonella enterica* in feed. Applied and Environmental Microbiology. 9, 2815-2822.
- Majjala R, Ranta J, Seuna E, Pelkonen S and Johansson T, 2005. A quantitative risk assessment of the public health impact of the Finnish *Salmonella* control program for broilers. International Journal of Food Microbiology, 102, 21-35.
- Wales AD, Allen VM and Davies RH, 2010. Chemical treatment of animal feed and water for the control of *Salmonella*. Foodborne Pathogens and Disease, 7, 3-15. 10.1089/fpd.2009.0373
- Wierup M and Häggblom P, 2010. An assessment of soybeans and other vegetable proteins as source of *Salmonella* contamination in pig production. Acta Veterinaria Scandinavica, 52, 15