Update on the annual reporting of STEC in the EU and on EFSA activities for molecular typing data collection for food and animal isolates

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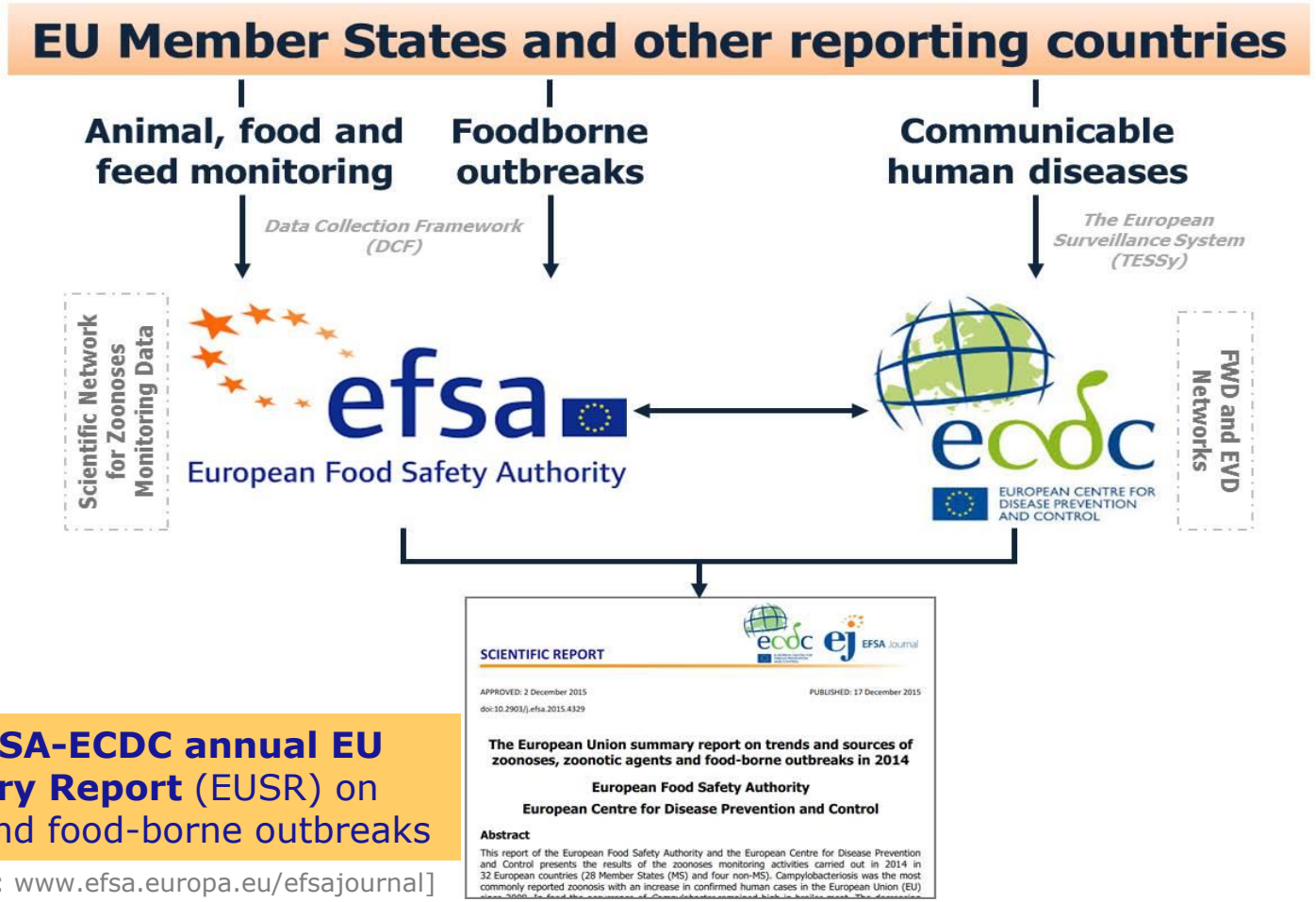
OUTLINE

- **Update on the annual reporting of STEC in the EU**
- **EFSA activities for molecular typing data collection for food and animal isolates**
- **EFSA's activities on WGS**

BACKGROUND



Joint EFSA-ECDC annual EU Summary Report (EUSR) on zoonoses and food-borne outbreaks

[Available online: www.efsa.europa.eu/efsajournal]

- Data on VTEC in food and animals are reported annually on a mandatory basis (**Directive 2003/99/EC**)

WHAT IS NEW IN THE EUSR2014

- **Previous EU Summary Reports**
  - ✓ Descriptive analysis of the data reported for certain food categories and animal species (any VTEC, O157)
  - ✓ Summary of the reported information on VTEC serogroup

- **EU Summary Report 2014**
  - ✓ Descriptive analysis of the data reported for certain food categories and animal species (any VTEC, O157)
  - ✓ Detailed analysis of the VTEC serogroup 2011-2014
  - ✓ Detailed description of the different analytical methods used and evaluation of possible impact on the distribution of VTEC serogroups

VEROTOXIGENIC *ESCHERICHIA COLI*

➢ **Important note for data analysis and interpretation**:

Different investigations are **not necessarily directly comparable** owing to **differences in sampling strategies and the analytical methods applied**

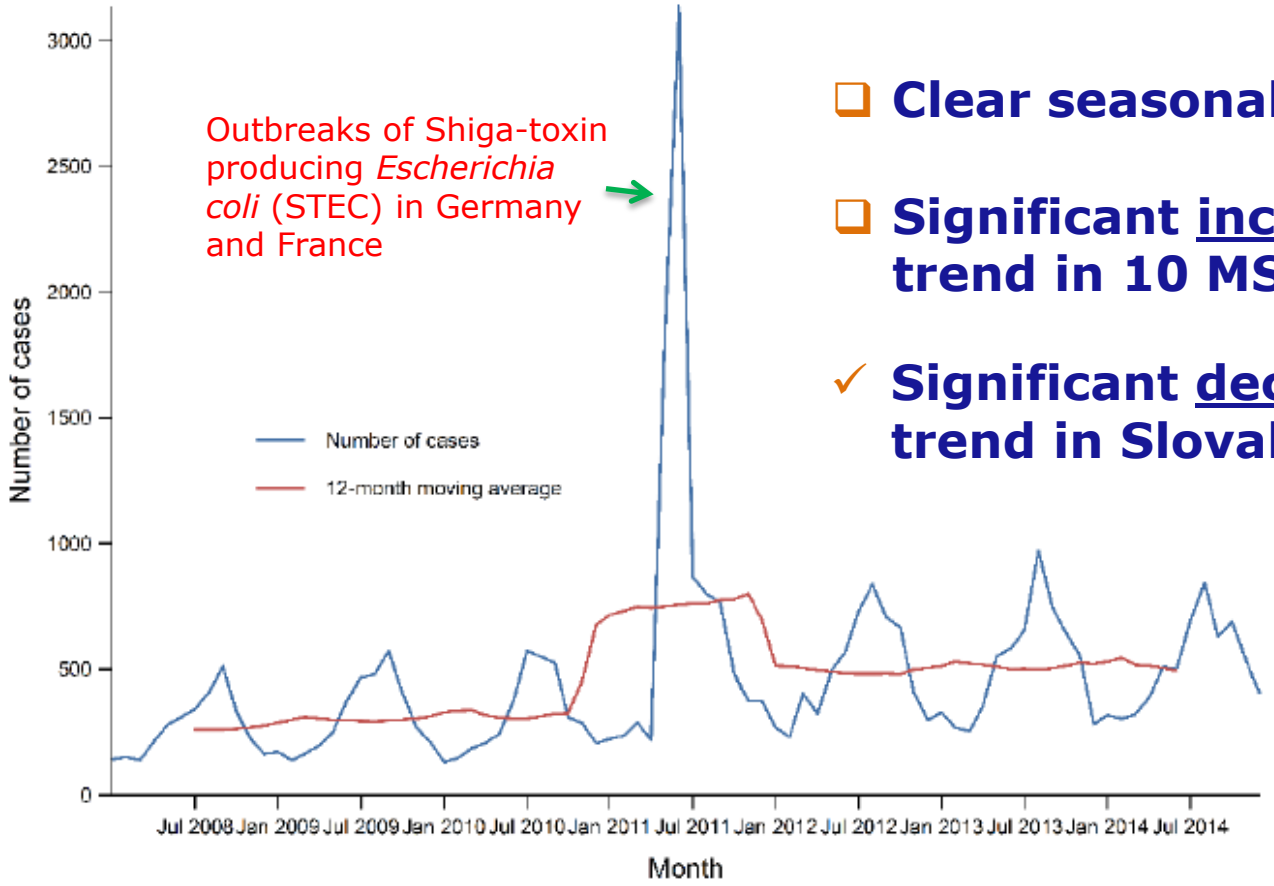
Two main categories of **analytical methods** used:

1. **Aiming at detecting any VTEC**, regardless their serotype, including: ISO/TS 13136:2012, other PCR-based methods, and also methods based on the detection of verocytotoxin production by immunoassays.
2. **Designed to detect only VTEC O157**, such as the method ISO 16654:2001 and the equivalent NMKL 164:2005. Focus has traditionally been on VTEC O157 in many of the MS surveillance programmes → impact on prevalence and frequency distribution of VTEC serogroups

VTEC IN HUMANS *(EUSR2014)*

Trend in reported confirmed cases of human STEC infections in the EU/EEA, 2008-2014

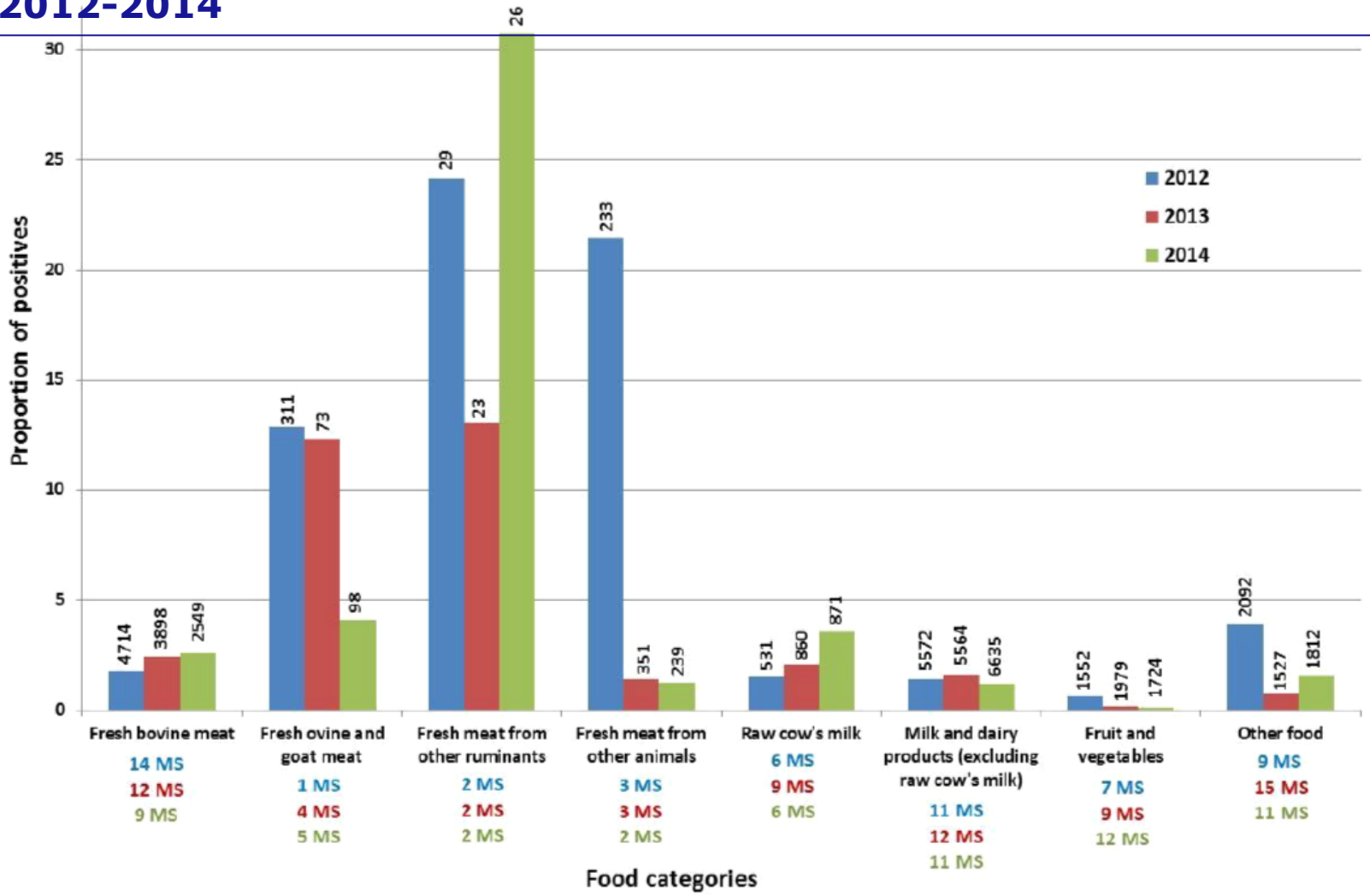
- In 2014, 6,013 cases of STEC infections, of which 5,955 confirmed reported in the EU → slight decrease compared with 2013



- **Clear seasonal trend**
- **Significant <u>increasing</u> trend in 10 MS**
- ✓ **Significant <u>decreasing</u> trend in Slovakia**

VTEC IN FOOD

The proportion of VTEC-positive samples in the main food categories, regardless the analytical method employed, in the reporting MSs, 2012-2014



VTEC IN FOOD

ANALYSIS OF VTEC SEROGROUPS IN FOOD

1. Relative frequency of each serogroup in the different food categories

→ *by using all data on the VTEC serogroups reported for food samples (any method)*

- In total 12 MSs provided information on **VTEC serogroups** in 226 VTEC isolates. For 53 isolates, only the information 'non-O157 serogroup' was reported.
- Most frequently reported serogroups:
  1. **VTEC O157** (58 isolates, 33.5% of the 173 strains with identified serogroup), prevalence influenced by MS-specific results (2 MS). Main sources: bovine meat, other meat, pig meat and raw milk
  2. **O26** (8.7%), main sources: milk and dairy products, followed by bovine meat
  3. **O103** (6.9 %) reported in both meat and milk & dairy products
  4. **O113** (6.4 %), **O146** (4.6 %), **O174** (4.6 %), and **O91** (4.0 %): mainly reported in isolates from meat products
  5. **O145** (2.9 %) reported in both meat and milk & dairy products
  6. **Others**: VTEC O8, O21, O22, O43, O55, O74, O88, O130, O139, O142, O15O, O153, O176, O182, O183

VTEC IN FOOD

ANALYSIS OF VTEC SEROGROUPS IN FOOD (cont.)

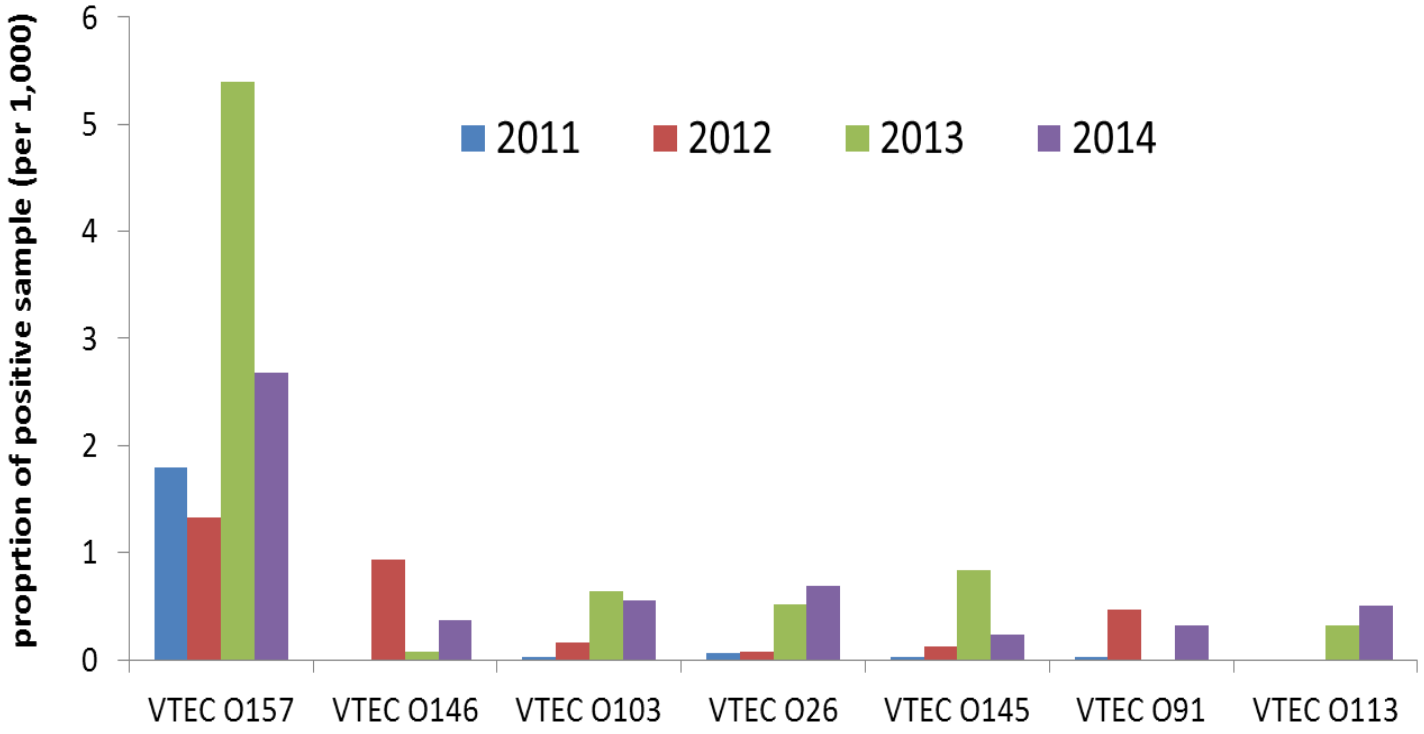
2. Proportion of positive samples for any VTEC and VTEC belonging to the "top-5" serogroups in food categories in Member States and non-Member States, 2014

→ ***Only samples tested by the ISO/TS 13136 method*** *or other Real Time PCR-based methods employing similar reagents and protocols were considered.*

Food category	Samples tested by ISO/TS 13136: 2012	Samples positive for											
		any VTEC		O157		O26		O145		O103		O111	
	n	N pos	% pos	N pos	% pos	N pos	% pos	N pos	% pos	N pos	% pos	N pos	% pos
Bovine meat [a]	2,522	75	3.0	3	0.1	5	0.2	3	0.1	5	0.2		
Ovine and goat meat [a]	21	3	14.3							1	4.8		
Other ruminants meat [a][b]	40	13	32.5										
Pig meat [a]	841	10	1.2	1	0.1								
Other meat [a][c]	786	13	1.7			1	0.1						
Mixed meat [a]			0.0										
Milk and dairy products [d]	2,182	13	0.6			4	0.2			3	0.1		
Raw milk [e]	410	13	3.2	1	0.2	4	1.0	2	0.5	2	0.5	1	0.2
Fruit and vegetable	1,150	1	0.1										
Seeds [f]	799												
Other food	217												
Total	8,968	141	1.6	5	0.1	14	0.2	5	0.1	11	0.1	1	0.0

VTEC IN FOOD

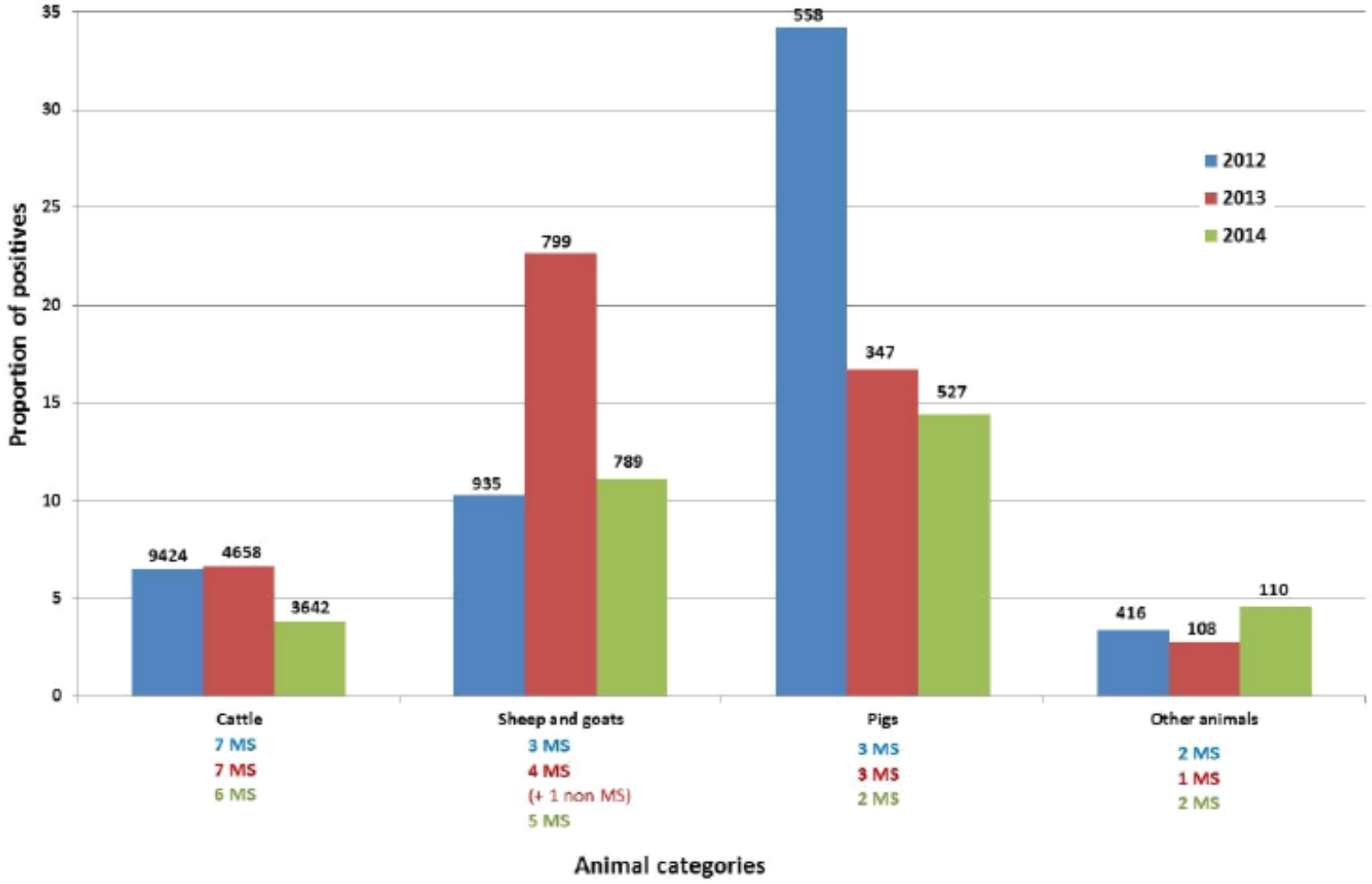
Proportion of food samples positive for the most frequent VTEC serogroups (per 1,000 samples tested), reported by MS and non-MS between 2011 and 2014.



An *increasing trend* of reporting in food was observed for **VTEC O26** and **VTEC O103**, two serogroups strongly associated with severe human infections in the EU.

VTEC IN ANIMALS

Proportion of VTEC-positive samples in the main animal categories, regardless the analytical method employed, in the reporting MS, 2012-2014



'Other animals' include: cats, dogs, horses, donkeys, turkeys, and other animals.

VTEC IN ANIMALS

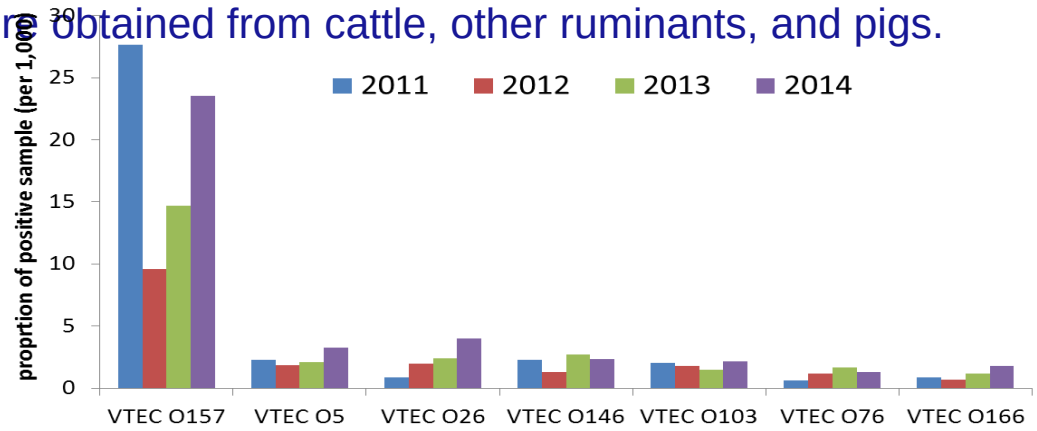
ANALYSIS OF VTEC SEROGROUPS IN ANIMALS

In total, 9 MS provided information on the serogroups of 303 VTEC isolates obtained from animal samples. Most isolates where from cattle and goat and sheep

Overall, the most frequently reported serogroup (using any analytical method) was **VTEC O157**, followed by **VTEC O26** → both mainly reported in cattle, but also in other species

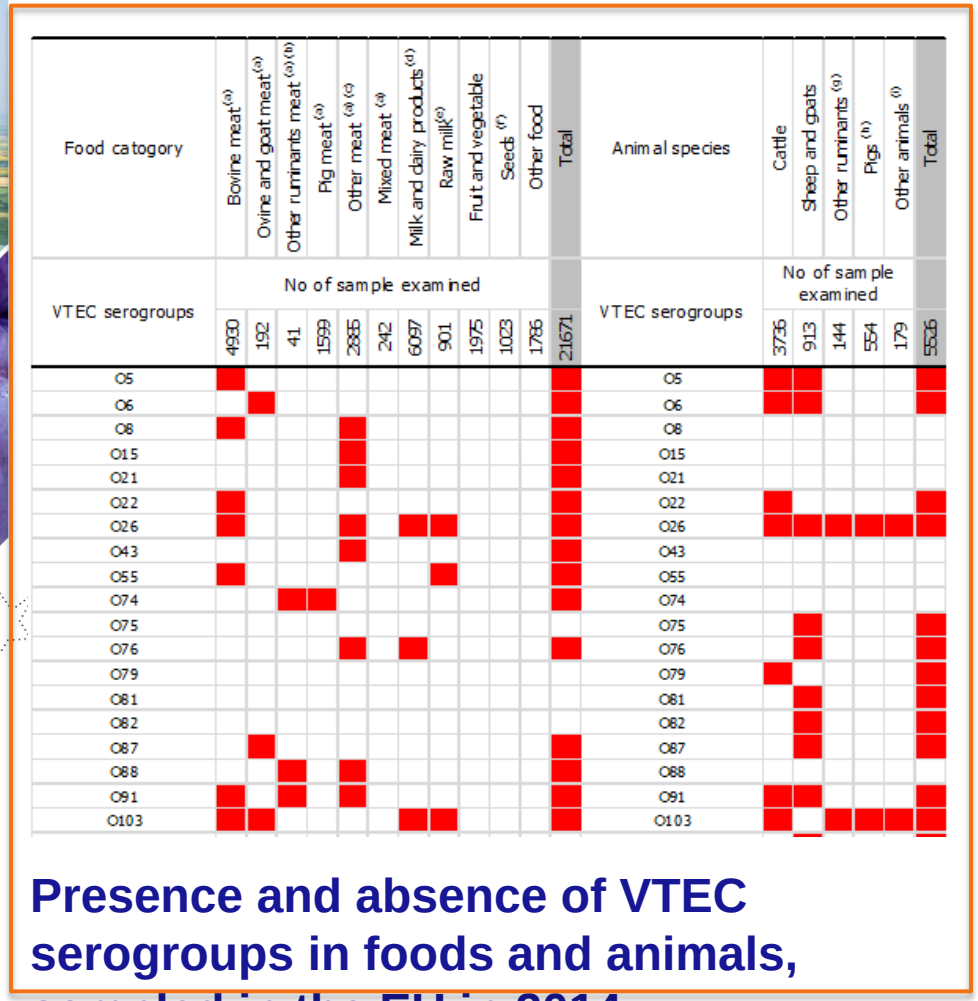
Other serogroups:

- **O146** only detected in sheep and goat
- **O103** isolated from all the species but sheep and goat
- **O113** and **O91** found in cattle as well as in sheep and goats
- few isolates belonging to the "top 5" serogroups O111 (0.7 %), and O145 (0.7 %) were obtained from cattle, other ruminants, and pigs.



VTEC IN FOOD AND ANIMALS

Atlas of VTEC serogroups reported in food & animals in EU



Presence and absence of VTEC serogroups in foods and animals, sampled in the EU in 2014

Trends in the presence of the different VTEC serogroups in food and animals reported in the EU between 2011 and 2014.

Food	2011	2012	2013	2014	Animals	2011	2012	2013	2014
	No of samples tested					No of samples tested			
VTEC serogroups	32767	25547	25008	21671	VTEC serogroups	8392	10134	6683	5526
O1					O1			■	
O2			■		O2	■		■	
O5				■	O5	■	■	■	■
O6				■	O6	■	■		■
O8	■			■	O8			■	
O13					O13	■			
O15		■	■	■	O15		■		
O17		■			O17	■			
O21		■		■	O21				
O22		■	■	■	O22	■	■	■	■
O26	■	■	■	■	O26	■	■	■	■
O27	■	■			O27		■		
O36		■			O36		■		
O39					O39	■	■	■	
O43				■	O43		■		
O46					O46		■		
O50					O50			■	
O51		■			O51				

MAIN CONCLUSIONS (NEW ASPECTS)

- **Analytical method** reported by most reporting countries. However, for 14% of the food samples and 46.5% of the animal samples tested the method used was not reported.
- **Highly variability** in the **number of samples tested** by country for each food and animal category → possible **bias** in the estimates of VTEC prevalence or VTEC serogroup distribution.
- In food, contamination reported for **meat from wild ruminants, ovine and goat meat**, **milk**, and **fresh bovine meat**. VTEC were also reported in **cheese samples**, in particular those made from sheep's and goats' milk
- **Contamination was rare in ready-to eat food of vegetal origin**. **No VTEC-positive** samples reported for **spices and herbs** as well as for **sprouted seeds**, the sole food category for which microbiologic criteria for VTEC have been established in the EU.

MAIN CONCLUSIONS (NEW ASPECTS)

A wide range of VTEC serogroups was reported, with VTEC O157 being the most frequent in both food and animal samples.

→ However, many of the MS' surveillance and monitoring programmes are traditionally focused on this serotype and this may have introduced a **bias in the estimates of the frequency of VTEC serogroups** → interesting to note that serogroups O26 and O103 were reported more frequently than O157 in food samples tested using the ISO/TS 13136:2012 standard method, which is able to detect any VTEC regardless its serotype

VTEC O26 was the second most reported serogroup in both food and animal samples (as well as in humans), with an increasing trend between 2011 and 2014

VTEC serogroups most frequently found in **food** samples (**O157, O26, O103, O113, O146, O91, O145**) are those most commonly reported in **human infections** in the EU/EEA in 2014 and previous years

MOLECULAR TYPING DATABASE

- The Standing Committee on Food Chain and Animal Health (representing all EU Member States) approved in December 2012 the *Vision paper on the development of databases for molecular testing of food-borne pathogens in view of outbreak preparedness*

- Request for technical assistance:
  - ECDC to collect molecular typing data from food-borne pathogens isolated from human cases (**TESSy**)
  - EFSA to collect similar data from food, feed and animal isolates, in close collaboration with relevant EURLs (**EFSA database**)
  - Regular joint data analyses of the data in the **joint EFSA-ECDC database** (hosted in ECDC), where curation of molecular typing data is carried out by the relevant curators (EURLs).

- The data collection to cover initially:
  - *Salmonella*, VTEC and *Listeria monocytogenes* with PFGE and MLVA (*S*. Typhimurium) methods.

MOLECULAR TYPING DATABASE

- To guarantee **data confidentiality** only a subset of the METAdata stored in the EFSA database will be sent to ECDC for storage in the joint EFSA-ECDC database.
- The **visibility of data** in joint EFSA-ECDC database depends on the **type of data** (sensitive or non-sensitive) and the **users**.

Data shared in the joint database:

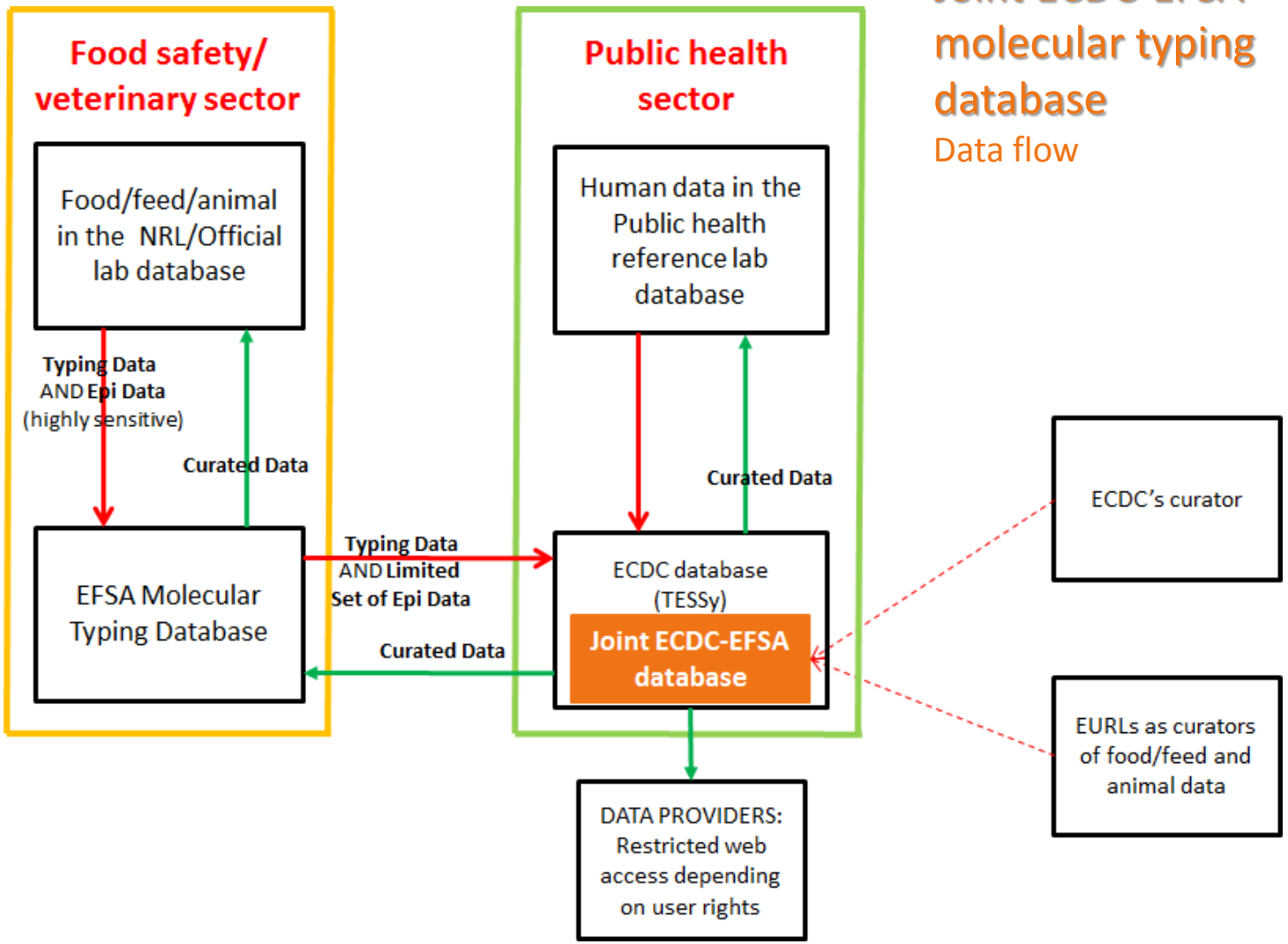
Non-sensitive data:
Microbiological Data, limited to *Molecular Typing Data* and other typing data (*Salmonella* serotype, *Listeria* serotype and STEC serogroup). EFSA Isolate Id, date of sampling, date of receipt of isolate in the reference lab, type of sample (e.g. 'animal', 'food', 'feed', 'environment')

Sensitive data:
Country of sampling, laboratory identification code

MOLECULAR TYPING DATABASE

Joint ECDC-EFSA molecular typing database

Data flow



SUPPORTING DOCUMENTS

Technical documents have been prepared to support the design and development of the database and the production of analytical results.

- EFSA Technical report on technical specifications for the pilot phase[1] (*ad hoc WG*).
- External Scientific Reports on SOPs for molecular typing data (PFGE, MLVA) production and interpretation for *Salmonella*, VTEC and *Listeria monocytogenes*[2] (*EURLs*).

1. http://www.efsa.europa.eu/en/supporting/pub/712e
2. *Salmonella*: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/703e.pdf
   VTEC: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/704e.pdf
   Listeria: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/702e.pdf

COORDINATION ACTIVITIES

Collaboration Agreement

- Collaboration Agreement on the **management of data on molecular testing** of food, feed and animal isolates of selected food-borne pathogens and their use together with molecular typing data on isolates from human infections **for public health purposes**
- It covers issues with regards to **data ownership**, **availability**, **access**, **use**, **publication** and **confidentiality**
- The implementation of this agreement will be supervised by a **Steering Committee**
- The Agreement has been signed by the Parties:
  - EFSA, ECDC, EURL *Salmonella,* EURL *Listeria monocytogenes,* EURL *E. coli*
- The **Appendix 1**: agreement with Member States

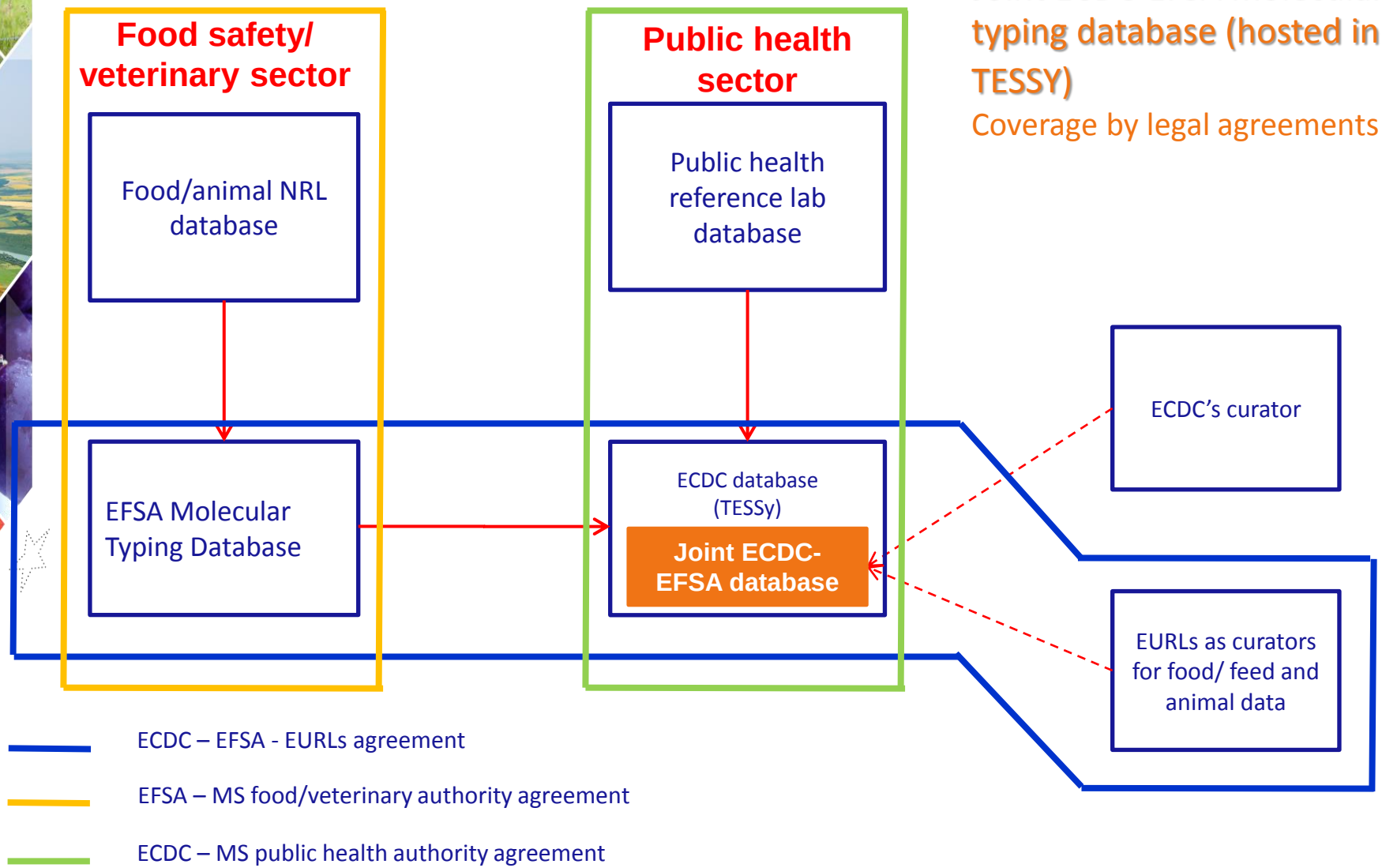
APPENDIX 1: AGREEMENT WITH MS

- **Appendix 1**: Member State food/feed NRLs and other official control laboratories and institutes **agreement** on the collection of data on molecular testing in food, feed and animal isolates of food-borne infections
  - I agree to provide data to EFSA for the collection of molecular typing data … in accordance with the terms set out in this collaboration agreement.
  - I agree that the data are submitted to the Joint database for analysis together with human origin data.
  - I agree that the data submitted may be utilised to assess exposures and to characterise risks related to zoonoses and zoonotic agents.
  - I **declare that Data Owners have given their consent on the reproduction and use of the Data**.

COLLABORATION AGREEMENT

Joint ECDC-EFSA molecular typing database (hosted in TESSY)

Coverage by legal agreements



COORDINATION ACTIVITIES

Set up of the Joint EFSA-ECDC Steering Committee

- ToR:
  - Development of standard operating procedures for data analyses → SOP for the analysis of data in the joint EFSA-ECDC molecular typing database
  - Monitoring and evaluation of the whole pilot phase
  - Identification of needs for revision of the data collection system
  - Communication on the pilot activities
- Members:
  - EFSA, ECDC, EURLs (EFSA' curators), ECDC's curator, (EC as an observer)

COORDINATION ACTIVITIES

SOP for the analysis of MT data

Objective

- To **describe the process of analysis of the molecular typing data stored in the joint EFSA-ECDC molecular typing database** for the **purpose of multi-country outbreak detection and assessment**.
- To allow the **identification of microbiological clusters of public health relevance** and support epidemiological investigation.

Scope

- PFGE data: for *Salmonella*, *Listeria monocytogenes* and VTEC
- MLVA data: for *S.* Typhimurium and *S*. Enteritidis

Process

- Microbiological cluster definition
- Data analysis process in EPIS-FWD platform

COORDINATION ACTIVITIES

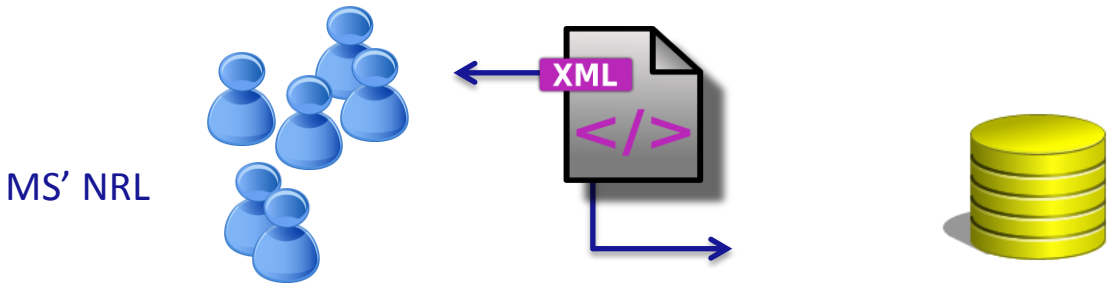
Communication activities on the pilot

- EURL annual meetings of the NRLs' networks (*Salmonella*, *Listeria monocytogenes*, *E. coli*)
- PAFF meeting – Section on Biological Safety of the Food Chain & Controls and Import Conditions
  - On 13 July 2015
  - General information on the project
- PAFF meeting – Working Group on Microbiological Criteria
  - On 30 October 2015
  - Detailed presentation of the project (3 hours)
  - Circulation of Collaboration Agreement and SOP for data analysis
- PAFF meeting – Section on Biological Safety of the Food Chain
  - 14 June 2016

ON-GOING ACTIVITIES

- Execution of the data collection

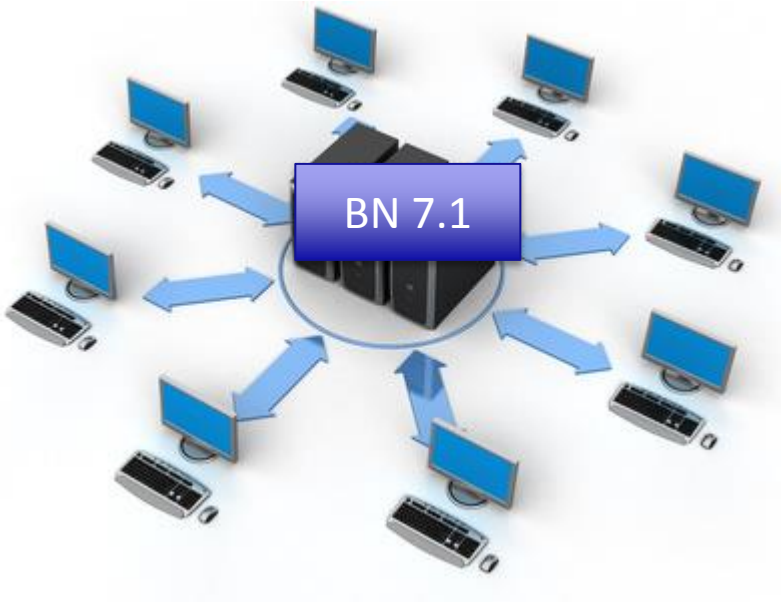
- Laboratories will be able to:
  - Retrieve their data curated by the EURLs
  - Search the joint database and perform cluster analysis on the information accessible based on the access rights (see collaboration agreement)



- During the pilot data collection phase, EFSA supports data providers in the data model mapping exercise.

SYSTEM DESCRIPTION

EFSA Molecular Typing database is at present based on **BioNumerics version 7.1 (or higher).**



- Integration of **Data Collection Framework** (DCF) as a unique entry point for data submission.
  - Impact on users: submission process will accept XML open format

LABORATORY ENGAGEMENT PROCEDURES

An informative document has been produced to involve laboratories in the Data Collection.

Type of activity

- Voluntary submission of molecular typing data

Type of data

- Nature of data: molecular typing results obtained through PFGE and MLVA (only for *Salmonella* Typhimurium)
- Source: isolates from food, feed, animal and food/feed processing environment
- Context: strains isolated and typed during outbreak investigation or during routine activities of the laboratory
- Time period of interest: available historical data and new data
- Frequency of submission: free, but suggested submission on weekly basis for real time results.

LABORATORY ENGAGEMENT PROCEDURES

Prerequisites

- Laboratories willing to participate to the EFSA Molecular Typing Data Collection must be compliant with the following prerequisites:
  - The laboratory is an **NRL or official control laboratory** for *Listeria monocytogens*, *Salmonella* or *E. coli*.
  - The laboratory owns **BioNumerics (Applied Maths) version 7.1** or higher or is able to submit data through the **EFSA's Data Collection Framework** (DCF).
  - The laboratory submits the data according to the **EFSA data model** as described in Technical Specifications document.

LABORATORY ENGAGEMENT PROCEDURES

Official nomination

- The countries willing to participate in the data collection have to:
  - officially nominate their representatives for submitting molecular typing data to EFSA and communicate them to Commission;
- The nominated users (or representative of their Institute) have to:
  - sign the Appendix 1 of the Collaboration Agreement
- Current engagement
  - 11 Member States nominated their representatives

FUTURE ACTIVITIES

Support to laboratories

- Web meetings organised upon request to support the laboratories during the data submission process

Extension of the data collection to WGS data

- Under discussion with European Commission

EFSA INTEREST ON WGS FOR FOOD SAFETY

EFSA is interested in using WGS for:

- Source attribution
- Outbreak detection and investigation
- Common source trace back investigations
- Detection and surveillance of emerging pathogens
- Monitoring of antimicrobial resistance

Our main interest is to use the data generated by new Sequencing technologies (WGS, Metagenomics) for Food Safety and Public Health Protection

ACTIVITIES ON WGS

- Procurement: Closing data gaps for performing RA on *L. monocytogenes* in "Ready to Eat Foods" (RTE): "Molecular characterisation employing WGS of strains from different compartments along the food chain and from humans", LISEQ
- Grant: Comparative genomics of quinolone-resistant *Campylobacter jejuni* of poultry origin from major poultry producing European countries – GENCAMP
- Questionnaire on the availability of Whole Genome Sequencing (WGS) methods for food- and water-borne pathogens isolated from animals, food, feed and animal/ food/ feed environmental samples EUSurvey
- Advisory Board WGS EU funded project (COMPARE, Effort, ECDC's projects..)

WGS TO SUPPORT THE EUSR ON AMR

EFSA Journal 2015;13(2):4036

SCIENTIFIC REPORT OF EFSA AND ECDC

EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013[1]

Selection of isolates:

- Emerging resistances
- Detection of clones
- Discrepancies

- Confirmation of results
- Ask MSs for the isolates
- Perform:
  WGS, MIC re-testing

WGS analyses support phenotypical AMR data?

THEMATIC GRANTS, PROMOTE NETWORKING

"Molecular approaches for identifying and characterising microbial foodborne pathogens, specifically using Whole Genome Sequence (WGS) analysis"

WGS generated data could be a powerful tool for Risk assessors i) genetic diversity, ii) epidemiological relationships, iii) putative markers conferring advantages.

BUT integration of WGS in microbial food safety routine needs:

i) time; ii) proofs of principle; iii) transnational collaboration/scientist coordination (One Health approach); iv) new analysis tools; v) translation of results into 'plain language'.

Granted projects

- INNUENDO (University of Helsinki): 2.5 years
  - *Salmonella/ Campylobacter/ Yersinia/* **VTEC**
- ENGAGE (Danish Technical University): 2 years
  - *Salmonella* (including AMR)/ ***E. coli*** **(including AMR)**

THEMATIC GRANTS ON WGS

Expectation from the Projects funded:
Applicability and integration of WGS methods for identification and characterisation of microbial foodborne pathogens.

- Provide proofs of principle
- Establish transnational collaboration/scientist coordination: One Health Approach
- Develop new analysis tools
- Translation of results into plain language

THANKS FOR YOUR ATTENTION!

EFSA is committed to:

Excellence, Independency, Responsiveness and Transparency

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Acknowledgements:
BIOCONTAM Unit
DATA Unit
ECDC
EC – SANTE G4
Zoonoses Monitoring Data Network
Steering Committee members

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