

12<sup>th</sup> Annual Workshop of the NRLs for *E. coli* in the EU

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# An outbreak of STEC O26 linked to water

Analysis on food, animal faeces and water

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# Aim of the presentation

- To describe the laboratory analysis to investigate STEC O26 in foods, animal faeces and water, sampled in the area of a small municipality of Bolzano province, within the epidemiological survey on HUS cases occurred between June and July 2017



# Methods

## Food samples

- ISO/TS 13136:2012 (25gr/ mL)

**SAMPLING** performed by  
Veterinary Service and Hygiene  
PH Service of the Local Health  
Unit of Bolzano

## Water samples

- One liter of water, filtered through membranes (0.45 µm)
- Membranes transferred in 100 mL BPW (18-24h at 37°C)
- Nucleic acid extraction and detection according to ISO/TS 13136:2012

## Fecal samples

- 10 gr of faeces, pre-enrichment at 37°C in 90 mL BPW for 18-24h
- Nucleic acid extraction from 1 mL of microbial enrichment with Instagene Matrix (Biorad)
- Nucleic acid diluted 1:20 prior to real time amplification
- Detection of serogroup O26 gene according to ISO/TS 13136:2012
- For O26 positive samples, detection of *stx1/stx2/eae* genes according to ISO/TS 13136:2012
- For STEC O26 positive samples, isolation of microorganism according to ISO/TS 13136:2012

# Sampling at

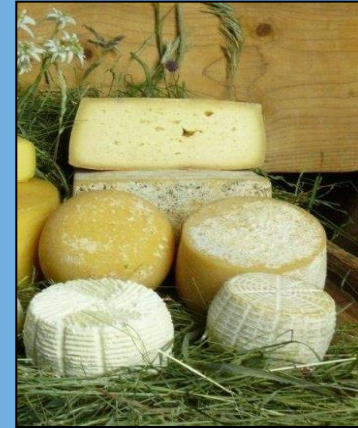
## MOUNTAIN PASTURE FARM with small dairy factory and B&B (1930 m asl)

### FOOD (July)

- 1 bulk milk sample
- 7 dairy products



NO STEC isolation



### FECAL SAMPLES

#### Sampling at MP farm (August)

- 24 individual fecal samples (animals from 3 different farms)



Isolation of *E. coli* O26 stx2 eae from 1/24 cows (herd 1)

#### Sampling at original farms (September)

(after the animals came back from the summer pastures)

- 39 fecal samples from herd 1
- 6 fecal samples from herd 2
- 9 fecal samples from herd 3



Isolation of *E. coli* O26 stx2 eae from 5/39 animals (2 calves and 3 heifers) of herd 1 only

Presence of O26 and stx genes in other 7 animals of herd 1 only

# Sampling at ALPINE DAIRY FARM with B&B (1300m asl)

## FOOD (July)

- 1 bulk milk sample
- 3 ice cream samples (not homemade)  
(5 more ice creams sampled by the same local producer)



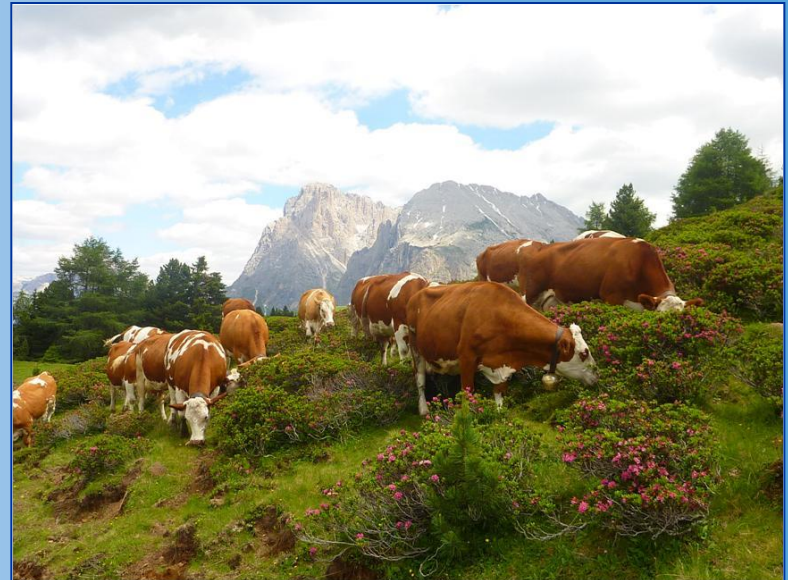
**NO STEC isolation**

## FECAL SAMPLES (July)

- 45 individual bovine fecal samples
- 2 individual goat fecal samples

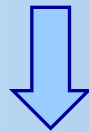


**NO STEC isolation**



## Further sampling (July - September)

- **3 SMALL TRADITIONAL DAIRY FARMS** (July- September):  
a total of 31 bovine fecal samples and 3 bulk milk samples
- **6 bovine cow pats** collected in the close proximity of a small stream (September)
- **WILD LIFE** (September): **3 red deer fecal samples**



**NO STEC isolation**



## Water samples (end of July)

11 WATER SAMPLES FROM MOUNTAIN SPRINGS, TANKS,  
FOUNTAINS AND AQUADUCTS

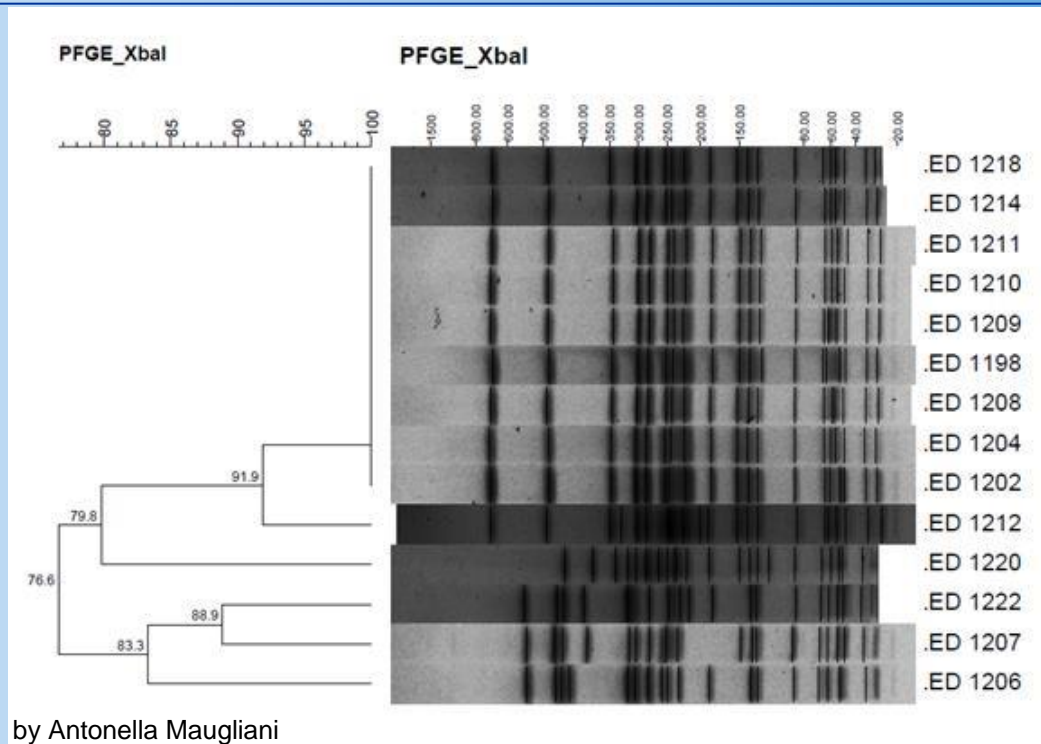
### RESULTS

**STEC were isolated from 4/11 water samples:**

- **E. coli O26 stx2 eae** from 2 water samples from the same mountain area with forest (one from a public aquaduct and one from a private spring close to **alpine dairy farm with B&B**), apparently not connected
- **E. coli O26 stx1 eae** from 1 water sample collected from a fountain near a small dairy farm
- **STEC stx1 stx2** from 1 water sample from a spring near the **mountain pasture farm with B&B**



PFGE at EU-RL highlighted 100% omology between the profile of *E. coli* O26 stx2 eae strains isolated from the 2 water samples and the human strains (isolated by AGES-Austria and ISS and SouthTirol Labs) apart from 2 (one of which anyway was very similar)



**Bovine strain O26 stx2 eae** from mountain pasture farm with B&B was not related with HUS strains



## In summary we tested:

- 156 bovine fecal samples
- 2 goat fecal samples
- 3 red deer fecal samples
- 11 water samples
- 20 food samples



## Conclusions



- STEC were isolated from 4/11 water samples
- 2 STEC O26 isolated from water with 100% omology with human STEC O26 HUS strains
- Bovine strain STEC O26 from mountain pasture farm with B&B was not related with STEC O26 HUS strains
- Cluster of STEC O26 cases apparently linked to water
- Further investigations are required

## Discussion: possible points raised....

- Analytical methods for STEC isolation from environment
- Mountain water springs contamination and risk of infection for local population and tourists, considering in particular young children
- Management of water resources and contamination prevention
- Possible source of STEC O26 strains... domestic/ wild ruminants, (humans)?
- Role of cattle as a reservoir of STEC O26 and STEC O26 ecology within the farm
- Need of prompt pathogen detection and source identification
- Importance of communication and cooperation among LNRs and National Authorities of different countries (good communication between LNR Austria and Italy)
- Need of continuous exchange of information and cooperation among Local and National Health Authorities involved in clinical diagnostics, microbiological and epidemiological surveys



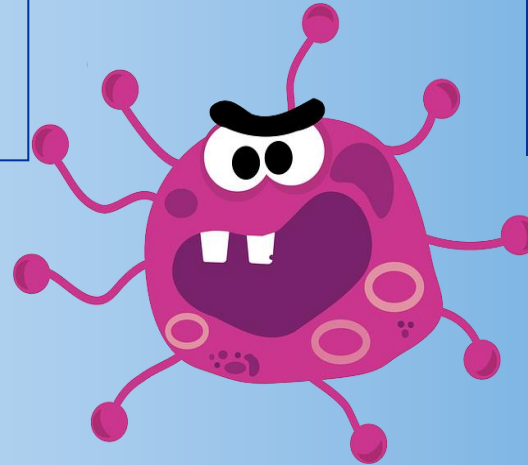
# Aknowledgements

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AUTONOME PROVINZ  
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**Dott.ssa MG Zuccaro**

**and their staff**

# ***Thank you!***

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