- **Epidemiological investigation:**
  - On 16 June, the family visited a didactic farm where children had contact with animals and their environment and were directly involved in a demonstration of cheese production
- **The NRL alerted the local health authorities and the regional Veterinary Public Health Institute (IZS Venezie)**

- **Feces and serum samples sent to the NRL: VTEC O26 (*vtx2*+) isolated; serum positive for Abs to the LPS of *E. coli* O26**

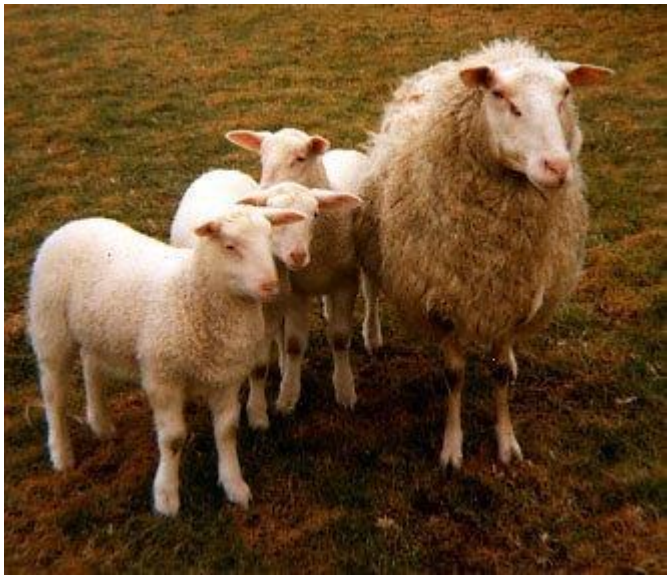
IZSVe: Microbiological investigation on the didactic farm

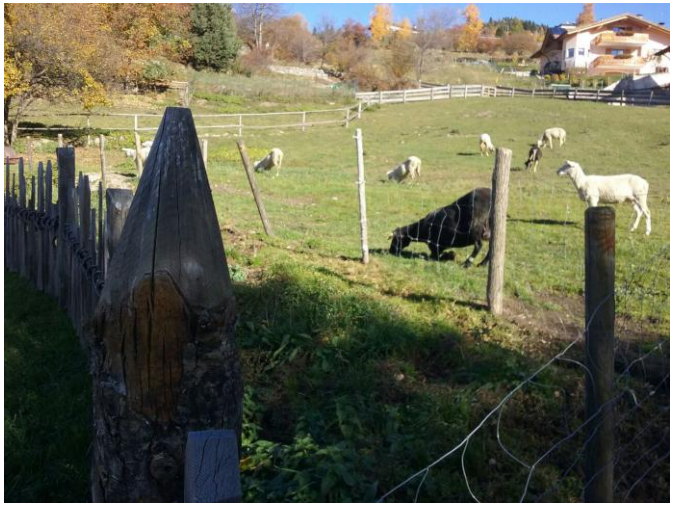
IZSVe: Investigation on the didactic farm: THE FARM

- Farm open to the public, hosting organized visits in different periods of the year (tourists, school-children, elderly)
- Located in the Prealps (about 1050 meters a.s.l)
- Family-run operation: different roles of each member
- Small dairy, completely separate from the farm and the milk tanks room.
- Production of bovine, ovine, goat cheese, ice-creams, yogurt
- Meals served mainly with own products

IZSVe: Investigation on the didactic farm: THE FARM

- **Small farm with**
  - **dairy cows (20),**
  - **sheep (60, Freisian cross-breed)**
  - **pigs (3)**
  - **goats (11): in a different separate premise (milk transported to the farm for cheese production)**
- **Cattle and sheep in warm months go outside during the day, grazing on separate pastures. Kept inside at night: two different areas of the same building separated by a central alley**





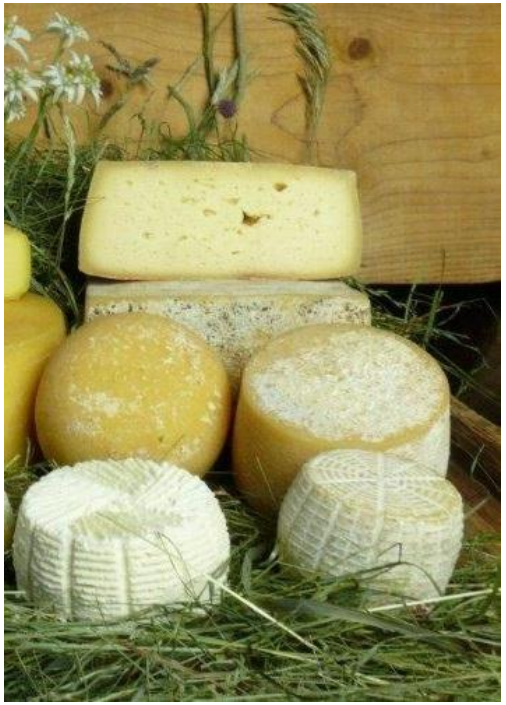
1st SAMPLING: milk and dairy products (6th July)

SAMPLING

- 3 bulk milk (bovine, sheep and goat milk)
- 3 cheese samples (bovine, sheep and goat cheese), (Reg. 2073, 5 Sample Units); lots of production close to the child visit
- METHOD: ISO/TS 13136 (25g)

RESULTS

- No VTEC isolated from all the milk and cheese samples
- Milk: - bovine: positive for vtx2 gene
  - ovine: positive for eae
- Cheeses: all positive for eae gene
  - bovine: negative for vtx genes
  - ovine: 1/5 S.U. positive for vtx1 gene
  - goat's: 5/5 S.U. positive for vtx1; 2/5 S.U. also positive for O145 gene serogroup
- Rather high Coagulase Positive Staphylococci (process hygiene criteria) and *E. coli* counts for all the cheeses



1st SAMPLING: fecal samples (7th July)

SAMPLING

- 20 faecal samples from sheep (9 rectal samples; 11 fresh fecal droppings)
- 20 rectal samples from cows
- 3 from pigs

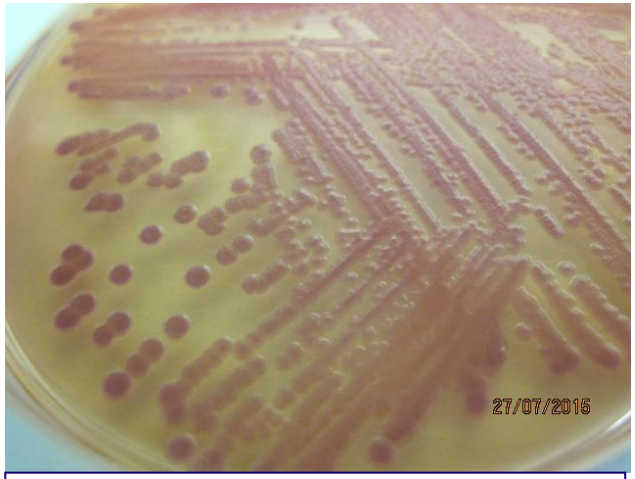
- METHOD: according ISO/TS 13136 (10gr samples)

RESULTS

- Most enrichment cultures from bovine ad ovine faeces tested positive for vtx genes (cattle: 17/20; sheep: 19/20), often in association with eae gene
- No O26 gene in cattle and pig samples
- 9/20 sheep samples PCR positive for O26, vtx1, vtx2 and eae genes
- VTEC O26, vtx2+ eae+ (4 isolates) from 2 of the 9 PCR-positive sheep samples
- Only enrichments plated onto CT-SMAC yelded VTEC O26 isolates; CT-SMAC suggested by NRL-ISS after successful isolation from child faecal sample (no from TBX, RMAC)
- Subtyping: STx2a

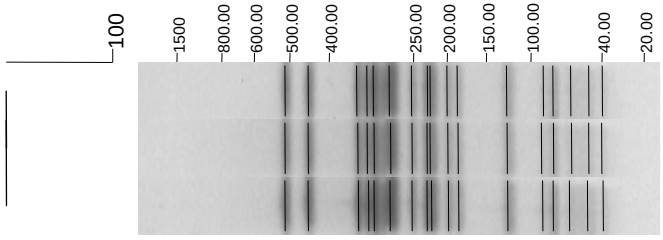
Human and animal strains comparison

- **4 isolates (respectively 3 from one sheep and one from another one) sent to NRL - Rome**
- **PFGE analysis showed an identical profile for the HUS human case and the sheep strains**



Sheep O26 VTEC isolate on CT-SMAC

PFGE_XbaI



ED 1032	O 26	ANIMAL (ovine)	2015
ED 1031	O 26	ANIMAL (ovine)	2015
ED 1027	O 26	HUMAN	2015

Joint inspection: Local Veterinary Service and VPH Laboratory (IZS Venezie)

Evidence of some critical points:

- **Animal management and milking performed by the same person with some lack of hygiene (workwear and boots)**
- **Possible cross-contamination between clean and dirty areas during the public visits**
- **Presence of fresh sheep faeces on a slope near the visitors gathering area**
- **Information to the public on possible risks from contact with animals and prevention given only orally; no signals**
- **Handwashing facilities well-organized but not immediately close to the food consumption area**
- **Cheese-production shown to the children, that sometimes taste the fresh cheese picking it by their hands**

Control Measures

- **General and detailed instructions were given by Veterinary Service on:**
  - GHP in dairy and farm environment (rooms, workers, cloths, equipment) and GMP
  - Need of strict separation for some activities (i.e. milking area, room exclusively for changing clothes and personal hygiene before dairy production)
  - Separation between different animal species
  - No visitor contact with sheep
  - Need of Manual for visitors with Access rules (forbidden and controlled areas)
  - No consumption of food used for demonstration
  - Communication and signage for visitors

- **Other actions:**
  - Further sampling to monitor animals and food safety
  - Specific education within courses for farmers who manage open/didactic farms
  - Update education for Veterinary Services involved in inspections on open farms

2nd and 3rd sampling visits on the farm

2nd and 3rd sampling: faecal samples, milk filters, water

- **No VTEC O26 isolated**

2nd - 27th July:

- **21 fecal samples collected from sheep (20 by rectal retrieving and 1 at necropsy).**

  Samples included the 2 previously positive sheep (one dead) with negative results for O26 gene.
- **8/21 were positive for O26, vtx1,vtx2 and eae genes.**

3rd - 28th September:

- **20 fecal samples collected from the same sheep.**
- **3/20 were positive for O26, vtx1, vtx2 and eae genes (2 positive at the previous visit)1**

- **Sheep milk filters (2):**

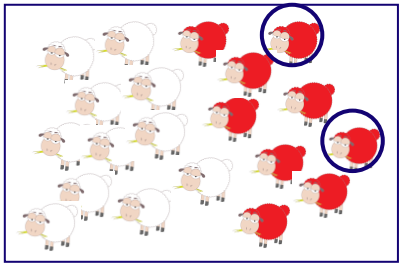
  positive for O26, vtx1,vtx2 and eae genes in both the visits

- **Water-trough samples (4):**

  always negative for vtx genes; one positive for O26 and eae genes

- **4th visit planned in November: it will include also cattle sampling**

Evolution of O26 shedding along the three sampling visits

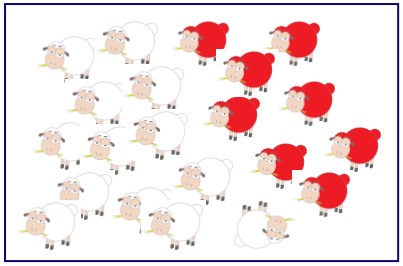


1° sampling (7th July)

vtx, eae and O26 genes from 9 animals

VTEC O26 strains isolated from 2 animals

9+/20



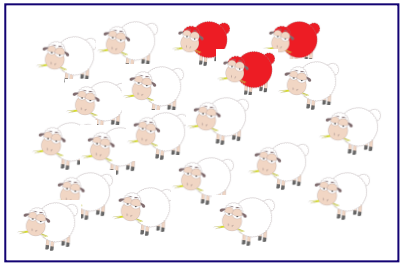
2° sampling (27th July)

vtx, eae and O26 genes from 8 animals

No VTEC O26 isolates

8+/21

(1 dead)



3° sampling (28th September)

vtx, eae and O26 genes from 3 animals

No VTEC O26 isolates

3+/20

What can we expect in the future samplings?

Conclusions

- **Prompt pathogen detection and source identification due to collaboration and communication among public health institutions, NRL, Veterinary service and labs → enforcement of preventive measures on the farm!**

  ONE HEALTH!

- **Need of Training and Education on risks of infection in open farms directed to farmers, visitors, PH Officials has emerged**

- **Interesting aspects to clarify:**
  - **VTEC O26 in sheep only**
  - **No O26 gene detection in cattle samples**
    somehow surprising in a small farm (box separation but possibility of indirect contacts)

E.coli O26 *vtx2* +: which is the reservoir ??

VTEC O26 in cattle

- ***E. coli* O26 *eae*+, *vtx*-: frequent (3% - 24% of samples)**
- **VTEC O26: rare (0.2% - 1% of samples)**
- ***E.coli* O26 *vtx2* +: very rare**

Stromberg et al. Foodborne Pathog Dis. 2015 Jul;12(7):631-8.
Bonardi et al. Vet Rec Open. 2015 Jan 20;2(1)
Dewsbury,et al. Foodborne Pathog Dis. 2015;12:726-32.
Paddock,et al. Foodborne Pathog Dis. 2014;11:186-93.
Bibbal et al. Appl Environ Microbiol. 2015 Feb;81(4):1397-1405.

E.coli O26 *vtx2* +: which is the reservoir ??

VTEC O26 in sheep

- ***E. coli* O26 *eae*+, *vtx*-: frequent (5% - 19% of samples)**
- **VTEC O26: rare (0.2% - 1.1% of samples)**
- ***E.coli* O26 *vtx2* +: possibly rare (0.1% - 0.5% of samples)**

Sekse et al.. Appl Environ Microbiol. 2011;77: 4949-58
Evans et al. J Med Microbiol. 2011;60: 653-60.
Blanco et al.. J Clin Microbiol. 2003; 41: 1351–1356

E.coli O26 *vtx2* +: which is the reservoir ??

E. coli O26 *vtx2*+ in cattle:

1 out of 26 VTEC strains reported (3,8%)

Stromberg et al. Foodborne Pathog Dis. 2015 Jul;12(7):631-8.
Bonardi et al. Vet Rec Open. 2015 Jan 20;2(1)
Dewsbury,et al. Foodborne Pathog Dis. 2015;12:726-32.
Paddock,et al. Foodborne Pathog Dis. 2014;11:186-93.
Bibbal et al. Appl Environ Microbiol. 2015 Feb;81(4):1397-1405.

E. coli O26 *vtx2*+ (vtx1+/-) in sheep:

7 out of 17 VTEC strains reported: (41,2%)

(4 vtx2, 3 vtx1 + vtx2)

Sekse et al.. Appl Environ Microbiol. 2011;77: 4949-58
Evans et al. J Med Microbiol. 2011;60: 653-60.
Blanco et al.. J Clin Microbiol. 2003; 41: 1351–1356

Aspects to clarify

- *E. coli* O26 vtx2+: which is the main reservoir?
- How/when was introduced into the farm? Contribution of trading animals?
- Other possible reservoirs, vehicles or source of contamination in this open farm?
- O26 persistence in animals and shedding evolution? The role of farm environment in O26 maintenance?

Acknowledgements

- The IZSVe – Pordenone lab staff
  - A. Pierasco
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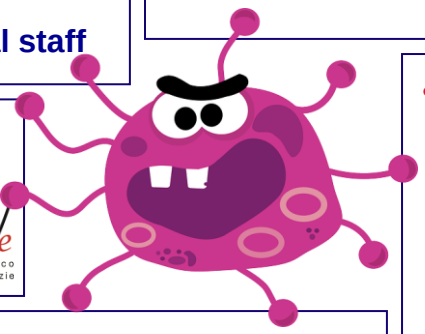

Thank you for your attention!