Enhanced diagnostic protocol to identify *E.coli* VTEC from milk filters

Claudia Gusmara, Francesca Albonico, Monica Loiacono, Theo Gugliotta, Michele Mortarino & Alfonso Zecconi

INTRODUCTION

The EHEC (enterohemorrhagic *E. coli*) are a subgroup of VTEC with strong pathogenicity. The most well-known EHEC serotype is E. coli O157:H7, which has been implicated in many large outbreaks of deadly human diseases. However, EHEC strains of other serotypes have increasingly been implicated in sporadic cases and outbreaks of serious illness in humans, e.g., serotypes O26, O45, O103, O111, O121 and O145. Epidemics studied from 1982 to date have shown that ruminants, and in particular bovine, appear almost always involved in the transmission of these bacteria to humans through direct or indirect fecal contamination of foods. Unpasteurized milk and milk products are considered minor, but important sources of infection. The possible ways to the entrance of VTEC in milk are fecal contamination and mammary excretion during *E.coli* mastitis. Between the two ways, the first is considered much more frequent in practice, but it cannot be excluded that a small part of EHEC found in milk resulting from mammary gland.
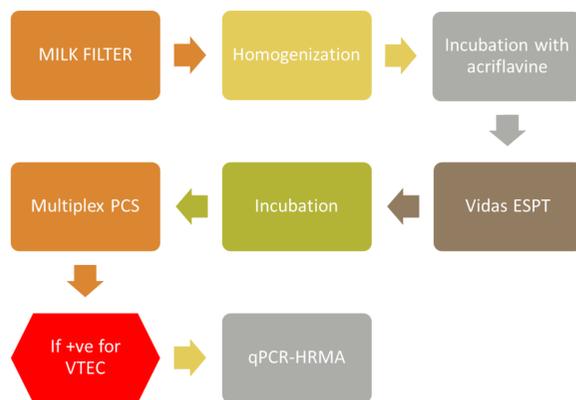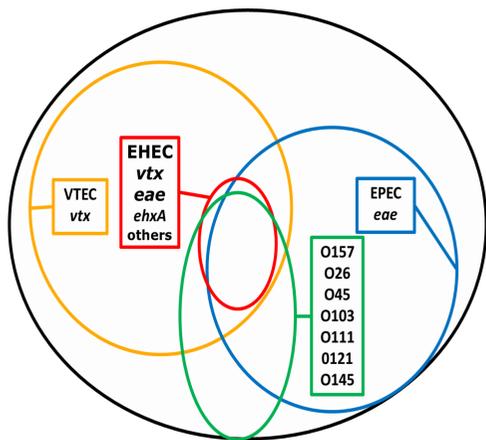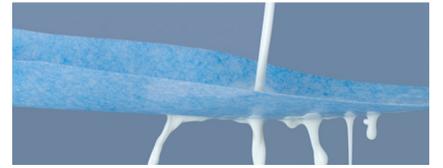
Previous investigation suggested that milk filters used in milking machines could be a useful control point to identify the presence of EHEC in dairy herds. However, conventional methods to identify the presence of EHEC have a poor sensitivity due the high content of fecal bacteria of these filters.

VTEC and EHEC

Relationship among the different serotypes and pathotypes of *E.coli* involved in enterohemorragic diseases

MATERIALS & METHODS

In order to set up a monitoring scheme to identify herds at risk, we developed and tested a diagnostic protocol involving VIDAS® UP *E.coli* serogroups (ESPT) which is a method using phage recombinant proteins for the immuno-concentration (IC) of *E.coli* serogroups O157, O26, O103, O111, O145, O45 and O121 from food, multiplex PCR and high resolution melting analysis (HRMA).

Practically, bulk tank milk or washing solution obtained from milk filters after stomacher mixing were analyzed by Vidas ESPT. After incubation, the solution obtained was analyzed by multiplex PCR based on serotype-specific primers coding for O-antigen regions of the seven major VTEC serogroups available in literature. If PCR was positive for any of the seven serogroups, a HRMA-based protocol to detect virulence-predictive SNPs, as discovered by Norman et al., 2012, was applied to confirm the presence of a EHEC strain.

The protocol was preliminary validated by inoculation of milk and milk filters with a known concentration of the seven EHEC serotypes (O26, O45, O103, O111, O121, O145, O157). The results confirmed that this protocol was able to identify as low as 10^1 UFC in both milk and milk filters.
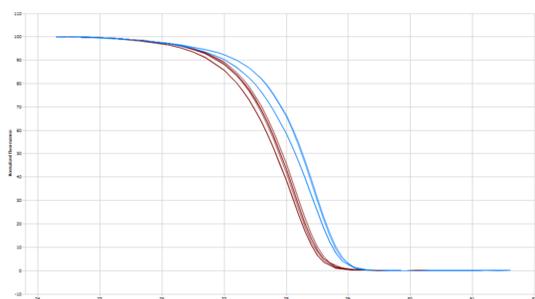

Work flow

HRMA for EHEC confirmation

Melting curves from isolates with or without the virulence predictive SNP.

O26 red (A) { Stx1 +ve / Stx2 +ve

O26 blue (G) { Stx1 −ve / Stx2 −ve

HRMA results

The tables reports the proportion of *E.coli* strains confirmed to harbor EHEC virulence genes based on their serotype

Serogroup	N°	EHEC	%
O157	10	10	100%
O103	3	1	33%
O26	5	0	0%
O45	2	2	100%
O145	2	2	100%
O121	4	2	50%
Total	**20**	**17**	**65%**

	Samples	+ve	%	Serogroup	N°	%
Milk filters (Lombardy region)	47	14	29,8	O157	7	14,89
				O103	1	2,13
				O26	3	6,38
				O26+O121	1	2,13
				O157+O121	1	2,13
				O157+O26+O103	1	2,13
Milk filters (Trentino region)	20	6	30,0	O145	2	10,00
				O45	2	10,00
				O103	1	5,00
				O121+O157	1	5,00
Total	67	20	29,9	O157	10	14,93
				O103	3	4,48
				O26	5	7,46
				O45	2	2,99
				O145	2	2,99
				O121	3	4,48

VTEC isolated and their serogroups

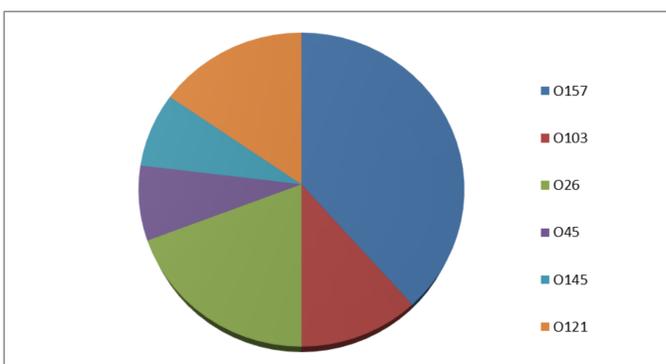### Proportion of serogroups among VTEC positives

CONCLUSIONS

The developed protocol and the technologies applied showed to have a good sensitivity and to be relatively easy to apply under field conditions.

Data confirmed that milk could be a source of VTEC and, in absence of proper heat treatment, this could increase the risk for foodborne diseases.

The serotypes isolated from milking machine filters are 6, suggesting that the protocol applied in North America targeting 7 different serotypes is appropriate to identify major EHEC involved in human diseases (Top 7). However, not all the VTEC identified confirmed to be EHEC, supporting the need to confirm the presence of a EHEC when a suspected strain is isolated.

Milk filters confirmed to be a very useful critical detection point to identify herd at risk for presence of VTEC and they could be a starting point to identify cow at risk, by individual sampling.