Enhanced diagnostic protocol to identify E.coli VTEC from milk filters
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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 epidemics, 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 milk products are considered minor, but important sources of infection. The protocol was preliminary validated by inoculation of milk and 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-serotypes 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.

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, O111, O145, O45 and O121 from food, multiplex PCR and high resolution melting analysis (HRMA).

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 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.

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.

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