



SHIGA TOXIN-PRODUCING ESCHERICHIA COLI CONTAMINATION OF RAW MILK CURDS PRODUCED IN ALPINE DAIRIES OF THE PROVINCE OF TRENTO, ITALY

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Aim. In recent years, raw milk cheeses produced in alpine dairies in the Province of Trento (Northern Italy) have occasionally tested positive for Shiga toxin-producing *Escherichia coli* (STEC), pathogens capable of causing severe illness, including hemolytic uremic syndrome. Specific self-monitoring protocols have been implemented as part of a comprehensive STEC surveillance strategy encompassing the entire dairy supply chain, including alpine facilities. This study assessed STEC occurrence in raw cow milk curds produced in alpine dairies in the Province of Trento during the summer seasons.

Methods. Between June and October 2023, 2024, and 2025, curd samples (n = 1,331) were collected from 32 alpine dairies located between 1,208 and 2,087 m above sea level, representing approximately one-third of the local alpine dairies. Samples were collected during curd extraction from the cheese vat. STEC detection was conducted according to ISO/TS 13136:2012 and the ISS EU-RL VTEC 4, Revision 2 protocol (2021), with initial screening for stx1, stx2, and eae genes by real-time PCR. Following a positive PCR result for at least one of the two stx genes, culture-based confirmation (CBC) was either performed or omitted, depending on the surveillance protocol (SP). Under the STEC risk management strategy adopted by the Provincial Health Authority and the economic impact of implementing control measures for the detection of STEC, food business operators could choose among six SPs. SP1 required PCR analysis of all curds produced during the alpine grazing season, followed by CBC for PCR-positive samples; SP2 included PCR analysis of all curds produced during the first three weeks of the season, followed by CBC for PCR-positive samples; SP3 required PCR analysis of all curds produced during the first

three weeks, without CBC; SP4 included weekly PCR analysis of one curd for five consecutive weeks, without CBC; SP5 applied to cheeses intended for ripening for at least 12 months, for which curd testing was not required; SP6 included alternative sampling plans (with or without CBC) and sampling frequency defined by the food business operator based on its own risk assessment. PCR-negative samples for stx genes were classified as compliant (C). PCR-positive samples not subjected to CBC were classified as non-compliant (NC). Finally, PCR-positive samples undergoing CBC were classified as NC if viable STEC were isolated or as C if CBC yielded negative results.

Results. Overall, 100 of the 1,331 samples (7.51%) were PCR-positive: 66 carried stx1, 19 stx2, and 15 both genes. Two PCR-positive samples not subjected to CBC were classified as NC because they were analyzed under SP4 and SP6. Among the remaining 98 PCR-positive samples, CBC confirmed 23 NC samples: 10 carrying stx1, 9 stx2, and 4 both genes. Among the confirmed NC samples, seven also carried the eae gene (6 in combination with stx2 and 1 with stx1). Including the two PCR-positive samples not subjected to CBC, a total of 25 NC samples (1.88%) were identified.

Conclusions. The occurrence of STEC in curds produced by alpine dairies was consistent with values reported in the literature in other raw milk production systems, with NC samples detected in a limited number of dairies. Nevertheless, the microbiological hazards associated with raw milk products should not be underestimated, and continued surveillance together with ongoing awareness and training activities for food business operators should be pursued.