



DETECTION AND CHARACTERIZATION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI SEROGROUP O103 FROM OVINE RAW MILK CHEESES AT DIFFERENT STAGES OF RIPENING

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Aim. The aim of this study was to evaluate the presence of the main Shiga-toxin-producing *Escherichia coli* (STEC) serogroups in differently ripened ovine raw milk cheese samples.

Methods. During the period between May and December 2025, a total of 168 ovine raw milk cheese samples, obtained from bulk milk and ripened at different stages, were collected from 7 different small and medium-sized food enterprises (SME₁₋₇) located in the provinces of L'Aquila, Teramo and Pescara (Abruzzo region, Italy). Each SME was visited 8 times during the study period, and at each visit, one cheese sample (75.0 g), from the same cheese wheels, was aseptically collected for each ripening interval: day 3 (t₃), 15 (t₁₅), and 30 (t₃₀). This resulted in 56 samples for each ripening interval, gaining a total of 168 samples across all standardized commercial productions. The experimental design adopted a culture dependent approach primarily aimed at STEC detection, also including quantitative microbiological analyses as hygienic indicators. Selective STEC detection was performed according to the ISO 13136:2013. For each sample, 25.0 g of cheese (including rind, medium and core) were homogenized in 225 mL of buffered peptone water (BPW) and incubated at 37.0 °C for 24 hours. Following enrichment, a loopful was streaked onto Tryptone Bile X-glucuronide (TBX) agar and incubated at 44.0 °C for 24 hours. Suspected positive blue/green colonies were subcultured on nutrient agar and subjected to preliminary biochemical identification using the Vitek 2 system. DNA was extracted from isolates and initially screened by multiplex PCR for virulence genes (eae, stx1 and stx2). These qualitative results were followed by the real-time PCR assays targeting both virulence genes and O-antigen genes specific for STEC sero-

groups [rfbE (O157), wbdL (O111), wzx (O26), ihp1 (O145), and wzx (O103)] (expressed as genome copies per millilitre - GC/mL), as described in the Annex E - ISO 13136:2012. Amplicons were sequenced and a phylogenetic tree was constructed. Additionally, quantitative microbiological parameters, such as Enterobacteriaceae (EBC) and *E. coli* (ECC), were assessed in parallel as hygienic quality indicators.

Results. At stage t₃, 9/56 (16.07%) samples presented typical blue-green colonies on TBX agar plates coming from 6 SME (SME₁₋₄, SME₆₋₇). At stage t₁₅, 4/56 (7.14%) still grew suspected positive ones (SME₁, SME₃₋₄, SME₇), and at stage t₃₀, 1/56 (1.78%) was still positive from SME₄. Seven out of nine (77.78%) strains harbored the stx1, stx2 and eae virulence genes with different GC/mL amounts (average values: 10⁴ GC/mL for stx1, 10¹ stx2, and 10⁴ GC/mL eae), according to the real time PCR assays. All the *E. coli* belonged to the O103 serogroup. In summary, 7/168 (4.17%) of raw milk cheese were positive for STEC. From a ripening perspective, 4/7 (57.14%) STEC were isolated at t₃ from SME₁, SME₃, SME₄, and SME₇, 2/7 (28.57%) at t₁₅ from SME₃ and SME₄, and 1/7 (14.28%) at t₃₀ from SME₄. The EBC, in the screened raw milk cheese samples, ranged between 4.0 to 7.0 log₁₀ CFU/g, with a mean value of 5.2 log₁₀ CFU/g, while the ECC ranged between 2.0 - 6.0 log₁₀ CFU/g with an average value of 2.9 log₁₀ CFU/g.

Conclusions. These results emphasize the food safety risks associated with STEC O103 contamination in raw ovine milk cheese. The detection and suboptimal hygienic indicators reinforce the need for strict microbiological surveillance to ensure consumer protection.