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Sabine Delannoy, Mai-Lan Tran, Patrick Fach. New molecular methods to detect pathogenic STEC in dairy products. VTEC 2023 - 11th International Symposium on Shiga Toxin (Verocytotoxin) Producing Escherichia coli Infections, May 2023, Banff, Canada. anses-04206789

HAL Id: anses-04206789

<https://anses.hal.science/anses-04206789>

Submitted on 14 Sep 2023

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New molecular methods to detect pathogenic STEC in dairy products

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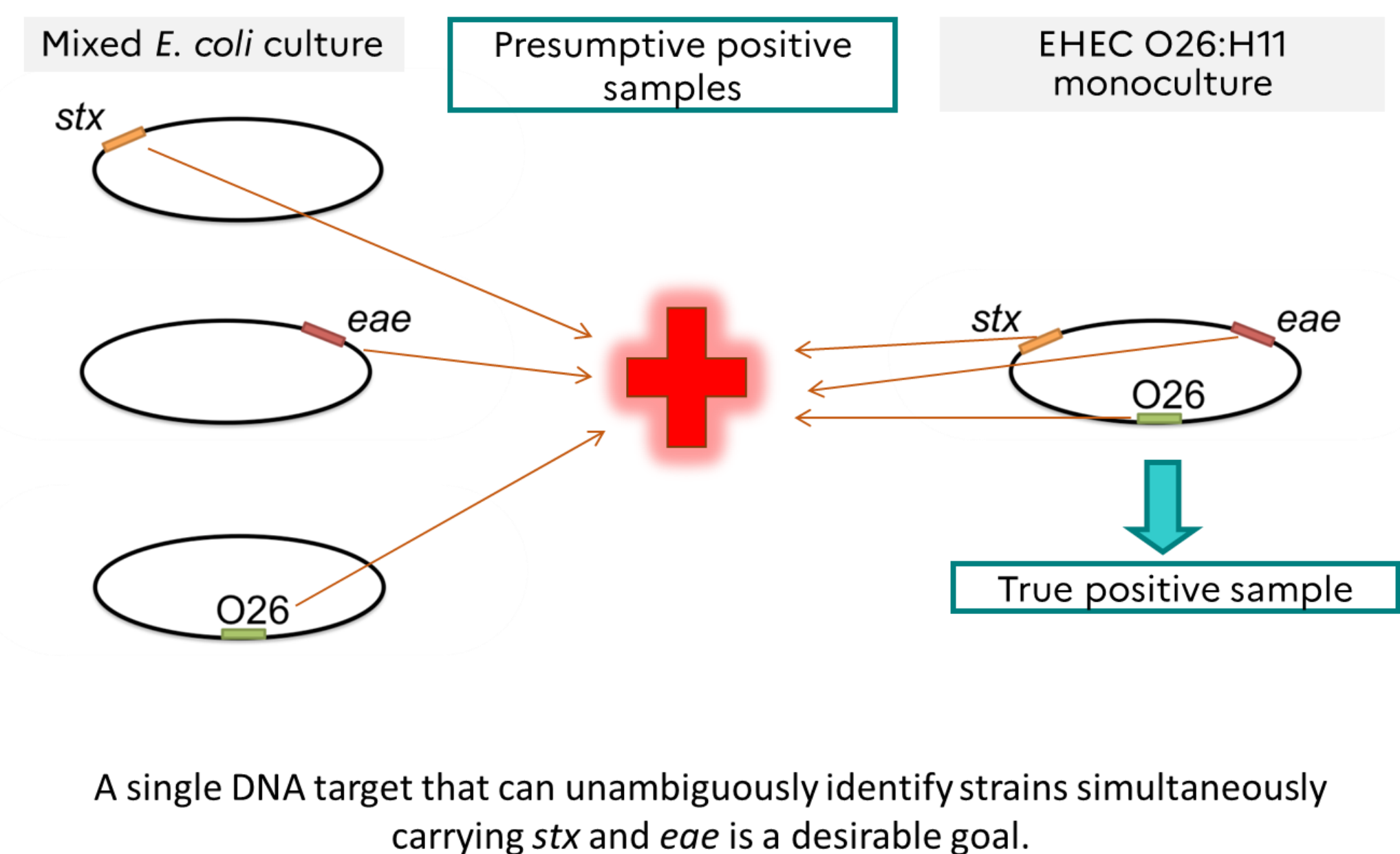


Introduction

- Molecular methods for screening pathogenic STEC in dairy products typically rely on the molecular detection of *stx*, *eae*, and the Top5 or Top7 EHEC serogroups in mixed bacterial enrichments (ISO/TS 13136:2012 and MLG 5C).
- We previously identified five genetic markers (*espK*, *espV*, Z2088, *ureD* and CRISPR_O26), which are preferentially associated with typical EHEC (*E. coli* strains positive for the *stx* and *eae* genes, irrespective of their serotype), and demonstrated that they could reduce, at the first screening step, the number of presumptive positive samples in beef enrichments by 48.9%.
- Here, high throughput qPCR was used to assess the contribution of these genetic markers in the dairy sector in the reduction of the number of presumptive positive samples after the first phase of screening by *stx* and *eae*.

Challenge in interpreting PCR results in polymicrobial samples

Genetic markers when detected in foods (mixture of bacteria) may derive from several clones (each being non-pathogenic)



Materials & Methods

The sampling scheme was designed to test the efficacy of the alternative screening method in reducing the number of samples presumptive positive for pathogenic STEC. It does not reflect prevalence of STEC in the French dairy sector.

A total of **4691 DNA extracts** from raw milk and raw milk products enrichments (from the goat, sheep and cow dairy sectors) were received from routine testing of raw milk products (Table 1).

The Biomark™ (Fluidigm, USA) was used for **high-throughput microfluidic real-time PCR** amplification using 192.24 dynamic arrays following a pre-amplification step. All samples were tested in duplicate.

Table 1. Dairy samples (n=4,691) selected in this study. Distribution of the number of samples according to the animal species (cow, goat and sheep), the dairy matrices (milk, cheese) and the IMS pre-treatment of the enriched culture.

Animal species	Dairy matrix	With IMS pre-treatment	Without IMS pre-treatment
Goat samples (N=1,601)	Milk	165	1025
	Cheese	408	3
Sheep samples (N=965)	Milk	331	7
	Cheese	13	614
Cow samples (N=2,125)	Milk	926	77
	Cheese	304	818

Results

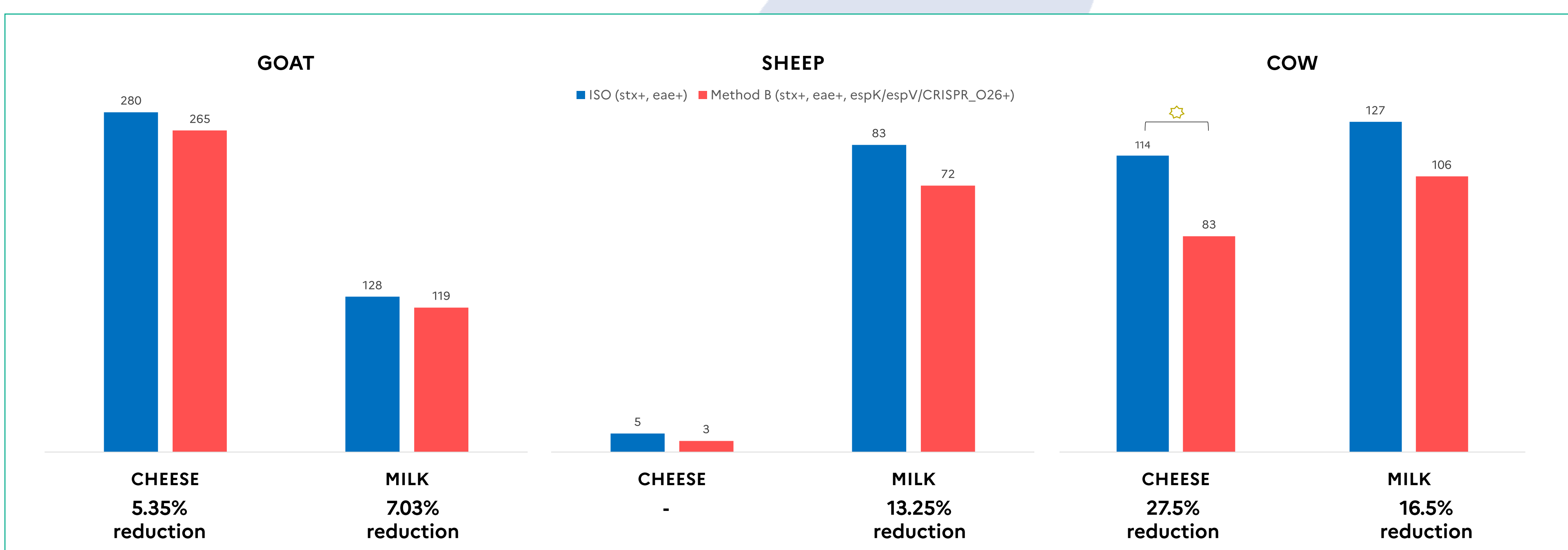


Figure 1. Number of presumptive positive dairy samples pre-treated **with IMS** using the ISO/TS 13136:2012 method (*stx*+, *eae*+) or the alternative method B (*stx*+, *eae*+, *espK/espV/CRISPR_O26*+).

Percentage of reduction of presumptive positive samples is indicated below each chart. The star indicates a significant reduction, $p < 0.05$.

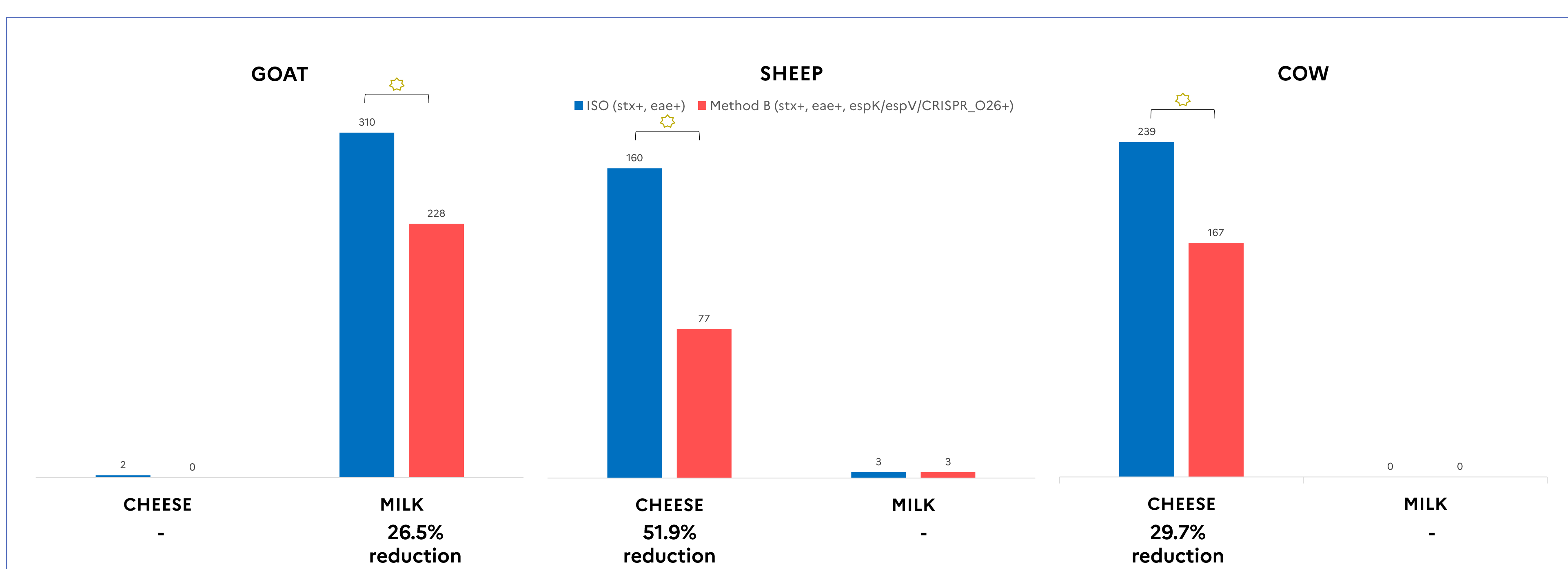


Figure 2. Number of presumptive positive dairy samples treated **without IMS** using the ISO/TS 13136:2012 method (*stx*+, *eae*+) or the alternative method B (*stx*+, *eae*+, *espK/espV/CRISPR_O26*+).

Percentage of reduction of presumptive positive samples is indicated below each chart. The star indicates a significant reduction, $p < 0.05$.

EHEC strains (n = 297) were isolated from these samples by the routine testing laboratories during the confirmation step. All but three were isolated from samples positive with method B, showing that these markers can be used with high confidence in the dairy industry.

Conclusion

Introduction of the new EHEC markers provided a significant reduction of the number of *stx*+/*eae*+ samples that require a second screening step for serogroup determination according to the ISO/TS 13136:2012 reference method.