# Identification of virulotypes and serotypes of enteropathogenic (EPEC) and Shigatoxigenic (STEC) *Escherichia coli* from healthy cattle at slaughterhouses in Wallonia.

Takaki S.[1,2], Duprez J.N.[1], Fakih I.[1], Korsak N.[3], Thiry D.[1], Mainil J.[1]

[1] Bacteriology, Department of Infectious Diseases and [3] Food Inspection, Department of Food Science, Faculty of Veterinary Medicine and Centre for Fundamental and Applied Research in Animal Health (FARAH), University of Liège, Belgium; [2] Department of Veterinary Medicine, Faculty of Agriculture, University of Miyazaki, Japan.

## INTRODUCTION

*Escherichia coli* producing the attachment-effacement (AE) lesion (EPEC) and/or Shiga toxins (STEC) cause enteritis and (bloody) diarrhoea in young calves and in humans, and are also present in the intestines of healthy cattle. Besides the O157:H7 serotype, which has been the main serotype causing STEC outbreaks in the world, EPEC and STEC can belong to dozens of O serogroups. Of them, 9 have been frequently identified worldwide: O5, O26, O103, O104, O111, O118, O121, O145 and O165.

## AIM

The aim of this study is to identify the virulotypes and serotypes of EPEC and STEC isolated from healthy cattle at slaughterhouses in Wallonia by DNA-DNA colony hybridization and multiplex PCRs.

## MATERIALS and METHODS

Faecal samples from 216 <1-year-old bulls, 25 cows and 4 heifers collected between April and June 2014 in 2 slaughterhouses in Wallonia were grown overnight at 37°C in Lauryl sulfate *Enterobacteriaceae* selective broth. The enrichment broths were assayed with an *stx1*, *stx2* (Shiga toxins) and *eae* (AE lesion) triplex PCR. Positive broths were inoculated onto 4 plates: McConkey's agar, Chromagar ES, Chromagar ES with tellurite, and Chromagar STEC.

A total of 2542 coliform isolates were subcultured and tested by the colony hybridization assay with gene probes targeting the *stx1*, *stx2* and *eae* genes The triplex PCR was again performed on all probe-positive isolates. The PCR-positive *E. coli* were subsequently assayed with two pentaplex PCR targeting the specific genes coding for the ten O serogroups listed above.

## RESULTS: COLONY HYBRIDIZATION

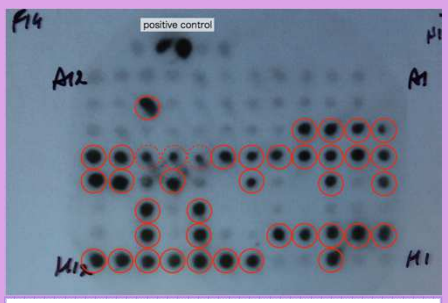


**Figure 1**: Filter of colony hybridization. The black dots with red circles correspond to probe-positive isolates.

- 744 out of the 2542 coliform isolates were positive with at least one gene probe: stx1, stx2 and/or eae;
- these 744 probe-positive isolates originated from 69 out of the 245 animals sampled.

**Table 1**: Number of probe-positive isolates after DNA-DNA colony hybridization.

| | eae | stx1 | stx2 | eae, stx1 | eae, stx2 | stx1, stx2 | eae, stx1, stx2 | TOTAL |
|---|---|---|---|---|---|---|---|---|
| MC (n=690) | 52 | 0 | 3 | 3 | 0 | 12 | 0 | 70 |
| ES (n=680) | 56 | 6 | 3 | 0 | 0 | 8 | 0 | 73 |
| ES tell (n=483) | 98 | 5 | 57 | 32 | 3 | 7 | 2 | 204 |
| STEC (n=689) | 229 | 18 | 49 | 59 | 13 | 23 | 6 | 397 |
| TOTAL (n=2542) | 435 | 29 | 112 | 94 | 16 | 50 | 8 | 744 |

MS: McConkey's agar, ES: Chromagar ES, ES tell: Chromagar ES with tellurite, STEC: Chromagar STEC.

## RESULTS: TRIPLEX PCR

- The positive isolates of colony hybridization were tested by the triplex PCR for confirmation;
- 611 out of the 744 probe-positive isolates gave identical results with the triplex PCR and the colony hybridization assay. The rate of concordance is 82,1%;
- another 67 isolates gave partially corresponding results and 66 isolates were PCR-negative. These isolates are being tested once more with the triplex PCR.

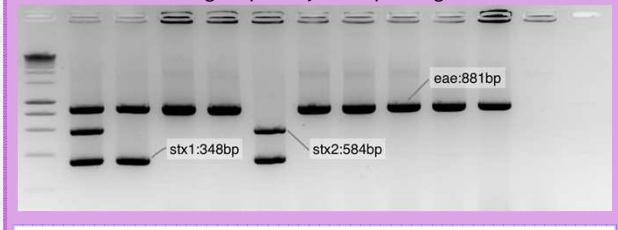


**Figure 2**: *stx1*, *stx2* and *eae* triplex PCR on 8 probe-positive isolates. The first two wells are positive controls and the last two wells are negative controls.

**Table 2**: Number of positive samples after triplex PCR.

| | eae | stx1 | stx2 | eae, stx1 | eae, stx2 | stx1, stx2 | eae, stx1, stx2 | TOTAL |
|---|---|---|---|---|---|---|---|---|
| MC (n=70) | 38 | 0 | 2 | 4 | 0 | 11 | 0 | 55 |
| ES (n=73) | 42 | 6 | 3 | 0 | 0 | 8 | 0 | 59 |
| ES tell (n=204) | 86 | 2 | 56 | 36 | 3 | 6 | 0 | 189 |
| STEC (n=397) | 202 | 14 | 55 | 67 | 2 | 21 | 13 | 374 |
| TOTAL (n=744) | 368 | 22 | 116 | 107 | 5 | 46 | 13 | 677 |

MS: McConkey's agar, ES: Chromagar ES, ES tell: Chromagar ES with tellurite, STEC: Chromagar STEC.

## RESULTS: PENTAPLEX PCR

- All of triplex PCR-positive isolates are right now being tested with the two pentaplex PCR to detect the specific genes encoding 10 most frequent and/or pathogenic O-serogroups in humans and/or cattle: O5, O26, O103, O104, O111, O118, O121, O145, O157 and O165.

## DISCUSSION and CONCLUSION

These results confirm that EPEC and STEC, which could represent a public health hazard, are observed in healthy cattle at slaughterhouse in Wallonia. The colony hybridization and the triplex PCR results show over 80% of concordance.

The colony hybridization is useful as a first step assay in large-scale studies and can improve the field surveillance and/or monitoring programs in combination. It can also help to predict the prevalence of EPEC, STEC, and AE_STEC in healthy cattle and humans and to trace the source of an infection alon with the different multiplex PCR assays.

Further studies are necessary to compare EPEC and STEC from young calves, healthy cattle and humans in order to identify host- and/or age-specific properties.

## REFERENCES

***Mainil, J. et al. (1993).*** Association between the effacing (*eae*) gene and the Shiga-like toxin (SLT)-encoding genes in *Escherichia coli* isolates from cattle. *American Journal of Veterinary Research*, 54, 1064-1068.

***Mainil J. and Daube G. (2005).*** Verotoxigenic *Escherichia coli* from animals, humans and foods: Who's who? *Journal of Applied Microbiology*, 98, 1332–1344.

***Mekata H. et al. (2014).*** Identification of O Serotypes, Genotypes, and Virulotypes of Shiga Toxin-Producing *Escherichia coli* Isolates, Including Non-O157 from Beef Cattle in Japan. *Journal of Food Protection*, 77, 1269-1274.

***Fakhi I. et al. (2016).*** Identification of Shiga toxin-producing *Escherichia coli* (STEC) in diarrhoeic calves and comparative genomics of O5:H- bovine and human STEC, *Veterinary Microbiology*, 10.1016/j.vetmic.2016.02.017.