Range of O-serogroups of Shiga toxin-producing (STEC) and enteropathogenic (EPEC) *Escherichia coli* in cattle in Wallonia

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INTRODUCTION

Escherichia coli producing Shiga toxins (STEC or ShigaToxigenic *E. coli*) and/or the attachment-effacement (AE) histological lesion (EPEC or EnteroPathogenic *E. coli*) cause enteritis with (bloody) diarrhoea in humans and young calves and the haemolytic uremic syndrome (HUS) in humans. Infection of humans through consumption of foodstuffs (in)directly contaminated by cattle faeces (which are healthy intestinal carriers) is proved for STEC and suspected for EPEC. Besides the O157:H7 serotype, STEC and EPEC can belong to more than sixty O serogroups. Of them, 10 are regularly identified in humans and/or calves worldwide: O5, O26, O45, O103, O104, O111, O118, O121, O145 and O165. This study aimed at identifying the O serogroups of STEC and EPEC isolated from (i) diarrheic calves and (ii) healthy bovines at 2 slaughterhouses in Wallonia.

MATERIALS and METHODS

(i) 233 enterohaemolysin-positive *E. coli* were isolated at ARSIA from diarrhoeic calves after growth on EHLY Medium. They all were tested with a triplex PCR targeting the *stx1*, *stx2* (Shiga toxins) and *eae* (AE lesion) genes (Fig. 1; Bardiau et al., 2010).

(ii) large intestine contents were sampled at 2 slaughterhouses in Wallonia from (165 <1 year-old bulls, 23 cows and 4 heifers) and grown overnight at 37° C in Lauryl sulfate *Enterobacteriacae* selective broth. The enrichment broths were assayed with the *stx1*/*stx2*/*eae* triplex PCR. Positive broths were inoculated onto agar plates (McConkey's, Chroagar ES with tellurite and Chromagar STEC, Fig. 2) and 10 colonies from each plate were picked up for testing with the same triplex PCR.

(iii) all triplex PCR-positive *E. coli* were further assayed with a multiplex PCR targeting the specific genes coding for the O serogroups listed above (Mekata et al., 2014). (Fig 3)

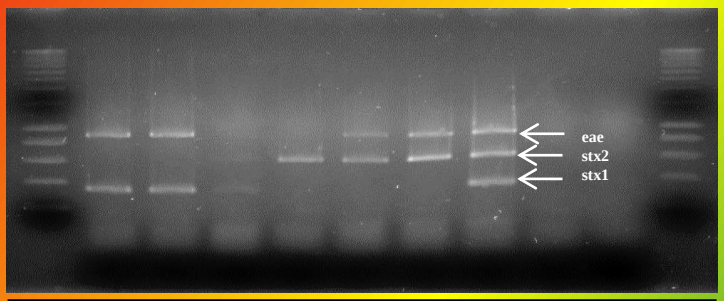

Fig. 1: triplex PCR targeting the *stx1*, *stx2* and/or *eae* genes

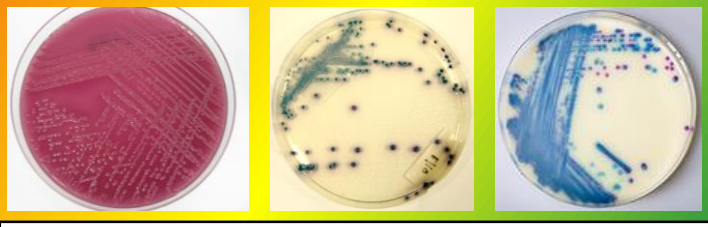

Fig. 2: Coliform in Mac conkey agar, tellurite resitant *E.coli* in Chromagar ES with tellurite , STEC (pink) and no pathogen *E.coli* (blue) in Chromagar STEC

RESULTS

(i) the triplex PCR was performed in duplicate by two different manipulators on the E. coli isolated from diarrheic calves: 69 tested positive with the PCR for the *stx1* and/or *stx2* and/or *eae* genes (Table 1) and 81 negative for the 3 genes. 83 strains gave conflicting results and are being checked.

TABLE 1: No. *E. coli* positive with the triplex PCR for ...

1 gene			2 genes			3 genes
stx1	*stx2*	*eae*	*stx1/stx2*	*stx1/eae*	*stx2/eae*	*stx1/stx2/eae*
47	1	7	3	9	1	1

(ii) 69 enrichment broths of slaugtherhouse samples tested positive with the PCR for the *stx1* and/or *stx2* and/or *eae* genes (Table 2). The PCR-positive broth are being inoculated onto the 3 agar plates and 10 colonies from each plate are being picked-up and stored before further testing.

TABLE 2: No. enrichment broths positive with the triplex PCR for ...

Origin	1 gene			2 genes			3 genes
	stx1	*stx2*	*eae*	*stx1 stx2*	*stx1 eae*	*stx2 eae*	*stx1/stx2 /eae*
Bulls	12	5	6	5	9	11	12
Cows	4	0	1	3	1	0	0
Heifers	0	0	0	0	0	0	0

(iii) Right now, STEC and EPEC from diarrheic calves are being tested with the PCR for the O serogroups. (

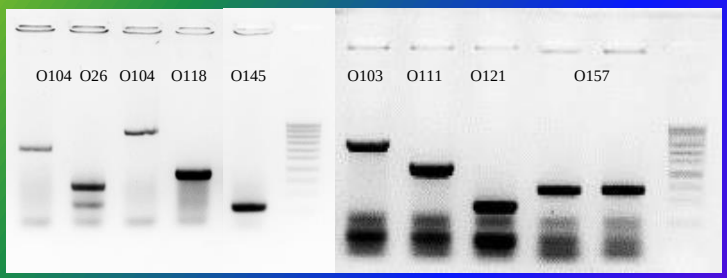

Fig.3: 2 pentaplex PCR targeting O 5, 26, 103, 104, 111, 118, 121, 145, 157 and O165 (no control strain) serogroups genes

DISCUSSION

- Animals for slaughter are, to some extent, holders of EPEC and STEC strains and thus represents a potential public health hazard.
- Moreover, there is no full correlation between the enterohaemolysin positive phenotype and the presence of the eae gene and / or those encoding the Shiga toxin. The use of enterohaemolysin agar might not be the best diagnostic test to isolate STEC and EPEC from diarrheic calves.

REFERENCES

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