Post-weaning diarrhoea in the Czech Republic, Hungary, Poland and Romania: characterization of *Escherichia coli* virulence factors

Nechvatalova, Katerina[1]; Kern, Primoz[2]; Biksi, Imre[3]; Jędryczko, Roman[4]; Kucharovicova, Ivana[5]; Simek, Bronislav[5]; Bunea, Cristina Rodica[6]

[1]Elanco Animal Health, Czech Republic, [2]Elanco, Animal Health, Slovenia, [3]SCG Diagnosztika Kft., Hungary, [4]VETlab group - WDL, Poland, [5]State Veterinary Institute Jihlava, Czech Republic, [6]Synevovet SRL, Romania

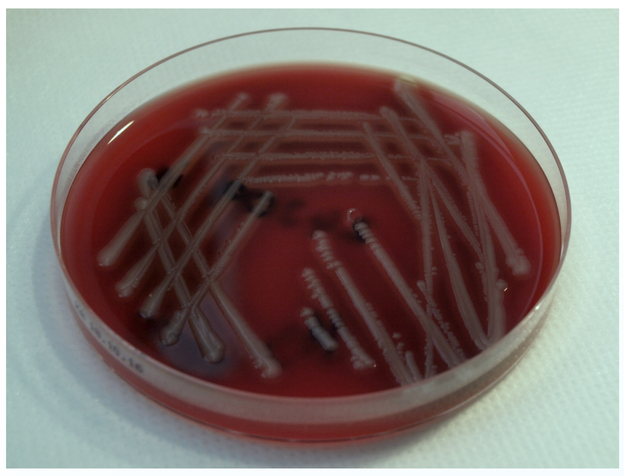
INTRODUCTION

Post-weaning diarrhoea (PWD) remains an important swine disease resulting in economic losses due to mortality, morbidity, decreased growth rate and cost of medication (Fairbrother et al., 2005). PWD typically causes mild to severe diarrhoea after weaning, which can be associated with marked dehydration, loss of performance and increased mortality.

PWD is mostly caused by enterotoxigenic *Escherichia coli* (ETEC), a pathotype characterized by the production of fimbriae that elicit colonization and enterotoxins that disrupt fluid homeostasis in the small intestine. F4 and F18 fimbriae are the types that are most frequently detected in ETEC isolates from cases of PWD (Fairbrother et al., 2005).

The objective of our study was to determine the prevalence of ETEC and its virulence factors in PWD cases in the Czech Republic (CZ), Hungary (HU), Poland (PL) and Romania (RO).

Photo 1. *Escherichia coli* colonies on a blood agar plate
(State Veterinary Institute Jihlava, Czech Republic, 2016)



MATERIALS AND METHODS

The study was conducted from September 2015 to December 2016. A total of 79 pig herds (22 in CZ, 16 in HU, 35 in PL, 6 in RO) showing clinical signs of PWD were selected for the study. Rectal swab samples from diarrheic nursery pigs in acute phase (usually 5 animals per farm), preferably less than 48 hours following the outbreak of diarrhea and before any antimicrobial therapy, will be collected and submitted to the laboratory. Samples will be kept on ice or between 2°C and 8°C before analysis. The samples will be plated on both Trypticase Soy Agar (TSAII) or Blood Agar with 5% sheep blood and MacConkey agar for isolation of colonies (Photo 1). Plates will be incubated overnight (12-18 hours) at 37±1°C. Three colonies identified as *Escherichia coli* by colony morphology and standard biochemical methods will be selected and transferred into a tube containing 5 ml of LB broth and incubated overnight (12-18 hours) at 37±1°C. The DNA template of the colonies will be collected and tested for the presence of genes encoding for adhesins (especially F4, F18) and toxins (especially LT, STa, STb) using a multiplex PCR (Photo 2).

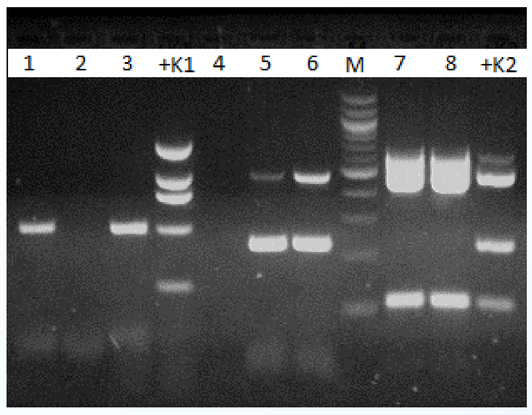
RESULTS

Escherichia coli isolates were identified in 94% samples, ETEC isolates that carried genes for both fimbriae and toxins (LT, STa, STb or EAST1) were detected in 43 herds with PWD (54.4%).

In 9 herds F4-ETEC was found as the cause of diarrhoea (20.9% of ETEC positive farms), whilst in 25 herds (58.1% of ETEC positive farms) *Escherichia coli* isolates were classified as F18-ETEC. In 9 herds (20.9%) ETEC carrying both F4 and F18 genes was detected (Table).

The most commonly detected ETEC virotypes were F4, STb, LT and F18, Sta, Stb.

Photo 2. Agarose gel showing results of multiplex PCR assays for detection of *Escherichia coli* virulence genes.
(State Veterinary Institute Jihlava, Czech Republic, 2016)



K positive controls
respective bands from the top to the bottom:
+K1 - F41, F4, F6, F18, F5
+K2 - LT, STb, Sta, EAST1
M size marker 1500-100 bp

1,3 colonies with genes for F18
2 colony without genes for fimbrial antigens
4 colony without genes for toxins
5,6 colonies with genes for STb and Sta
7,8 colonies with genes for LT, STb and EAST1

Table: Frequency of occurrence of respective fimbrial antigen (F4, F18) in ETEC positive farms

	Number of positive farms in respective countries			
	CZ	HU	PL	RO
F4-ETEC	3	2	3	1
F18-ETEC	6	5	12	2
F4 + F18-ETEC	1	2	3	3
Sum of ETEC positive farms	10	9	18	6

CONCLUSION

From our study we can conclude that both F4-ETEC and F18-ETEC were identified as a cause of PWD, but F18-ETEC isolates were more prevalent in these four monitored European countries. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of different *Escherichia coli* isolates in PWD outbreaks and initiate appropriate preventive and control measures.

References:
Fairbrother, J.M., Nadeau, É., Gyles, C.L. (2005). *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Animal Health Research Reviews 6, 17-39.