INVESTIGATION OF THE ECOLOGICAL NICHE OF BLOWN PACK SPOILAGE ORGANISMS

E. Esteves[1]*, D. Bolton[1], P. Whyte[2]

[1]Department of Food Safety, Teagasc Food Research Centre, Ashtown, Dublin 15

[2]UCD Centre for Food Safety, UCD, Belfield, Dublin 4

*Email: eden.esteves@teagasc.ie

Abstract - The aim of this research was to investigate the environmental niches of blown pack spoilage (BPS) *Clostridium* spp. on beef farms. Faecal, silage, soil, bedding straw, air, drinking and puddle water samples were taken on 2 beef farms and tested for *Clostridium* spp. *Clostridium estertheticum* and *Clostridium gasigenes* using culture and molecular based techniques. This study found BPS species were commonly distributed in the beef farm environment.

Key words: beef spoilage, blown pack spoilage, psychrophilic *Clostridium* spp.

I. INTRODUCTION

Psychrophilic *Clostridium spp.* cause spoilage of vacuum packed red meat at correctly stored temperatures resulting in 'blowing' of the pack, a foul smelling odour, a metallic sheen on the surface of the meat with primarily carbon dioxide production [1]. *C. estertheticum* and *C. gasigenes* are the main species causing blown pack spoilage (BPS) [2, 3]. These bacteria have been reported in various animal samples including the hide and the faeces of cattle [4]. The aim of this study was to identify the ecological niches of BPS *Clostridium spp*. on beef farms using culture and molecular based methods.

II. MATERIALS AND METHODS

Environmental samples were collected from 2 beef farms during Spring. Exactly, 10 fresh bovine faecal samples and 5 soil, silage, bedding straw, drinking water, puddle water and air samples were collected. These were tested for *Clostridium* spp. including *C. estertheticum* and *C. gasigenes.* Briefly, solid samples were supplemented with Peptone Yeast Glucose Starch (PYGS) broth and 5ml of each sample and broth suspension was treated with 5ml of absolute ethanol (1 hour at 4°C). Post incubation 0.1ml was transferred onto freshly prepared Columbia Blood Agar (CBA) supplemented with 5% defibrinated horse blood. CBA plates and the remaining PYGS suspension were incubated for 3 weeks at 4°C. DNA extractions were carried out using the DNeasy Blood and Tissue kit (Qiagen Ltd, Crawley, UK) on enriched and plated sample as per the manufacturer's instructions. The samples were tested using both conventional and real time PCR methods.

III. RESULTS AND DISCUSSION

The results are presented in Table 1. All samples on farm 1 were negative for *C. estertheticum* but *C. gasigenes* was detected in faecal, soil, bedding straw and puddle water samples. On farm 2, *C. estertheticum* and *C. gasigenes* were detected in all samples using real time PCR and culture based methods, respectively. The highest concentration of cells detected were approximately 5 $\log_{10}$ cfu/ml in soil, bedding straw and puddle water samples (data not shown). Overall, this study showed high prevalence of *C. gasigenes* on beef farms. Interestingly, Moschonas et al. [4] also reported high prevalence of this organism in beef abattoirs.

Table 1: The presence of *C. estertheticum* and *C. gasigenes* in the environmental farm samples as determined using culture and molecular based methods

Sample type (number of samples)	Conventional PCR		Real time PCR		Direct plating	
	C. est[1]	C. gas[2]	C. est	C. gas	C. est	C. gas
	Farm 1					
Bovine faeces (10)	-	-	-	-	-	+
Soil (5)	-	-	-	-	-	+
Bedding straw (5)	-	-	-	-	-	+
Silage (5)	-	-	-	-	-	-
Drinking water (5)	-	-	-	-	-	-
Puddle water (5)	-	-	-	+	-	+
Air (5)	-	-	-	-	-	-
Farm 2						
Soil (5)	-	-	-	-	-	+
Bedding straw (5)	-	-	+	-	-	+
Silage (5)	-	-	+	+	-	+
Drinking water (5)	-	+	+	+	-	+
Puddle water (5)	-	-	+	+	-	+
Air (5)	-	+	+	+	-	+

[1]C. est = *C. estertheticum*

[2]C. gas = *C. gasigenes*

IV. CONCLUSION

This study concluded that BPS *Clostridium* spp. were widely distributed on the 2 beef farms in this study.

ACKNOWLEDGMENTS

This study was funded by Teagasc.

REFERENCES

1. Yang, X., Gill, C. O., & Balamurugan, S. (2010). Products of glucose and lactate fermentation, and utilization of amino acids by Clostridium estertheticum subspp. laramiense and estertheticum growing in meat juice medium. *Journal of Food Protection*, *73*(7), 1348–52.
2. Adam, K. H., Flint, S. H., & Brightwell, G. (2010). Psychrophilic and psychrotrophic clostridia: Sporulation and germination processes and their role in the spoilage of chilled, vacuum-packaged beef, lamb and venison. *International Journal of Food Science and Technology*.
3. Bolton, D. J., Carroll, J., & Walsh, D. (2015). A four-year survey of blown pack spoilage Clostridium estertheticum and Clostridium gasigenes on beef primal cuts. *Letters in Applied Microbiology*, *61*(2), 153–157.
4. Moschonas, G., Bolton, D. J., Sheridan, J. J., & McDowell, D. A. (2009). Isolation and sources of blown pack spoilage clostridia in beef abattoirs. *Journal of Applied Microbiology*, *107*(2), 616–624. http://doi.org/10.1111/j.1365-2672.2009.04229.x