# THE PREVALENCE OF BACTERIAL AND PROTOZOAN FOODBORNE PATHOGENS IN ABATTOIR WASTES INTENDED FOR USE ON AGRICULTURAL LAND

R. Pepperell<sup>1</sup>, J. Massanet-Nicolau<sup>2</sup>, V. M. Allen<sup>1</sup>, S. Buncic<sup>1</sup>

<sup>1</sup>University of Bristol, Department of Clinical Veterinary Science, Division of Farm Animal Science, Bristol, UK, BS40 5DU. <sup>2</sup>Thames Water Utilities Ltd., Spencer House Laboratories, Manor Farm Road, Reading, Berks, UK, RG2 0JN.

### Background

A significant quantity of organic waste produced by commercial abattoirs in the UK is currently disposed of via direct application as fertilisers to agricultural land. Despite this common practice, information regarding the prevalence and levels of pathogenic enteric microorganisms within this material is scarce, and it is potentially the case that food-borne pathogens could be "recycled" within the food chain. The affects of storage time, quantities and condition on the microbial populations in this waste material is also not known. By surveying the current handling procedures of waste prior to disposal, and by determining the prevalence of potential pathogens in this material, our work should assist in an informed assessment of the potential risk posed to human health by these practices.

#### Objectives

The main objectives of this study were a) to conduct a comprehensive survey into the current commercial practices with regard to the disposal of abattoir waste products, and b) to establish the prevalence of pathogens in these wastes by employing microbiological sampling. Finally, an assessment of the health risk presented by the practices observed will be made.

#### Methodology

Survey of commercial practice: A total of 28 abattoirs were included in the survey, selected in order to give an accurate representation of all current practices and waste types. The survey was carried out by personal visits, inspection and interviews with technical staff at each location. *Microbiological sampling program:* 12 sites were chosen from the 28 originally surveyed in order to cover all observed practices. Waste samples were collected from each site at 4 points over a 12 month period in order to assess any potential seasonal variation in pathogen levels – sampling for the final season (spring) in currently ongoing. All samples were tested for the presence of the following bacterial pathogens – *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria* spp. and *Campylobacter* spp., and for the protozoan pathogens *Cryptosporidium parvum* and *Giardia Lamblia*. Presence of the bacterial pathogens was determined using a filtration capture technique and an organism-specific resuscitation step, followed by enumeration with specific chromogenic media. Confirmation of presumptive positive results was performed using immunological (*E. coli* O157, *Salmonella* spp., *Campylobacter* spp.) and biochemical (*Listeria* spp.) techniques. Recovery of *Crypt. parvum* and *G. lamblia* was performed by capture with organism-specific Immunomagnetic separation, followed by staining with 4',6-diamidino-2-phenylindole (DAPI) and Propidium Iodide (PI). Slides were then examined and enumerated using epiflourescent microscopy.

#### **Results and Discussion**

Methods of waste disposal and types of waste applied to land were found to vary greatly from plant to plant (Figure 1), and between species. Blood and lairage-based waste was most commonly applied to land from cattle abattoirs, whereas application of lairage and stomach contents was more frequently observed from sheep and pig plants. Waste from poultry plants was almost exclusively blood-based. Treatment of this waste prior to disposal (i.e. Length of storage time, mixture with other waste types) was also found to vary greatly between each site. Microbiological analysis of the waste collected revealed low levels of all pathogens under investigation (Table 1). Over the course of the first 3 seasons sampling, lairage material from a single red meat abattoir was found to be positive for the presence of Campylobacter spp. during the summer and winter seasons, with counts ranging from  $5.4 \times 10^1 - 1.06 \times 10^4$  cfu/g waste being recorded. Lairage waste material from the same red meat abattoir also tested positive for the presence of L. ivanovii (8.08 x 103 cfu/g) during the autumn sampling season. No further samples from red meat abattoirs were found to be positive for any of the other pathogens under investigation. In the case of the poultry abattoirs a single blood sample obtained during the autumn sampling season was seen to be positive for the presence of Campylobacter spp-(1.6 x 10<sup>3</sup> cfu/g). Analysis of the samples for protozoan parasites revealed the presence of these pathogens in all waste types far more frequently than the bacterial pathogens investigated (see table 1). A majority of lairage samples tested positive for the presence of both Crypto. parvum (66.6%) and G. lamblia (88.9%), although levels of both varied greatly. In the case of Crypt. parvum, levels of between 2.67  $oocysts/g - 6.42 \ge 10^3$  oocysts/g were observed in lairage material, with between 2.64 cysts/g - 5.87 x  $10^3$  cysts/g being seen for *G. lamblia*. Similarly the bacterial pathogens, no significant correlation between pathogens' numbers and season was observed. Further sampling for all the pathogens (the final spring season) is currently ongoing.

#### **Concluding remarks**

Overall, the prevalence of foodborne pathogens was found to be low in the abattoir wastes tested, and enumeration of parameters in positive samples revealed that levels of these organisms were variable. Waste material from lairage areas was found to contain the highest number of pathogens, both in the case of enteric bacteria, and particularly in the case of protozoa, where up to 90% of samples taken during a single season were found to be positive for the presence of *G. lamblia.* This research has given a first insight into the current commercial practices with regard to the disposal of abattoir waste products, and the ongoing sampling program will provide previously lacking information regarding the levels of potentially disease causing organisms within these materials.

#### Acknowledgements

The authors would like to acknowledge the Food Standards Agency (UK) for funding this research (Project B05008).

Figure 1: Application of wastes from red meat abattoirs to agricultural land



## Table 1: Incidence of Food-born Pathogens in Abattoir Wastes

Waste Type	Percentage Positive (Seasonal Variation)					
	E.coli O157	Salmonella spp.	Listeria spp.	Campylobacter spp	Crypt. parvum	G. Lamblia
	ek én res					i yang pana bara ba
	Red Meat abattoirs (n=9)			Red Meat Abattoirs (n=10)		
and the second second						routions (n° 10)
Lairage	0	0	4.2% (0%-12.5%)	8.3% (0%-12.5%)	66.6% (66.6%)	88.9% (77.8%-100%)
Lairage/Stomach Contents	0	0	0	0	0	0
Stomach Contents	0	0	0	0	22.2% (0%-33%)	11.1% ((0%-33.3%)
Blood	0	0	0	0	0	16.6% (0%-50%)
Effluent	0	0	0	0	33.3% (33.3%)	66.6% (66.6%)
	White Meat abattoirs (n=3)				White Meat Abattoirs (Not Tested)	
						(
Lairage	0	0	0	0	NT	NT
Stomach Contents	0	0	0	0	NT	NT
Blood	0	0	0	33.3% (0%-100%)	NT	NT
Effluent	0	0	0	0	NT	NT