IN VITRO GROWTH RATE OF *CAMPYLOBACTER JEJUNI* NCTC 11168 IN CAECAL CONTENTS SUPPLEMENTED WITH (-)-NOREPINEPHRINE, (-)-EPINEPHRINE, CORTICOSTERONE AND CORTISOL

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Abstract – Human campylobacteriosis is among the most common foodborne diseases worldwide, commonly originating from the consumption of chicken meat. The chicken caecum is the organ where the highest concentration of *Campylobacter* is found in birds. It provides the ideal atmosphere and conditions of temperature, nutrient availability and osmotic pressure for the pathogen to grow. Physiological stress and the stress hormone norepinephrine have been previously linked to the increased growth of enteric pathogens including *Campylobacter jejuni*. The objective of this study is to characterize the growth of *Campylobacter jejuni* subs. *jejuni* NCTC 11168 in caecal contents and in Brain-Heart Infusion broth in the presence of the neuroendocrine stress hormones (-)-norepinephrine, (-)-epinephrine, corticosterone and cortisol.

Key Words – Broiler stress, campylobacteriosis, growth rates in vitro.

I. INTRODUCTION

The main source of campylobacteriosis in humans is infected animal meat, primarily poultry meat [1]. *Campylobacter* spp. colonises the broiler chicken intestine, reaching highest counts in the caecum [2]. The microbiome present in the avian gut may be exposed to small concentrations of neuroendocrine hormones such as epinephrine, norepinephrine, corticosterone and cortisol during stressful circumstances such as thinning and transportation [3, 4]. Evidence indicates that norepinephrine and epinephrine promote the growth and pathogenicity of *Campylobacter* spp [4-6]. There is no existing knowledge on the impact of other physiological stress effectors on the growth and pathogenicity of *Campylobacter*.

This study will provide insight into the effects of stress-associated neuroendocrine hormones on the growth of *Campylobacter jejuni* NCTC 11168.

II. MATERIALS AND METHODS

Flocks were randomly selected to collect the caeca from 1,500 broiler chickens between February and March 2017. The caecal contents were aseptically extracted and pooled into 600g portions, vacuum packed and sterilized by gamma irradiation at a dose of 10kGy. Once the absence of *Campylobacter* spp. was confirmed, the samples were separated into 30g portions that were inoculated with *C. jejuni* NCTC 11168 at a concentration of 1 $\log_{10}$ cfu/g. Norepinephrine, epinephrine, corticosterone and cortisol (Sigma Aldrich, UK) were added in duplicate to samples at 100 μM. As a control, the same was added to 30ml of Brain-Heart Infusion broth (Oxoid Ltd) also at a cell concentration of 1 $\log_{10}$ cfu/ml.

The samples were incubated at 42ºC in a microaerophilic atmosphere. Sampling occurred at time points 0, 3, 6, 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69, 75, 81, 87 and 93 hours. Viable *C. jejuni* were detected via direct plating using the method outlined in ISO 10272. Enrichment was performed incubating 1g or 1ml of sample in Bolton Broth (Oxoid Ltd) from each time point at 42ºC for 48h before plating as outlined in ISO 10272.

Generation times were calculated using the formula G=t/3.3 logb/B; where t=time interval in hours, b=number of remaining bacteria at the end of the time interval, and B=number of bacteria at the start of the time interval. Lag times and umax were calculated using MicroFit Software. Statistical comparisons were performed in GENSTAT by Anova.

III. RESULTS AND DISCUSSION

All samples were inoculated to a level of approximately 4.5 $\log_{10}$ cfu/g, which was maintained in the control samples (irradiated caecal contents without hormones) throughout the experiment. Studies have shown that caecal contents sustain the growth of *C. jejuni* in vivo and in vitro [1], which is reflected in these results.

A similar pattern was observed with norepinephrine. Existing data shows that norepinephrine promotes the growth of *Campylobacter* [3, 4]. In contrast, treatment with epinephrine and corticosterone results in an immediate decrease in the Campylobacter population to below the level of detection which recovered to the inoculaton levels after 45 and 63h, respectively, and remained at this concentration. The initial *C. jejuni* concentrations also decreased immediately after inoculation in the cortisol treated samples, recovered by 51h (5.4 $\log_{10}$ cfu/g) and remained at this level for the remainder of the experiment.

The effect of stress on *Campylobacter* infection has been illustrated in literature that showed norepinephrine promotes the growth and the expression of virulence factors of *C. jejuni* [3, 4], epinephrine was also shown to promote the expression of virulence factors [5]. Although the physiological role of cortisol and corticosterone in broilers is well understood, research is needed to understand their effects on the growth and pathogenicity of the *Campylobacter* species.

IV. CONCLUSION

This preliminary experiment suggests that the hormones used as 100 μM did not promote the growth of *C. jejuni*. However, more in depth studies are ongoing using a range of inoculum levels, to establish whether or not this was a true effect of the hormone treatments.

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