

Does the Rearing Environment affect the Incidence of Zoonotic Infections in Pigs?

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This project aimed to address the question of whether differences in rearing environment on pig farms can affect incidence of zoonotic infections in pigs. Outdoor rearing of pigs has gained in popularity in recent years due to interest in animal welfare and an increase in the marketability of organic food. However, in the outdoor environment, pigs will be introduced to a wide range of bacteria that would not be present in indoor facilities. This will presumably have some effect on the colonic flora of the animals.

The aim of this project was to compare the gut microflora of pigs reared indoors with that of pigs reared outdoors, for the presence of human pathogens. The organisms of most interest were *Campylobacter jejuni*, *Clostridium difficile* and Verocytotoxigenic *Escherichia coli*.

Method Development

The gut microbiota of pigs has been well characterised by a number of studies during the last 40 years which have illustrated the diverse populations of the pig intestinal tract. In the method development stages of this project, a variety of bacterial growth media was used to isolate and enumerate commonly occurring bacterial groups using classical culture methods. The aim was to optimise methods for analysing bacterial populations in faecal samples from pigs.

Another aim of this project was to assess bacterial populations in the porcine gut using molecular methods such as amplification of target genes by Polymerase Chain Reaction (PCR) and population profiling by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. For both these methods successful extraction and amplification of bacterial DNA from faecal samples was critical. An accurate analysis of the bacterial populations present in a sample could only be made if all bacterial DNA was extracted and amplified. As such, considerable effort was spent on developing and trialling methodologies for these procedures.

By the end of the first year of this project we had developed and adapted traditional culture methods to identify and quantify bacteria that commonly occur in the porcine gut. To complement this, a new molecular method for profiling bacterial communities was developed with the potential to provide more information about the total populations of bacteria in the porcine gut. For molecular analysis, an effective DNA extraction method was developed that was able to isolate bacterial DNA from faecal samples and remove inhibitors of PCR. A PCR protocol for amplifying the 16S rDNA from faecal DNA extracts was optimised, as well as a protocol for detecting specific pathogens in faecal DNA. The development of these methods was required to enable the analysis of bacterial populations in porcine faeces in a number of experimental trials.

Pilot Study at University of Leeds Farm

The purpose of this pilot study was to assess the bacterial populations present in the gut of pigs reared either in indoor accommodation or outdoor accommodation on an individual pig unit. The aim was also to investigate whether rearing environment had an effect on the carriage of zoonotic bacteria in pigs. The first hypothesis of this study was that rearing environment would have an effect on the gut microbiota of the



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pigs, and in particular that the pigs reared outdoors would have a more complex gut microbiota. The second hypothesis was that outdoor-reared pigs would be more likely to carry zoonotic bacteria in their gut than indoor-reared pigs.

A variety of media was used in this study to isolate and enumerate four commonly occurring bacterial groups using classical culture methods, in an attempt to compare the gut microbiota of pigs reared indoors and outdoors. The groups chosen were enterococci, *Bacteroides* spp., lactobacilli, and coliforms. This study also used T-RFLP analysis to illustrate the complexity of bacterial populations present in faecal samples and assess differences in microbial diversity. A PCR-based detection method was used for the detection of three zoonotic bacteria – *Clostridium difficile*, *Campylobacter jejuni*, and verocytotoxigenic *Escherichia coli* O157:H7 – using specific probes.

Considerable variation was observed in the populations of the common gut bacteria between weeks, particularly around weaning age. However, there were few statistically significant differences between indoor and outdoor samples. Pigs reared exclusively indoors retained higher populations of lactobacilli than pigs reared outdoors which might confer a health benefit on indoor reared pigs. Good correlation was observed between the results of the classical culture methods and the T-RFLP analysis. There were no significant differences in the occurrence of zoonotic pathogens between environments. Minimal numbers of *C. jejuni* and VTEC O157 were detected, however a number of samples were positive for *C. difficile*. Interestingly, asymptomatic *C. difficile* colonisation was common in young pigs but decreased with age. Similar findings have been reported in humans.

National Survey

In order to make a comparison of indoor and outdoor environment on multiple units, a national study was designed to sample indoor and outdoor pig units from a variety of locations across the UK. T-RFLP analysis was used to assess the bacterial populations present in the faecal samples and PCR was used to detect the three zoonotic pathogens investigated in the pilot study.

Evidence for the presence of two zoonotic pathogens, *C. difficile* and VTEC O157:H7, were detected on a number of farms, while the third, *C. jejuni*, was not detected. It appeared that the rearing environment did not affect the incidence of *C. difficile* carriage as there was no statistically significant difference between the numbers of positive samples from pigs reared indoors compared with those reared outdoors. For VTEC O157:H7, a positivity rate of 5.6 % was observed in this study. There was a statistically significant difference ($p < 0.05$) between the numbers of positive samples from pigs reared indoors compared with those raised outdoors. It is worth noting, however, that the three farms from which the positive samples were obtained all had cattle facilities nearby. It is possible that the close proximity of cattle could increase the chance of VTEC carriage in pigs. Therefore this observation needs to be interpreted with caution, and these findings need to be investigated further.

With the T-RFLP analysis there were not obvious differences between the 'indoor' and 'outdoor' samples and similar groups of bacteria were identified in both sets. However, when the data was analysed using principal components analysis, separate clustering of the 'indoor' and 'outdoor' samples was observed suggesting that there were overall differences in the populations. Unfortunately, these differences could not be characterised using this analysis.

Asymptomatic Carriage of *Clostridium difficile* in Pigs

Clostridium difficile has been reported to be the most common cause of neonatal enteritis in pigs in the United States and there are a number of reports of its presence in pigs in Europe. In contrast, we had observed in the pilot study that the incidence of asymptomatic *C. difficile* carriage in pigs is common in young pigs but rare in pigs at slaughter age. The aim of this study was to observe the incidence of *C. difficile* carriage in piglets at weaning through to 18 weeks of age. We hypothesised that asymptomatic *C. difficile* carriage would be high in pigs at weaning, but would decrease with age. Our second hypothesis was that the addition of a therapeutic level of an antibiotic, phenoxymethyl penicillin, to the pigs diet would increase the incidence of *C. difficile* carriage. Antibiotic treatment is a risk factor for the onset of *C. difficile* associated infection (CDI) in humans.

Asymptomatic carriage of *C. difficile* was found to be common, but short-lived in piglets. All isolates of *C. difficile* from this study were ribotyped and found to belong to the 078 ribotype commonly associated with pigs. The inclusion of phenoxymethyl penicillin in the pigs' diet was found to have no effect on the carriage of *C. difficile*. The observations of this study were contradictory to reports from North America and parts of Europe, where *C. difficile* is claimed to cause symptomatic illness in young pigs. The phenomenon of asymptomatic carriage which decreases with age has been observed in other species, including humans and rabbits, but has not before been demonstrated in pigs. Further work is required to determine the reasons for this phenomenon, and to understand why *C. difficile* is a problem in North America, but not in the UK.

Conclusions

This work has shown that rearing environment may affect the gut microbiota in pigs, but other factors (in particular diet, and/or age) clearly also play important roles. It has been observed that pigs reared exclusively indoors retain higher populations of lactobacilli than pigs reared outdoors. This may confer a health benefit on indoor reared pigs. The national survey conducted as part of this project found no statistically significant difference in carriage of *C. difficile* and *C. jejuni* between indoor and outdoor farms, but a difference in the number of VTEC O157:H7 was observed. Differences were observed in the overall bacterial populations in the gut suggesting that environment does have some effect on gut microbiota. Further work looking at the incidence of *C. difficile* in young pigs has shown that asymptomatic carriage of *C. difficile* is common in young piglets but decreases with age. This is contradictory to reports on *C. difficile* carriage in other countries and needs to be investigated further.

It is hoped that this project will improve our understanding of how production environment can affect faecal microflora. This will enable the modification of production systems to optimise pig health and ensure consumer safety. This may provide numerous benefits to the industry by improving pig production and increasing consumer confidence in meat safety.