

A comparison of the gut microbiota in indoor and outdoor reared pigs

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Introduction The bacterial populations present in the porcine gut play an important role in pig health. A number of factors may influence these populations including diet, medication and environment. To identify what effects rearing environments have on the gut microbiota, this study compares the numbers of four common groups of gut organisms – enterococci, *Bacteroides* spp., lactobacilli and coliforms – in faecal samples taken from pigs reared either indoors or outdoors. The presence of three zoonotic bacteria – *Campylobacter jejuni*, *Clostridium difficile*, and Verocytotoxigenic *Escherichia coli* O157 - was also assessed to determine whether rearing environment might influence the threat of zoonotic infections.

Materials and methods Piglets (n=24) were farrowed either outdoors or indoors. At 4 weeks of age all piglets were weaned indoors where they remained until slaughter. Faecal samples were collected from 12 indoor pigs and 12 outdoor pigs at 4, 7, 14 and 23 weeks of age. Enterococci and lactobacilli plate counts were performed on kanamycin aesculin azide agar and LAMVAB agar respectively, and incubated aerobically for 48 h at 37°C. Plate counts of *Bacteroides* spp. were performed in an anaerobic cabinet on bile aesculin agar and incubated anaerobically for 48 h at 37°C. Coliform counts were carried out using the most probable number method which involved inoculation in MacConkey broth and aerobic incubation at 37°C for 24 h, followed by confirmatory subculture in brilliant green bile broth and aerobic incubation at 44°C for 48 h.

For molecular analysis of zoonotic bacteria, DNA was extracted from each sample using a phenol: chloroform based method. PCR amplification of specific genes was used to detect the three zoonotic bacteria in each sample. The genes targeted were: the *hipO* gene of *C. jejuni*, the toxin A gene of *C. difficile*, and the *eaE* gene of VTEC O157. Statistical analysis of the results was carried out with a Mann Whitney U test (SPSS version 11.0).

Results Considerable variation was observed in the populations of the common gut bacteria between weeks. However, there were few statistically significant differences between indoor and outdoor samples. 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* (table 1). These numbers decreased with age.

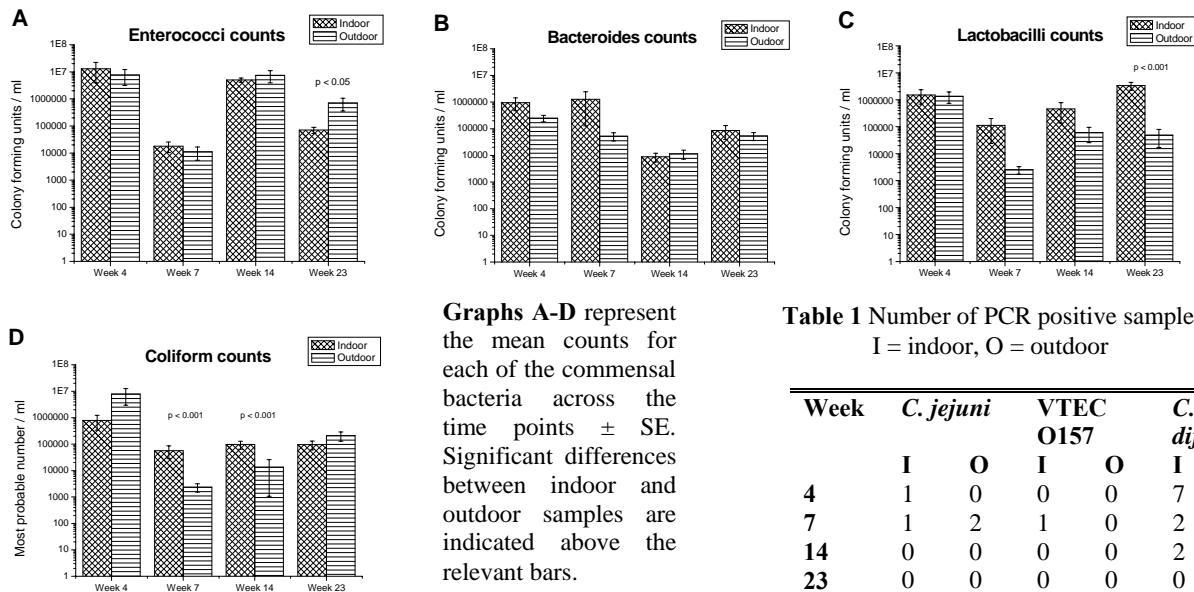


Table 1 Number of PCR positive samples
I = indoor, O = outdoor

Week	<i>C. jejuni</i>		VTEC O157		<i>C. difficile</i>	
	I	O	I	O	I	O
4	1	0	0	0	7	6
7	1	2	1	0	2	4
14	0	0	0	0	2	2
23	0	0	0	0	0	1

Table 2 Statistical significance of difference in counts between weeks. P<0.05 is significant. NS = not significant.
↑ = significant increase, ↓ = significant decrease

Weeks	Enterococci		Bacteroides		Lactobacilli		Coliforms	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
4 – 7	↓ P<0.001	↓ P<0.05	↑ P<0.05	↑ P<0.01	↓ P<0.01	↓ P<0.001	↓ P<0.001	↓ P<0.001
7 – 14	↑ P<0.001	↑ P<0.001	NS	NS	NS	↑ P<0.001	NS	NS
14 – 23	↓ P<0.001	↓ P<0.001	↑ P<0.05	NS	↑ P<0.001	NS	NS	↑ P<0.001

Conclusions Rearing environment may affect the gut microbiota in pigs, but other factors (in particular diet, and/or age) clearly also play an important role. Pigs reared exclusively indoors retained higher populations of lactobacilli than pigs reared outdoors. This may confer a health benefit on indoor reared pigs. Interestingly, asymptomatic *C. difficile* colonisation was common in young pigs but decreased with age. Similar findings have been reported in humans (Donta & Myers, 1982).

References

Donta, S. T. & Myers, M. G. 1982. *Clostridium difficile* toxin in asymptomatic neonates. *Journal of Pediatrics* 100, 431