A high-resolution investigation of multi-strain *Campylobacter* infection of European broiler flocks using parallel sequencing.

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Introduction

- Contaminated chicken meat remains the most common source of human *Campylobacter* infection in many countries, despite efforts to increase biosecurity on farms.
- *Campylobacter* is most commonly detected amongst broiler (meat) chicken flocks from 3 weeks of age using bacterial culture or qPCR.
- It is thought that broiler flocks become infected horizontally, when *Campylobacter* is introduced into chicken houses by personnel or machinery.

Methods

- Between 5 and 16 fresh faecal samples were tested from 54 broiler flocks in the UK, France and Switzerland at early (< 8 days of age) and late (>28 days of age) time points in the production cycle.
- Samples were tested individually for presence/absence of *Campylobacter* by conventional culture or qPCR methods, alongside 16S bacterial profiling and parallel sequencing of the *porA* locus, which encodes for the *Campylobacter* major outer membrane protein (MOMP).

Figure 1. Parallel sequencing method to detect the Campylobacter porA fragment directly from faecal samples

Results: *Campylobacter* DNA was detected from commercially reared chicks at an early age (< 8 days) in all flocks

- *Campylobacter porA* DNA was detected amongst samples from all of the flocks tested at both time points, irrespective of whether or not they tested positive for *Campylobacter* by conventional culture or qPCR. This usually represented <0.01% of the 16S bacterial profile.
- A single *porA* variant predominated in samples that tested positive by culture or qPCR. A more diverse pattern of *porA* variants was seen amongst samples that tested negative by culture or qPCR.
- It was not possible to distinguish *Campylobacter porA* populations by farm or parent flock identification using the single *porA* gene target.

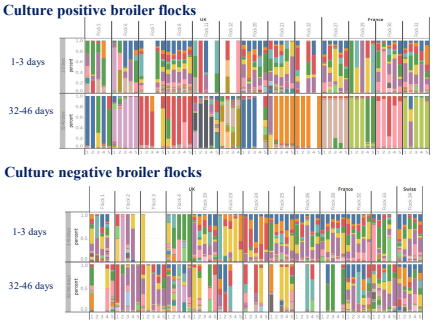
Table 1. Presence/absence of Campylobacter porA or 16S DNA in chicks <8 days of age versus end-point status.

Company	Farm/flock	*Campylobacter* presence/absence 16S 1 2 3 4 5	*porA*f2 1 2 3 4 5	Flock *Campylobacter* status by conventional means*
A	Farm 1 F1			Negative[a]
	Farm 2 F2			Negative[a]
	F3			Negative[a]
	Farm 3 F4			Negative[a]
	F5			Positive[a]
	F6			Positive[a]
	Farm 4 F7			Positive[a]
	F8			Positive[a]
B	Farm 5A** F9			Positive[b]
	F10	NT	NT	Positive[b]
	Farm 5B** F11			Positive[b]
	F12			Positive[b]
	Farm 6 F13			Positive[b]
	F14	NT	NT	Positive[b]
	Farm 7 F15			Positive[b]
	F16			Positive[b]

*Flocks were tested for *Campylobacter* by conventional culture[a] or qPCR[b] at end point

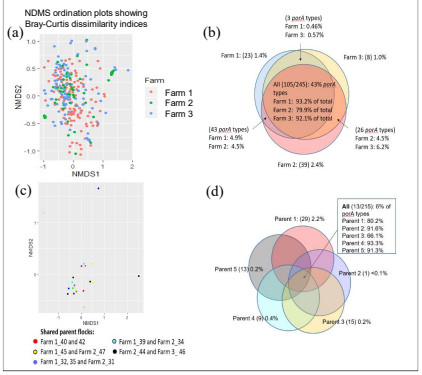
**Flocks 11/12 were placed after flocks 9/10 had left.

Figure 2. The breakdown of Campylobacter porA variants by sample; each variant is represented by a different colour.



Culture/qPCR negative samples: high diversity, <0.01% 16S profile
Culture/qPCR positive samples: low diversity, <0.1% 16S profile

Figure 3. Campylobacter porA populations were not distinguishable by farm (a,b) or parent flock id (c,d).



Conclusions

- Parallel sequencing of the *porA* target provides an efficient and sensitive tool for culture-independent detection of multiple strains of *Campylobacter*. Further development with additional loci is needed for more refined strain typing with greater resolution.
- *Campylobacter* is rarely cultured from commercial flocks < 3 weeks of age, but these results suggest that attention should be focused on younger chicks in order to reduce contamination. Further research is needed to determine why single *porA* variants become dominant in some flocks that become culture/qPCR positive and not others that remain culture/qPCR negative.

References: (1) Colles FM, Hedges SJ, Dixon R, Preston SG, Thornhill P, Barfod KK, Gebhardt-Henrich SG, Créach P, Maiden MCJ, Dawkins MS and Smith AL Appl Environ Microbiol (2021) 10:87(23):e0106021; (2) Colles FM, Preston SG, Barfod KK, Flammer PG, Maiden MCJ and Smith AL. Sci Rep (2019) 9(1):6204

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