Campylobacter jejuni transmission and colonisation in broiler chickens is inhibited by Faecal Microbiota Transplantation.

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ABSTRACT

BACKGROUND: *Campylobacter jejuni,* the most frequent cause of foodborne bacterial infection, is found on around 70% of retail chicken. As such there is a need for effective controls in chicken production. Microbial-based controls such as probiotics are attractive to the poultry industry, but of limited efficacy. Furthermore, as commercially-produced chickens have no maternal contact, their pioneer microbiome is likely to come from the hatchery environment. Early delivery of microbials that lead to a more 'natural avian' microbiome may, therefore, improve bird health and reduce susceptibility to *C.jejuni* colonisation.

A faecal microbiota transplant (FMT) was used to transfer a mature cecal microbiome to newly-hatched broiler chicks and its effects on *C.jejuni* challenge assessed. We used both a seeder-bird infection model that mimics natural bird-to-bird infection alongside a direct-challenge model. We used a 16S rRNA gene sequencing-based approach to characterize the transplant material itself alongside changes to the chicken microbiome following FMT.

RESULTS: FMT changes the composition of the chicken intestinal microbiome. We observed little change in species richness following FMT compared to untreated samples, but there is an increase in phylogenetic diversity within those species. The most significant difference in the ceca is an increase in *Lactobacilli,* although not a major component of the transplant material, suggesting the FMT results in a change in the intestinal milieu as much as a direct change to the microbiome.

Upon direct challenge, FMT resulted in lower initial intestinal colonisation with *C.jejuni.* More significantly, in a seeder-bird challenge of infection transmission, FMT reduced transmission and intestinal colonisation until common UK retail age of slaughter. In a repeat experiment, transmission was completely blocked following FMT treatment. Delayed FMT administration at 7 days of-age had limited effect on colonisation and transmission.

CONCLUSIONS: We show that transfer of a whole mature microbiome to newly-hatched chicks reduces transmission and colonisation of *C.jejuni.* This indicates that modification of the broiler chick microbiome can reduce intestinal colonisation of *C.jejuni* to levels projected to lead to lower the human infection rate. We believe these findings offer a way to identify key taxa or consortia that are effective in reducing *C.jejuni* colonisation and improving broiler gut health.

KEYWORDS: (3-10) *Campylobacter jejuni*, Faecal microbiota transplant, chicken intestinal microbiota, probiotics

77 **BACKGROUND**

78 *Campylobacter jejuni*, a highly motile Gram-negative proteobacteria, is the most frequent cause of human
79 bacterial foodborne gastroenteritis worldwide (1). There are currently estimated to be 9 million cases in the
80 European Union each year, amounting to a vast medical and productivity burden across many of the world's most
81 developed countries (2). The preparation and consumption of poultry meat continues to be the single largest source
82 of human infection, with over 70% of retail chicken carcasses within the EU showing *C.jejuni* contamination (3).
83 With current intervention strategies aimed at reducing *C.jejuni* burden within the commercial broiler (meat-
84 producing) chicken showing limited success, a pragmatic means of large-scale on-farm control continues to be a
85 key goal (4). The need to develop controls within poultry production without the use of antimicrobials is a public
86 health priority. However, unlike *Salmonella,* where vaccination has proved successful, the nature of both the
87 pathogen and host response to *Campylobacter* in the chicken make the development of vaccines challenging (3).
88 With the gut microbiome acting as the immediate biological barrier against *C.jejuni*, its manipulation could play
89 a key role in its reduction and control in chicken meat production.

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91 Manipulation of the microbiota in livestock has a long history (5). Animal husbandry practices including
92 transfaunication, the transfer or rumen content between cattle and the use of dietary products, particularly
93 probiotics and microbial products in poultry, to manipulate or modify animal intestinal microbiomes to improve
94 health, productivity and wellbeing have long been used (6). Early work using cultured avian intestinal flora to
95 reduce *Salmonella* colonisation in chicks by Rantala & Nurmi (1973) was the forerunner of many subsequent
96 studies on probiotics but the basis of how any manipulation of the microbiome is effective in reducing pathogen
97 load in chickens remains unclear, though broadly there would appear to be two main possibilities. Firstly, any
98 preparation may have a competitive exclusion (CE) effect, originally an ecological term, based around
99 competition for a niche and resources. We also now understand that intestinal tract bacteria such as *Firmicutes*
100 produce metabolites such as butyrates that can inhibit the growth of proteobacteria (7). Secondly, probiotics and
101 microflora preparations may drive immune development and immunity in the gut helping limit pathogen
102 colonisation (8).

103

104 Attempts at reproducing and improving such probiotic efficacy in reducing the colonisation of the avian
105 gastrointestinal tract (GIT) with *C.jejuni* has been of largely empirical nature with little evidence of a practical
106 industrial role (8,9). With oral probiotics, doses often provide a relatively low magnitude of microorganisms

107 compared to that found within the native microbiome making repeated administration necessary (5). Although
108 commonly derived from the avian intestinal tract, environmental adaptation during *ex vivo* culture may limit
109 beneficial impact of probiotic formulations which may no longer display the same phenotype as when in the gut
110 (5). Consideration of the gut microbiota as an entire system as opposed to the sum of individual entities offers
111 potential for a more viable solution to *C.jejuni* control. The use of more complex but undefined microbiota
112 preparations such as Aviguard® or Broilact® have been increasingly adopted in Europe, though these are cultured
113 products that are unlikely to contain the full complement of species or genera found in the healthy microbiome.
114 However, their undefined nature precludes their use in many countries such as the United States.
115
116 The introduction of a complete, stable gut microbiome from a healthy donor into a recipient through a Faecal
117 Microbiota Transplant (FMT) has recently been incorporated into the therapeutic treatment of an array of known
118 and idiopathic conditions (5). Perhaps the best described and most effective clinical use of FMT in human
119 medicine is to treat recalcitrant *Clostridium difficile* infection (CDI), a result of dysbiosis stemming from
120 antibiotic use, is one of the most notable examples of current therapeutic benefit. A study by Aas et al (2003)
121 presented a FMT treatment success rate exceeding 90% within trial evaluable patients, such findings being
122 reproducible throughout considerable further research (10,11). Although the scientific rationale behind its
123 efficacy remains somewhat elusive, the undoubtable success of FMT in the treatment of CDI warrants further
124 indication of multiple applications beyond current practice. While use of FMT is becoming progressively
125 disseminated throughout human clinical practice, FMT in a modern sense has not yet been adopted into
126 livestock. Here we transfer a faecal, or more strictly a cecal, microbiome transfer from eight-week old animals
127 to newly hatched chicks and using challenge models assess the effect of FMT on host susceptibility to *C.jejuni*
128 infection and its transmission. We determine how FMT alters the microbiome through 16S rRNA gene-based
129 sequencing, with a view towards a rational approach of determining individual bacterial taxa or consortia that
130 offer protection against *C.jejuni* infection.

131 **RESULTS**

132 **Early faecal transplant has significant impact on *C.jejuni* colonization of the Ceca and ileum following**
133 **experimental seeder infection.** Early faecal microbiota transplantation significantly reduced *C.jejuni* M1 load
134 in both the ceca and ileum following experimental seeder infection of broiler chickens (Table 1). Birds receiving
135 FMT showed a significant reduction in *C.jejuni* load within the ileum when compared to Hatchery control birds

136 in both seeder Experiments 1 and 2 (P=0.0007; P=0.0451). The impact was even greater in reducing
137 colonisation further along the tract in the ceca (P<0.0001; P<0.0001) (Figure 1**).** Using direct challenge, rather
138 than seeder-bird challenge, colonisation was significantly lower in the ceca and ileum of birds given FMT
139 (P=0.0035; P=0.0152) at 4dpi (days post-infection). However, at 10dpi there was no significant difference
140 between the treatment populations indicating initial inhibition but not prevention of colonisation (Figure S3 in
141 Supplementary Material).
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	EXPERIMENT 1		**EXPERIMENT 2**	
	Median *C.jejuni* M1 content at 14 dpi (CFU.g^{-1}) [IQR]		Median *C.jejuni* M1 content at 12 dpi (CFU.g^{-1}) [IQR]	
	Ceca	Ileum	Ceca	Ileum
FMT	4.40 X 10^4 [5.80 X 10^7]	0.00 [0.00]	0.00 [0.00]	0.00 [0.00]
Ext. Hatchery Control	1.70 X 10^{11} [1.10 X 10^{11}]	4.00 X 10^3 [2.42 X 10^4]	3.78 X 10^8 [1.56 X 10^9]	0.00 [6.15 X 10^3]

144
145 *Table 1*. Levels of *C.jejuni* M1 in ceca and ileum of broiler chickens under experimental conditions according to Experiment 1 and Experiment 2 protocols.
146
147 **Transmission of *C.jejuni* within an experimental broiler flock is delayed by early faecal microbiota**
148 **transplantation.** Between 2dpi and 14dpi cloacal swabs were used to determine the dynamics of *C.jejuni*
149 infection within each population of birds. The kinetics of transmission were considerably slowed within the
150 FMT group compared to that of the untreated hatchery group (Figure 2). Experiment 1 showed 18/19 hatchery
151 birds were shedding *C.jejuni* at 5dpi, whereas there was no detected shedding within the FMT population. All
152 19/19 birds were shedding *C.jejuni* M1 by 8 dpi within the Hatchery external control population and all birds
153 continued to shed until 12 dpi. There was no bacterial shedding within the FMT population until 12dpi, with
154 4/19 birds shedding *C.jejuni* by this time-point. The transmission dynamics within experiment 1 were similar
155 within experiment 2, with no *C.jejuni* shedding detected in the FMT group during the course of the trial.
156 Shedding was detected within the Hatchery group from 3dpi and by 10dpi 11/12 birds were shedding *C.jejuni*
157 (Figure 2).
158
159 **Faecal microbiota transplantation administration at 7 days of age has no significant impact on *C.jejuni***
160 **GIT colonisation and transmission.** Swabs taken between 3dpi and 10dpi in experiment 3 showed a slight

161 delay in transmission of *C.jejuni* within the FMT population compared to that of the Hatchery, however this was
162 not sustained. At 3dpi 1/15 birds was shedding *C.jejuni* within the group of birds given the FMT while this
163 number was 3/17 within the external control hatchery group. However, by 5dpi the difference in level of
164 shedding between the two groups was negligible, with this relationship continuing until swabbing at 10dpi
165 whereby all birds in both groups were shedding *C.jejuni*. There was no significant reduction in final levels of
166 *C.jejuni* colonisation within either the ceca or the ileum (P=0.2403; P=0.1268) at 12dpi of experiment 3
167 populations.
168
169 **Extra intestinal spread of *C.jejuni* may be reduced following early faecal transplant administration.** At
170 post-mortem examination, extra intestinal *C.jejuni* colonisation was present in all 3 experimental trials.
171 Experiment 1 showed *C.jejuni* within the liver tissues of 2/19 Hatchery birds and 1/19 birds given FMT. This
172 result was similar in experiment 2, with *C.jejuni* present in 2/12 liver and 1/12 spleen samples from Hatchery
173 birds. No *C.jejuni* colonisation was detectable within the FMT population of this experiment. Both Hatchery
174 and FMT populations of experiment 3 showed high liver (>75%) and spleen (>18%) infection. These results
175 further confirm the invasive ability of *C.jejuni* M1 to spread beyond the GIT (Humphrey *et al.,* 2014).
176
177 **Cecal microbiota contains similar bacterial species richness following FMT but phylogenetic diversity**
178 **within those species is increased.** Pre-infection microbiota samples taken 7 days post-hatch from Internal
179 control, Hatchery and FMT populations of experiment 1 were sequenced using Illumina MiSeq sequencing
180 protocols. Targets amplification of the hypervariable V3/4 region of the 16S rRNA gene was used and amplified
181 reads clustered into Operational Taxonomic Units (OTUs) based on 97% similarity. The number of unique
182 OTUs observed within each sample following filtering ranged from 538 to 1833 with a total of 2613 unique
183 OTUs observed across all tested samples. A core microbiome of 1874 shared phylotypes was present between
184 different treatment groups. When directly comparing all treatment groups, FMT and Hatchery external control
185 groups showed fewest shared observed OTU's (Figure 3a). Alpha rarefaction was performed on all samples,
186 with the depth based on the median count of sequences found per sample. Faith's phylogenetic diversity and
187 Chao1 index were used as measures of community evolutionary distance and predicted species richness
188 respectively within samples (Figure 3b, 3c).
189

190 Shannon diversity index was used to characterize this taxonomic diversity within each sample, with FMT and
191 Internal control populations showing significant difference in diversity (P=0.0357). FMT treated population
192 may therefore show a similar microbiome species richness compared to the Hatchery and Internal control
193 populations, but the phylogenetic diversity seen within the phylotypes present is greater.
194
195 **The taxonomic composition of FMT microbiota was distinct to that of Hatchery and Internal control**
196 **populations.** To identify possible variations in the community structure of the gut microbiota by treatment, we
197 calculated the beta-diversity of the samples using PCoA transformation of weighted UniFrac matrixes. The
198 FMT population showed significant clustering and distinct spatial separation from the Hatchery external control
199 and Internal control populations using ADONIS analysis (P=0.001, R^2=0.361) (Figure 4). These data suggest
200 that the provision of a faecal microbiota transplant immediately post-hatch alters the overall composition of the
201 gut microbial community compared to those not having received treatment.
202
203 To examine differential representation of taxa between our sample groups, we compared relative abundance at
204 multiple taxonomic levels. The 2613 phylotypes (OTUs) identified were classified into four known and one
205 unknown phyla, with *Firmicutes* predominating all samples at a relative abundance of >90%. Further taxonomic
206 classification at order level showed FMT samples had an average 4.50 fold increase in relative abundance of
207 *Lactobacillales* and an average 1.78 fold decrease in relative abundance of *Clostridiales* compared to both
208 Internal control and Hatchery external control treatment populations (Figure 5, Figure 6).
209
210 ***Clostridiales* formed the major taxa within administered FMT inoculum.** Samples of FMT inoculum were
211 taken for V3/V4 16S rRNA gene sequencing using Illumina MiSeq sequencing protocols as mentioned
212 previously. Comparing relative abundance of different taxa at multiple taxonomic levels found *Firmicutes* as the
213 predominant phyla at >95% abundance, as with samples taken from our treatment groups. Further classification
214 identified *Clostridiales* as having an abundance >86%, being the core taxa with samples of administered FMT
215 inoculum (Figure 5). The number of observed OTUs within the FMT inoculum itself was the same as that
216 culminated within the treatment group samples.
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220 **DISCUSSION**

221 Here we show that transplanting a whole developed intestinal microbiome from older birds to newly-hatched
222 chicks leads to the long-term modification of the intestinal microbiome, which decreases experimental
223 transmission of *C.jejuni* within a flock of broiler chickens. We propose that early administration of a complex
224 microbiota offers clear potential to reduce *C.jejuni* infection in chicken meat production. Whilst a transfer of a
225 whole microbiota may be impractical in commercial production where billions of birds are reared worldwide
226 each year, it does represent a significant tool in finding consortia of microorganisms that protect against
227 infection in a rational manner.

228

229 One fundamental aspect allowing for the potential success of microbiota-based interventions in commercial
230 chicken production is the very nature of large-scale poultry meat production itself. Unlike other livestock
231 species, commercially-produced poultry have no contact with their mothers and do not acquire a pioneer
232 microbiome through maternal transfer, but through the environment of the hatchery (8). This leads to a lack of
233 early diversity and a potentially 'humanised' microbiome via hatchery workers (8) . Provision of an early, more
234 'avian' microbiome could help drive gut and immunological development leading to a healthier gut and an
235 animal less able to be colonized by *Campylobacter*.

236

237 While no statistically significant differences in species richness were found between treatment groups, it is
238 notable that in terms of the cecal microbiota taxa observed there were marked differences between treatment
239 groups regarding phylogenetic diversity. It was found that chicks hatched and reared within our poultry unit as
240 internal controls showed closer phylogenetic similarity to hatchery obtained chicks when compared to FMT
241 treated chicks also reared within our unit. As such, it is likely to be the FMT that is contributing most to the
242 stable shift in microbiome composition within the FMT treatment population. Administration of transplant
243 material at a week-of-age had limited impact on transmission and colonization, suggesting that provision of an
244 early and diverse microbiome is important.

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246 It is also interesting that main difference in taxa is the increase in *Lactobacillus* in the transplanted birds,
247 although this taxon does not form a large part of the transplant material (~5%). This suggests that transplant
248 may as much change the intestinal milieu to a more beneficial one, rather than just simply form the basis of the
249 microbiome. The change towards a cecal microbiome rich in *Lactobacilli* is perhaps indicative of this as usually

these form a small part of the microbiome in the lower intestinal tract, though are considered beneficial to chicken gut health, being the basis of many probiotics. Moreover, several recent studies have indicated that low levels of *Lactobacilli* in the chicken intestinal tract are associated with an increased load of *Campylobacter,* with links being made to modulation of cytokine gene expression altering immune response or the production of organic acids and anti-campylobacter proteins (12–14). Here, we show higher levels of *Lactobacillus* following transplantation correlate with reduced levels of *C.jejuni* colonization or even exclusion from the ceca following FMT.

As yet, we have not defined the mechanism or mechanisms that reduce *C.jejuni* transmission following cecal transplantation. As discussed previously these are likely to be either competitive exclusion effects or enhanced immune protection. Future work will look assess avian immune response to FMT administration, further characterize microbiome shifts and alternative methods of FMT inoculation that could be utilised on a larger, industrial scale.

280 **CONCLUDING REMARKS**

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282 Together, our data indicate that at-hatch transplantation of an adult microbiome significantly delays *C.jejuni*
283 colonization and transmission at a flock level. The provision of a complete, rather than culturable 'chicken'
284 microbiome acts to improve chicken gut health and impede *C.jejuni* in a naturalistic model of infection. We
285 suggest that it is essential that microbiota administration occurs immediately post-hatch to replace the naïve and
286 dynamic chick microbiota with that of a stable 'chicken' microbiome. We believe this concept could offer an
287 effective, low-cost control strategy to *C.jejuni* within the poultry industry.

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310 **METHODS**

311 **Bacterial strains and culture conditions.** *C.jejuni* M1 was cultured from frozen stocks maintained at -80 °C on
312 Colombia blood agar supplemented with 5% defibrinated horse blood (Oxoid, Basingstoke, Hampshire,
313 United Kingdom) for 48 h in microaerobic conditions (80% N_2, 12% CO_2, 5% O_2, and 3% H_2) at 41.5 °C.
314 Liquid cultures were grown for 24 h in 10 ml of Mueller-Hinton broth (MHB) in microaerobic conditions at
315 41.5 °C and adjusted by dilution in fresh MHB to a final concentration required. All microbiological media
316 were purchased from Lab M Ltd. (Heywood, Lancashire, United Kingdom).
317

318 **Faecal microbiota preparation –** The microbiota was obtained from three *Campylobacter* free, 8 week old
319 Ross 308 birds, reared under bio-secure condition The birds were euthanised before caecal contents were
320 aseptically removed. Caecal contents were then diluted 1:20 in Phosphate Buffered Saline (PBS) solution,
321 filtered through a 25µM filter and stored at -80°C until use.
322

323 **Experimental animals.** All work was conducted in accordance with United Kingdom (UK) legislation
324 governing experimental animals under project license PPL 40/3652 and was approved by the University of
325 Liverpool ethical review process prior to the award of the licenses. All animals were checked a minimum of
326 twice-daily to ensure their health and welfare. For experiment 1 and 2, embryonated Ross 308 hens' eggs
327 were obtained from a commercial hatchery and incubated in an automatic roll incubator under standard
328 conditions for hen eggs. Chicks were removed from the incubator post-hatch and an inoculum of FMT was
329 administered to each chick within 4 hours of hatching. A small group (n=10) of hatched chicks were not
330 given any FMT inoculum to act as a separate Internal control group in experiment 1 and used solely for 16S
331 rRNA gene analysis. Age matched, 1 day-old mixed sex chicks of Ross 308 broiler chickens were obtained
332 from the same commercial hatchery and not given the FMT to act as a Hatchery external control group.
333 Chicks were housed in the University of Liverpool high-biosecurity poultry unit as with (1). At 7 days post-
334 hatch during experiment 1, a small number of chicks from the FMT (n=3) and Hatchery control (n=5),
335 alongside all Internal control (n=10) chicks were culled and microbiota snap frozen for use in 16S rRNA
336 gene sequencing protocols. For further clarification on each experimental protocol see Figure S1&2 in
337 Supplementary Materials.
338

339 Prior to experimental infection, all birds were confirmed as *Campylobacter* free by taking cloacal swabs,
340 which were streaked onto selective blood-free agar (modified charcoal-cefoperazone-deoxycholate agar
341 [mCCDA]) supplemented with *Campylobacter* enrichment supplement (SV59; Mast Group, Bootle,
342 Merseyside, United Kingdom) and grown for 48h in microaerobic conditions at 41.5 °C.
343
344 **EXPERIMENTS 1 & 2. Effect of faecal transplantation on seeder *C.jejuni* infection.** At 21 days post-
345 hatch, two birds from both the FMT (Experiment 1 n=19; Experiment 2 n=8) and the Hatchery control
346 (Experiment 1 n=19; Experiment 2 n=12) groups were orally infected with 10^6 cells of *C.jejuni* M1 in 0.2ml
347 of MHB. Challenge at 21 days of age has previously shown to be a robust model that mimics the situation in
348 the field in the UK, where birds typically become infected at around three weeks of age due to a 'lag phase'
349 considered to be a consequence of protection by maternal immunity (4).
350
351 At 2, 5, 8 and 12 (Experiment 1) or 3, 5, 7 and 10 (Experiment 2) days post-infection, cloacal swabs of all
352 birds were taken to assess within-group transmission. At 14 dpi (Experiment 1) or 12 dpi (Experiment 2), all
353 birds were culled via cervical dislocation. At post-mortem examination, samples of tissue and gut contents
354 were collected and processed for host bacterial enumeration. The presence/absence of hock marks and/or
355 pododermatitis also was recorded for every bird at post-mortem examination.
356
357 **EXPERIMENT 3. Effect of delayed administration of faecal transplant on seeder C.jejuni infection.**
358 Age matched, 1 day-old mixed sex chicks of Ross 308 broiler chickens were obtained from a commercial
359 Hatchery and housed as with experiment 1 and 2 in the University of Liverpool high-biosecurity poultry
360 unit. At 7 days post-hatch, a group of birds (n=15) were inoculated with FMT. The remaining birds (n=17)
361 were not given the FMT to act as a control group. At 21 days post-hatch, two birds from both the FMT
362 (n=8) and the Hatchery external control (n=12) groups were orally infected with 10^6 cells of *C.jejuni* M1 in
363 0.2ml of MHB. At 3, 5, 7 and 10 days post-infection cloacal swabs of all birds were taken to assess within-
364 group transmission. At 12 days post-infection birds were killed via cervical dislocation. At post-mortem
365 examination, samples of tissue and gut contents were collected and processed for host bacterial
366 enumeration.
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368 **Assessment of *C.jejuni* load.** To determine the level of *C.jejuni* intestinal colonisation within each group,
369 cecal and ileal content was collected from individual birds at necroscopy. This was diluted in 9 volumes of
370 maximal recovery diluent (Lab M, Heywood, Lancashire, United Kingdom [MRD]) with further serial 10-
371 fold dilutions being made of each sample in MRD. Using the method as described Miles & Misra (1938),
372 triplicate 20µl spots were plated onto mCCDA agar supplemented with SV59. The plates were incubated
373 under microaerobic conditions at 41.5°C for 48 h, and *Campylobacter* colonies were enumerated to give
374 colony forming units per gram (CFU/g) of cecal and ileal content. Liver and spleen tissue was also biopsied
375 at post-mortem to assess any extra intestinal spread of *C.jejuni* infection. Differences in final colonisation
376 levels between treatment groups were analysed for significance ($P<0.05$) using Mann-Whitney *U* tests in
377 GraphPad Prism version 7.00 software.
378
379 **Assessment of C.jejuni shedding.** Cloacal swabbing provided a non-sacrificial method of following
380 *C.jejuni* shedding within individual experimental groups. Cloacal swabs were briefly plated onto mCCDA
381 agar supplemented with SV59. Swabs were then eluted in 2ml modified 5% Exeter broth consisting of
382 1,100ml nutrient broth, 11ml lysed defibrinated horse blood (Oxoid, Basingstoke, Hampshire, United
383 Kingdom), *Campylobacter* enrichment supplement SV59 (Mast Diagnostics), and *Campylobacter* growth
384 supplement SV61 (Mast Diagnostics). Enriched swabs were then incubated at 41.5 °C for 48 h and re-plated
385 onto mCCDA agar and incubated for 48 h at 41.5 °C. Plates were assessed for *C.jejuni* positivity.
386
387 **DNA Extraction.** Cecal and ileal microbiota contents were collected from a random sample of birds in
388 experiment 1 and FMT alongside inoculum samples, snap frozen and stored at -80°C before DNA extraction.
389 Microbial community DNA was extracted from faecal samples using the Qiagen QIAamp® Fast DNA Stool
390 Mini (Qiagen, Hilden, Germany) following the protocol for the Isolation of DNA from Stool for Pathogen
391 Detection. DNA was eluted in 200µl of DNase/RNase Free Water and stored at -20 °C until further analysis.
392 Isolated DNA quality and integrity was assessed through 2.0% agarose gel electrophoresis and
393 concentration measured using a Qubit 2.0 Fluorometer (Life Technologies).

394 **Illumina MiSeq platform sequencing.** Extracted DNA was sent for llumina MiSeq sequencing of the V3/V4
395 hypervariable 16S rRNA gene at the Centre for Genomic Research (University of Liverpool). Sample library
396 preparation and amplification were performed according to the method previously described by D'Amore et al
397 (2016). Prior to data processing, all raw Fastq files were trimmed using Cutadapt version 1.2.1 (15) to remove

398 any Illumina adapter sequences. All reads were subsequently trimmed using Sickle version 1.200 (16) with a
399 minimum window quality score of 20 and any reads containing fewer than 10 base pairs were removed.

400 ***Sequencing data processing.*** Data processing was performed using the QIIME 1.9.1 pipeline (17). Forward and
401 reverse Fastq reads were joined, filtered and demultiplexed. FASTA file sequences were clustered using
402 USEARCH to create OTUs using a 97% identity threshold (18). Chimeric sequences were removed using
403 UCHIME and the most abundant sequence from each OTU used as representative (19). Taxonomic level for
404 each of these representative sequences was assigned against the Greengenes 16S rRNA gene database (20) and
405 aligned using PyNAST at a minimum identity of 75% (21). OTUs with a number of sequences less than 0.005%
406 of the total number of sequences were removed from further processing (22). A phylogenetic tree was generated
407 using FastTree (23). FMT inoculum samples were then filtered from treatment group samples and analysed
408 separately. Corresponding sequence files and metadata for all samples analysed using 16S rRNA MiSeq
409 sequencing have been deposited in Figshare and are included here as additional files 1 and 2 respectively.

410 Alpha and Beta diversity metrics were used to assess microbial composition within and between sample groups.
411 Shannon's Diversity Index, Faith's Phylogenetic Diversity and Chao1 metrics were all used to assess
412 community richness with Faiths Phylogenetic Diversity further incorporating phylogenetic relationships
413 between features. Alpha rarefaction curves were constructed based on Chao1 and Faith's Phylogenetic Diversity
414 metrics as a comparison between different treatment groups. A Venn diagram was created using MetaCoMET's
415 jvenn programme (24) to compare microbiome data from each treatment group with the core microbiome
416 represented within the shared overlapping regions between the circles. A breakdown of the taxa at an Order
417 level contributing to samples within each treatment group were created using MetaCoMET relating to their
418 relative abundance within those samples. Beta diversity principal coordinate analysis (PCoA) estimates were
419 created based on Weighted UniFrac distances (25) to identify similarities between samples under different
420 treatment groups.

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425 **LIST OF ABBREVIATIONS**

426 EU, European Union; CE, Competitive Exclusion; GIT, Gastrointestinal tract; FMT, Faecal Microbiota
427 Transplant; CDI, *Clostridium difficile* infection; MHB, Mueller-Hinton broth; PBS, Phosphate Buffered Saline;
428 UK, United Kingdom; dpi, days post-infection; mCCDA, modified charcoal-cefoperazone-deoxycholate agar;
429 MRD, Maximal recovery diluent; CFU/g, colony forming unites per gram; OTU, Operational Taxonomic Unit;
430 PCoA, Principal Coordinate Analysis.

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455 **DECLARATIONS**

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457 ***Ethics approval and consent to participate***. All work was performed in accordance with relevant UK legislation
458 of Animal Use (Animals [Scientific Procedures] Act 1986 under project licence PPL 40/3652 which required
459 Ethical Review by the University of Liverpool Animal Welfare and Ethical Review Body prior to its award.

460

461 ***Consent for publication.*** Not applicable.

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463 ***Availability of data and material.*** The 16S rRNA sequencing datasets generated and analysed during the current
464 study are available in Figshare as Additional electronic supplementary files.
465
466 https://figshare.com/s/469b48d2e022440ce2dc – Additional file 1: MiSeq 16S rRNA sequence files (2.29 GB).
467
468 https://figshare.com/s/ae6b0048285f9f2af5fb - Additional file 2: Metadata associated with sample sequences
469 analysed using 16S rRNA sequencing (1.3kB).
470

471 ***Competing interests.*** The authors declare that there are no conflicts of interest associated with this publication.

472

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480 manuscript

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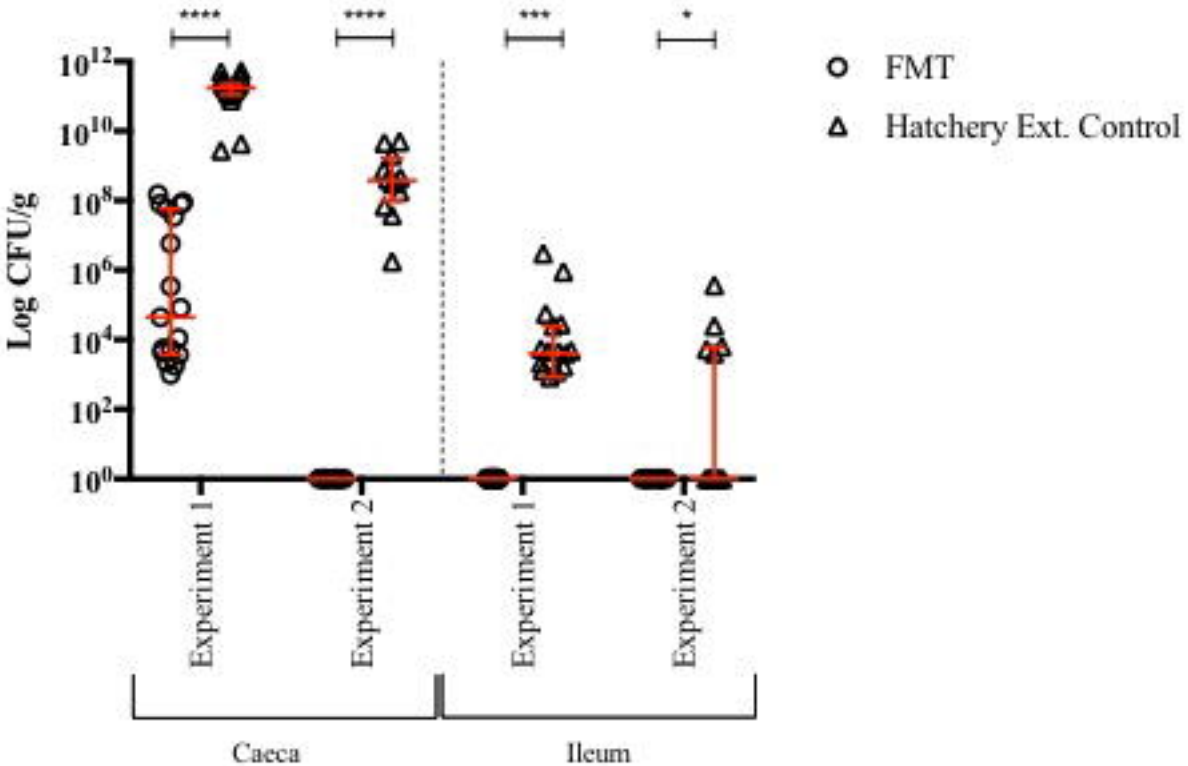

Figure 1. Levels of *C jejuni* M1 in the ceca and ileum of broiler chickens grown under experimental conditions based on protocols for Experiment 1 and Experiment 2. Each symbol represents *C jejuni* colonisation load for an individual sample. Results are also expressed as median values and associated IQR with significance determined using Mann Whitney-U analysis. Levels of significance given are at *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

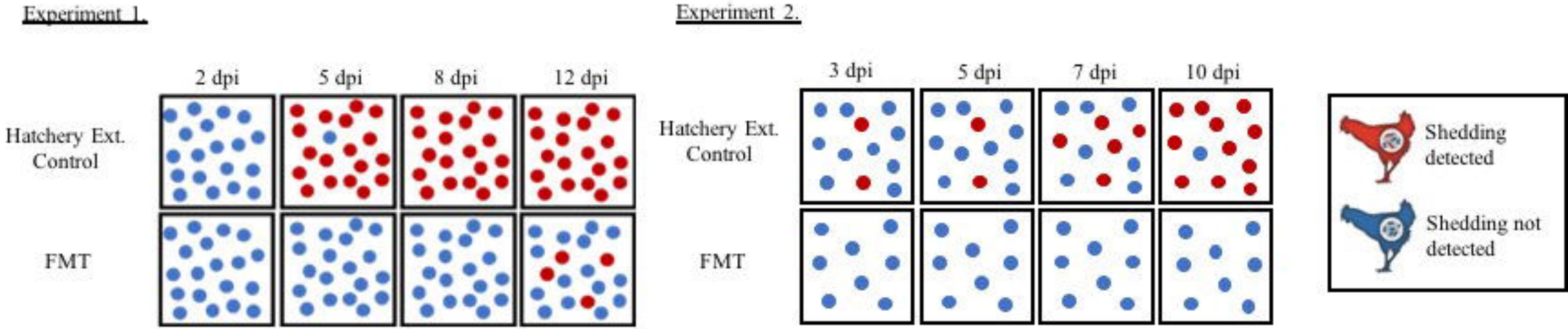

Figure 2. C. jejuni M1 transmission within broiler groups of Experiment 1 and Experiment 2 determined through cloacal swabbing. Red shapes show birds where bacterial shedding was detected while blue shapes show groups with no bacterial shedding.

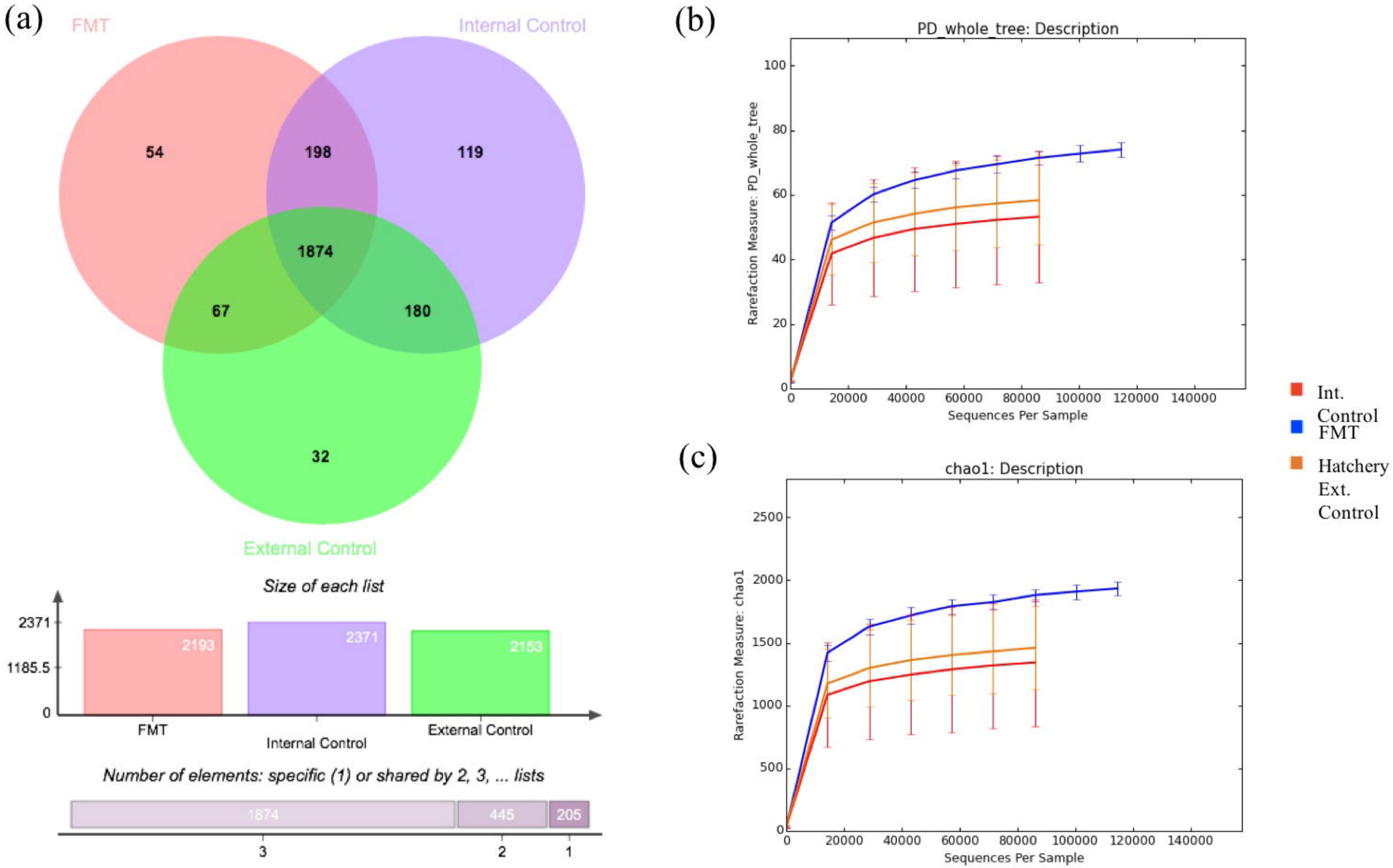

Figure 3. Venn diagram showing the shared gut microbiome (OUT's) between the 3 treatment groups of Experiment 1 pre *C.jejuni* infection (a). Alpha rarefaction curves were generated based on the OUT's identified using 97% sequence similarities for FMT, Hatchery External control and Internal control groups using a measure of Faith's phylogenetic diversity (b) and Chao1 (c).

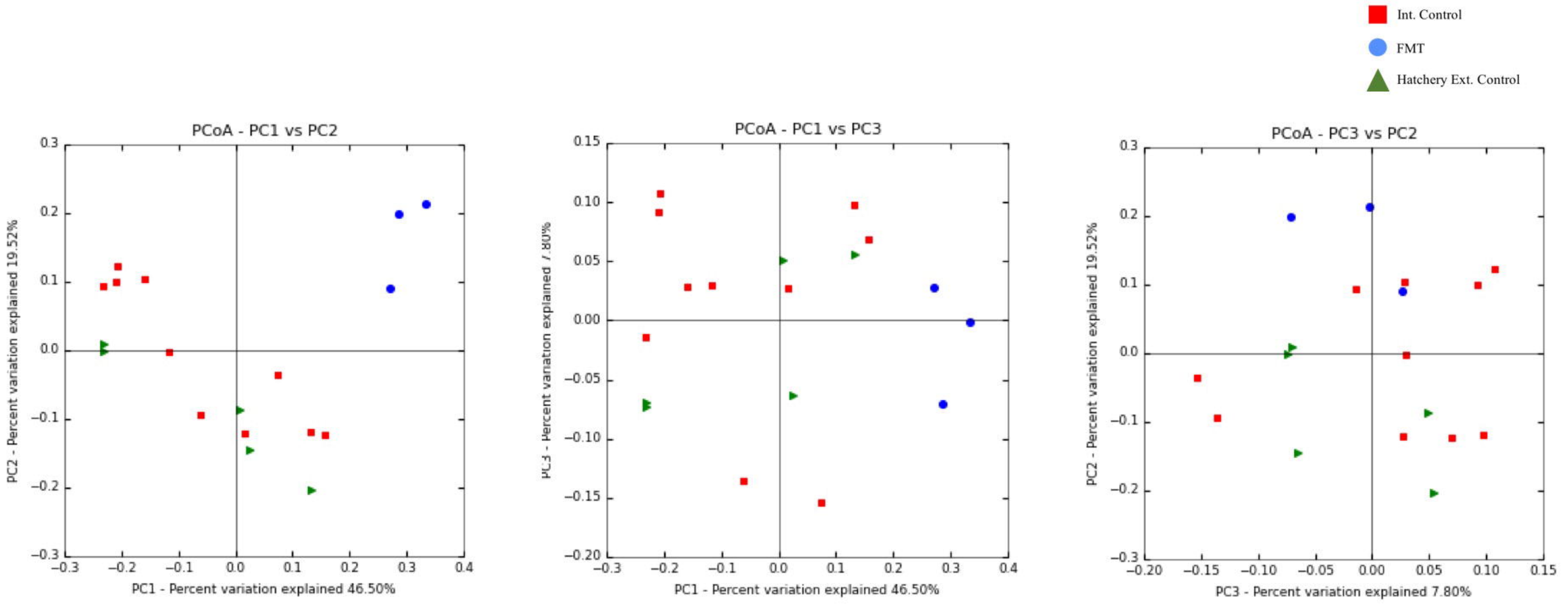

Figure 4. Principal coordinate analysis (PCoA) plot based on weighted UniFrac distances for FMT, Hatchery control and Internal control experimental populations from Experiment 1. Points represent individual samples in the data set. ADONIS testing revealed a significant clustering of samples ($P<0.05$). PC1 and PC2 explain >65% of the total variance within the dataset. Red data points: Internal control; Blue data points: FMT; Green data points: Hatchery external control.

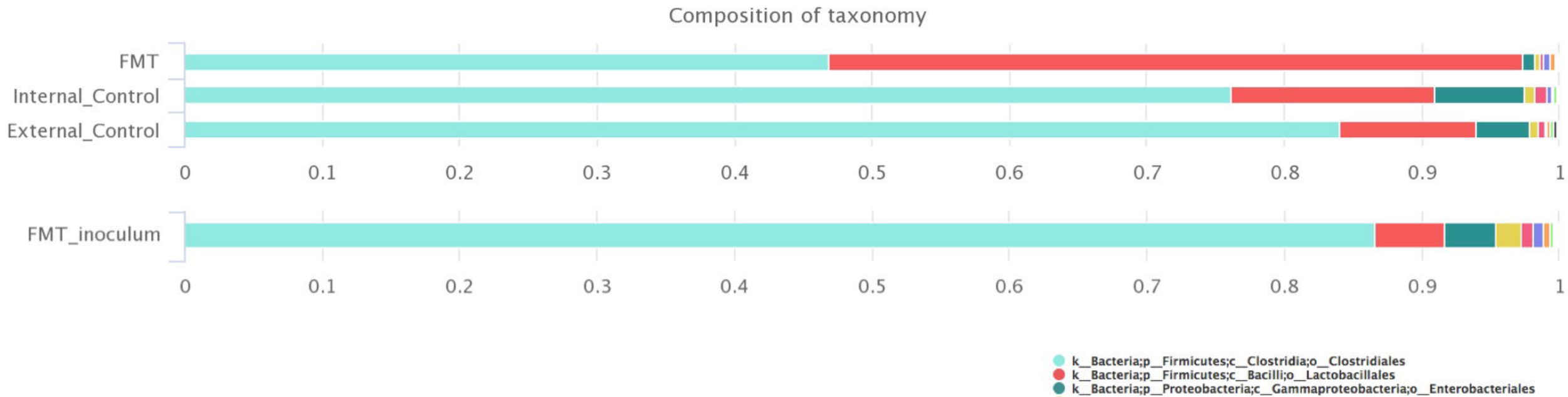

Figure 5. Microbial composition of samples from FMT, Hatchery external control and Internal control treatment populations following 16S rRNA gene sequencing. Included is detail on the composition of FMT inoculum material given to all birds within FMT treatment populations. Composition is displayed as relative percentage abundance of the common taxa (Order) within that treatment group. The 3 most abundant taxonomic groups within treatment samples and inoculum are listed.

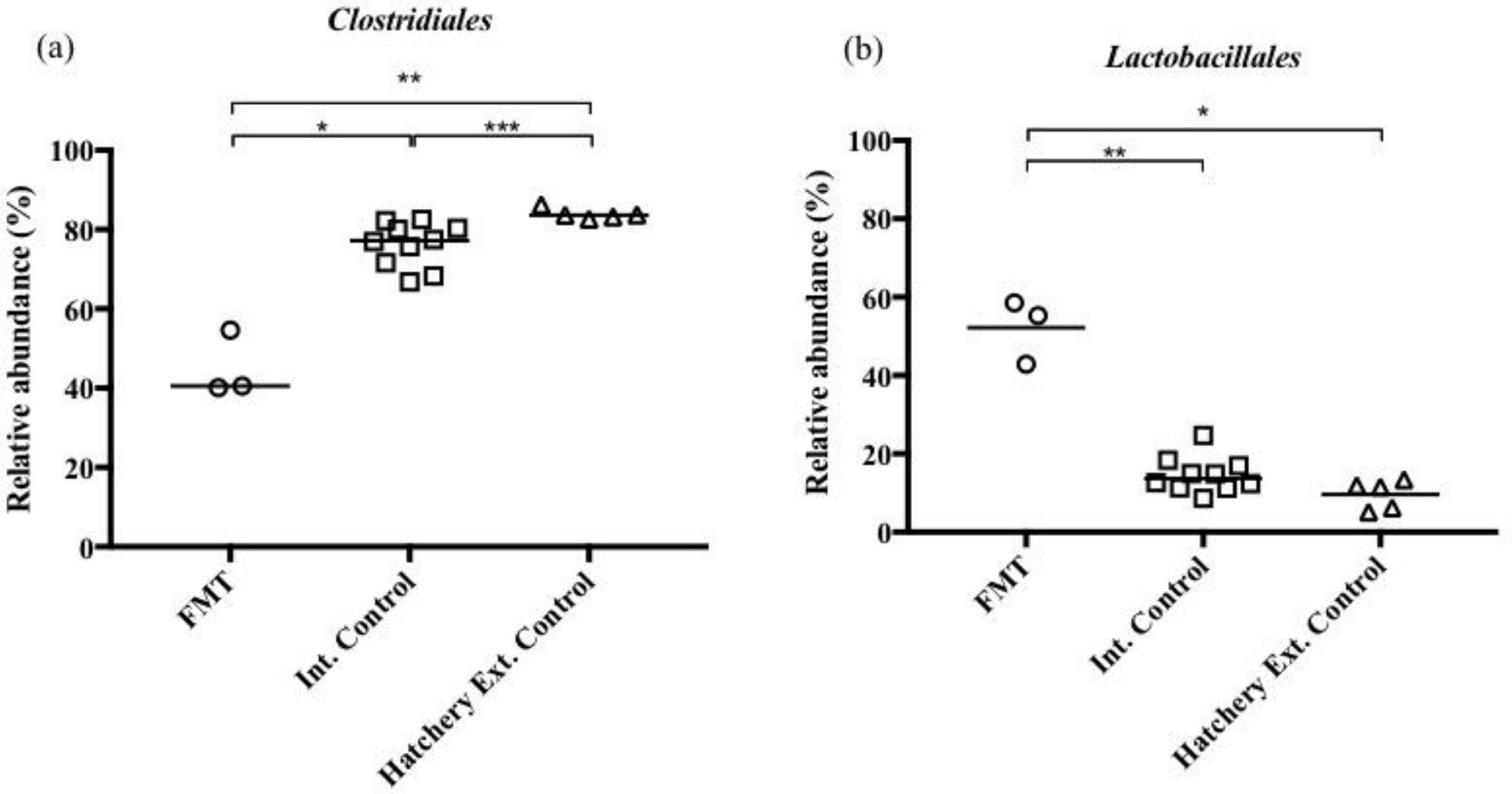

Figure 6. Relative percentage abundance of Clostridiales (a) and Lactobacillales (b) taxa within samples from each treatment group following 16S rRNA gene sequencing of samples from Experiment 1. Each symbol represents relative abundance within an individual sample. Results are also expressed as median values with significance determined using Mann Whitney-U analysis. Levels of significance are given at *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.