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# Changes within the intestinal flora of broilers by colonisation with *Campylobacter jejuni*

## *Veränderungen der Darmflora des Geflügels durch Besiedelung mit Campylobacter jejuni*

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**Summary**

In most European countries human campylobacteriosis is the most important bacterial zoonotic foodborne infection. Chicken meat is considered the main source of infection. Since most strategies assessed so far, in reducing *Campylobacter* colonization in chickens or in the reduction of human disease, have not been very effective, new knowledge regarding Campylobacter's interaction with the host is needed. In this study we analysed fecal and cecal samples of five chicken flocks of different Austrian farms for the occurrence of *C. jejuni* and *C. coli*, and analysed the intestinal microbiota by PCR-SSCP, cultural detection of lactic acid bacteria, *Enterococci, Staphylococci*, *Enterobacteriaceae*, *E. coli*, and total aerobic colony counts. Furthermore ten chicken samples of cecal content of a flock during colonization with *Campylobacter* spp. was analysed by high throughput sequencing. With all three methods used we could detect a change within the microbiota caused by *C. jejuni*. Enumeration of different bacteria was significantly lower in fecal samples positive for *C. jejuni,* pointing out that a higher water content and thus, a preliminary stage of diarrhea might appear during *Campylobacter* colonization. By PCR-SSCP analysis the microbiota composition differed between colonized and non-colonized chicken fecal samples. This could also be detected in community analysis by high throughput sequencing, but this difference was only a tendency and not statistically significant. It can be concluded that *C. jejuni* is interacting with the intestinal microflora in their respective hosts and hence, this has to be taken into account when providing new strategies to combat *Campylobacter* colonization and disease.

**Keywords:** *Campylobacter jejuni,* microbial community, PCR-SSCP, sequencing, chicken

**Zusammenfassung**

Die Campylobacteriose stellt europaweit die häufigste lebensmittelbedingte bakterielle Infektionskrankheit dar. Als Hauptursache dieser Infektion gilt nach wie vor Geflügelfleisch. Da alle bisherigen Bemühungen, eine Besiedelung von Geflügelherden mit *Campylobacter* zu verhindern oder humane Infektionsraten zu senken, fehlschlugen, gilt es neue Erkenntnisse zu gewinnen. In der vorliegenden Arbeit wurden fäkale und zäkale Proben von österreichischem Mastgeflügel auf das Vorhandensein von *C. jejuni* und *C. coli* untersucht und die intestinale Mikrobiota mit genetischen Methoden (PCR-SSCP) und kulturellen Nachweisen von Laktobazillen, Enterokokken, Staphylokokken, *Enterobacteriaceae*, *E. coli*, ebenso wie die Ermittlung der aeroben Gesamtkeimzahl, analysiert. Weiters sind zehn zäkale Proben, die während einer stattfindenden Herdendurchseuchung entnommen wurden, mittels einer „high throughput sequencing" Analyse untersucht worden. Mit allen drei Methoden konnte eine Änderung der mikrobiellen Flora durch eine Besiedelung mit *Campylobacter* nachgewiesen werden. In *Campylobacter* positiven Proben wurden signifikant weniger Mikroorganismen detektiert. Dies ist möglicherweise durch einen höheren Wassergehalt in den fäkalen Proben zu erklären, was auf die Vorstufe einer Diarrhö hinweisen könnte. Mittels PCR-SSCP

Analyse wurde eine Veränderung in der Zusammensetzung der Mikrobiota nachgewiesen, was auch mittels dem „high throughput sequencing" erkennbar war. Dieser Unterschied war nur als Tendenz erkennbar und nicht statistisch signifikant. Zusammenfassend konnte mit dieser Studie bestätigt werden, dass *C. jejuni* mit der intestinalen Mikroflora interagiert. Diese Erkenntnis sollte in die Planung neuer Bekämpfungsstrategien miteinbezogen werden.

**Schlüsselwörter:** *Campylobacter jejuni*, mikrobielle Flora, PCR-SSCP, Sequenzierung, Huhn

## Introduction

*Campylobacter (C.)* infections in humans are still on the rise in industrialized, emerging and developing nations worldwide (Lévesque et al., 2013). Chicken and chicken meat are considered the main source of human infection (EFSA, 2010). Strategies to limit *Campylobacter* colonisation in chickens can considerably lower the risk of human disease (Boysen et al., 2013). *C. jejuni* gets into the chicken stables by different means. Most often a flock colonizes between the third and the seventh week of fattening by contaminated working tools, cages, clothes, shoes or coats but *Campylobacter* may also be transferred by insects or rodents (Newell et al., 2011). The transfer between stables at the same farm is rather the rule than the exception. Most often a flock gets colonized when it is thinned for slaughter (part of the chickens are harvested for slaughter and the remaining chickens of the flock are fattened for a longer time) for the first time. Thinning is used to respond to consumer's needs – requests for whole chicken as well as chicken parts – and to stay to animal welfare regulations in regards to square meter per weight. With contaminated cages, chicken harvesters or bird catchers the remaining birds are colonized within a few days (Allen et al., 2008).

*Campylobacter* spp. are zoonotic pathogens, that can be found in the intestinal tract of most animal species. Despite that, specific species and genotypes predominate in different hosts (Wilson et al., 2008). The organism, particularly *C. jejuni*, is able to colonize various hosts. It can cause symptoms in humans and can colonize the lower intestinal tract of chickens without clinical signs of disease (Janssen et al., 2008). But likewise it colonizes the duodenum in cows without symptoms but may cause diarrhoe in calves (Klein et al., 2013). One specific feature of *C. jejuni* has been shown by Bereswill et al. (2011): Colonization resistance in mice is dependent on the intestinal microbiota. More precisely *C. jejuni* can colonize and even cause disease symptoms in mice with a completely eradicated or with a human like intestinal microflora. Thus, *C. jejuni*'s colonization ability and its aptitude to cause disease symptoms are dependent on the microflora of the specific host. Very recently Dicksved et al. (2014) demonstrated that colonization resistance in humans is attributed to a specific intestinal microflora but *C. jejuni* colonization leads to a change in the intestinal microbiota in humans as well.

During colonization of the chicken's ceca *C. jejuni* can reach levels of $10^9$ colony forming units (cfu) of bacteria/g in the cecal content (Shane, 1992). Therefore, a shift in the intestinal microbiota by *C. jejuni* infection is not surprising. Recently, a study revealed, by high throughput sequencing analysis, that the microbiota in chickens comprises four enterotypes and that *Campylobacter* spp. was observed to a lower extent in one of the four enterotypes (Kaakoush et al., 2014). Nevertheless flock and age of the birds contribute to the different flora composition.

In this study we used different methods like PCR-SSCP (Polymerase chain reaction single strand confirmation polymorphism) analysis and high throughput sequencing analysis of the 16S RNA gene as well as cultural methods to define the microbiota of fecal and cecal content of five flocks of different Austrian farms in correlation to *C. jejuni* colonization. Results revealed both, a specific microbiota, and a lower content of culturable microorganisms for *Campylobacter* colonized chickens.

## Material and Methods

### Sample collection

Samples were collected in Austria in the years 2003 to 2006 and in the year 2013. At all four different seasons (spring, summer, autumn, and winter) one flock was analysed. Fecal samples were drawn in fattening week 6 and 7 (sample number for each flock n = 36, in total n = 144). Ceca content was collected at slaughter (n = 8 for each flock, in total 32). One additional flock was sampled (cecal samples n = 31) in autumn in the year 2013 and ten samples of this flock were subjected to community analysis using high throughput sequencing. All flocks were kept under conventional husbandry conditions. Fractionated slaughter (the flock was slaughtered in two parts; interval: a week) was used.

### PCR-SSCP (Polymerase chain reaction single strand confirmation polymorphism)

#### *DNA-Isolation*

0.5–1 g of sample was extracted using the QIAamp® Stool Mini Kit (Qiagen Hilden, Germany) according to manufactures advice. Most samples were analyzed immediately at arrival; transportation to the laboratories was performed under cooling conditions at 3°C.

#### *PCR*

The 16S RNA gene variable region V4–5 was amplified using the COM primer set: 5'-cagcagccgcggtaatac-3' and 5'-ccgtcaattcctttgagttt-3' (Schwieger and Tebbe, 1998). Conditions. Denaturation 94°C 30", annealing 54°C 30", elongation 72°C 30" for 30 cycles (all PCR reagents: New England Biolabs, Ipswich, USA, Primer synthesis: Sigma Genosys – Proligo).

#### *Denaturation*

25 µl of the PCR product and 16 µl denaturation buffer (95% formamide, 10 mM NaOH, 0.25% bromophenol

blue and 0.25% xylene cyanol) were incubated at 95°C for 2 min and immediately set on ice (10 min) (Ott et al., 2004). The total volume of 41 µl per sample was loaded on PAG's slots.

*Polyacrylamide gel electrophoresis*

A 10% PAG using 1xTBE (Tris-Borate-EDTA) buffer, Acrylamide-Bis solution (37.5:1) (Serva, Heidelberg, Germany), APS (Ammoniumpersulfat, Serva), TEMED (N,N,N,N'-Tetramethylethylendiamine Sigma, St. Louis, USA) was casted between two glass plates (0.5 mm thick). Polymerization was carried out over night. The gel was used immediately or cooled at 7°C until use (max. 3 days of storage). The electrophoresis was carried out in 2xTBE buffer on a horizontal electrophoresis chamber (Bio Phoresis, BIORAD) under running conditions: 200V/const. for 700V/h (Power supply: Model 3000Xi, BIORAD). The samples were denatured immediately before they were loaded on the gel. For visualization silver staining of PAGs' was performed according to Heukeshoven and Dernik (1985). Cluster analysis was done using the Bio-Rad FingerprintingTM II version 3.0.

**Isolation of bacteria by cultural methods**

Each individual fecal sample and cecal content was serially diluted 1:10 in sterile physiological sodium chloride solution. A 10-fold dilution series from $10^{-2}$ to $10^{-10}$ was spread onto specific agar plates. Coli-ID-Agar plates (Bio Merieux, Marcy l'Etoile, France) were used to enumerate *E. coli* and were incubated for 24 h at 42°C. Typical colonies were counted. A random sample of these colonies was biochemically verified as *E. coli* by using the Api-20E (Bio Merieux, Marcy l'Etoile, France). For the detection of Lactobacilli afore mentioned serial dilutions were incubated on MRS-Agar-plates (Merck, Darmstadt, Germany) for 72 h at 30°C under aerobic conditions and enumerated. *Staphylococci* were analysed on Baird-Parker Agar (Merck, Darmstadt) supplemented with egg yolk emulsion (Merck, Darmstadt, Germany) at 37°C for 48 h under aerobic conditions. Serial dilutions for the detection of *Enterococci* were plated on Chromocult-*Enterococci*-Agar (Merck, Darmstadt, Germany) and aerobically incubated for 24 h at 42°C. Typical colonies were enumerated. *Enterobacteriaceae* were analysed on VRBD-Agar (Merck, Darmstadt, Germany) according to manufacturer's advice after aerobic incubation at 37°C for 24 h. Total aerobic colony counts were enumerated using serial dilutions $10^{-6}$ to $10^{-10}$ on Plate Count Agar (Merck, Darmstadt, Germany) incubated at 30°C under aerobic conditions and 48 h.

Thermophilic *Campylobacter* spp. were analysed according to ISO-10272-(2002). In brief: 15 g of fecal or cecal samples were pre-incubated in Bolton Broth (Oxoid, Basingstoke, United Kingdom) at 42°C for 48 h under microaerophilic conditions (10% $CO_2$, 5% $O_2$ and 85% $N_2$). The samples were then plated on two selective agars, modified CCDA (Oxoid, Basingstoke, United Kingdom) and CampyFoodAgar (Bio Merieux, Marcy l`Etoile, France) and incubated at 42°C for 48h under microaerobic conditions. Additionally, all fecal samples were directly streaked onto above mentioned selective agars (without prior enrichment). Typical colony morphology, aerobic incubation and a PCR (Linton et al., 1997) was used for identification and species differentiation between *C. jejuni* and *C. coli* as described.

**High throughput sequencing and data analysis**

DNA from cecal samples was isolated using a combination of mechanical and enzymatical lysis. Briefly, 175 mg cecal samples were homogenised in MagnaLyser Bead tubes using the MagnaLyser Instrument (Roche Diagnostics, Mannheim, Germany) according to standard procedures. Bacterial DNA was isolated with the MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) in a MagNA Pure LC 2.0 Instrument (Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions adding 100 µg lysozyme (Carl Roth GmbH, Karlsruhe, Germany) per 100 µl sample. High throughput sequencing was performed at the core facility molecular biology at the ZMF center of medical research Graz, Austria based on the 16S RNA gene variable region V1–2, 75 ng of total genomic DNA was amplified with the FLX 454 one way read (Lib-L kit, Primer A, Primer B, Roche 454 Life Science, Branford, CT, USA). Fusion primer F27-5'AGAGTTTGATCCTGGCTCAG3' and R357-5'CTGCTGCCTYCCGTA3' (Baker et al., 2003; Watanabe et al., 2001). Each PCR reaction was of 50 µl containing 1 x Fast Start High Fidelity Buffer (Roche Diagnostics, Mannheim, Germany), 2.5 U High Fidelity Enzyme (Roche Diagnostics, Mannheim, Germany), 200 µM dNTPs (Roche Diagnostics, Mannheim, Germany), 0.4 µM barcoded primers (Eurofins MWG, Ebersberg, Germany), PCR-grade water (Roche Diagnostics, Mannheim, Germany) and 75 ng DNA. PCR products were purified according to standard procedures and amplicon concentrations were determined using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, CA, USA) according to manufacturer's instructions. After quantification the barcode labeled amplicons were pooled equimolar and analyzed on a 2100 Bio Analyzer (Agilent Technologies, Waldbronn, Germany) using a DNA 7500 kit. Emulsion PCR with the GS Titanium MV emPCR Kit and method (Lib-L) (Roche 454 Life Science, Branford, CT, USA) was performed according to the manufacturer's instructions. Sequencing of the pool was performed using the GS FLX Titanium Sequencing Kit XLR70 (Roche 454 Life Science, Branford, CT, USA) according to the manufacturer's instructions.

**Data analysis and statistical analysis**

Raw sequence data generated by the Roche GS FLX sequencer was de-noised with Acacia 1.52 and processed for initial quality filtering with Qiime 1.7.0 (read length between 200 and 600, no ambiguous bases, no primer mismatches, minimum quality score of 25 and maximal number of homopolymers 6). For the assessment of operational taxonomic units, reads were clustered with uclust allowing for 3% sequence distance (97% sequence identity threshold). Representative sequences from each OTU cluster were aligned with PyNAST and taxonomy was assigned by using the RDP classifier with confidence score of 0.8. To remove PCR artifacts we used ChimeraSlayer for a chimera check on the aligned representative sequences. Phylogenetic trees constructed with FastTree were used to generate distance matrices with UniFrac. Beside phylogenetic distance metric we alternatively calculated Bray-Curtis distances. Species richness and diversity, including estimation indices, were calculated using the R statistical Software platform supplemented by the community ecology packages Vegan and BiodiversityR. In the beta diversity analysis, data normalization was carried out by downsampling (rarefaction) to the

smallest sample size (2950) of the OTU table. Principal coordinate analysis on the normalized data was performed by Qiime.

The relation of the presence and absence of *C. jejuni* and the detection of the amount of culturable microorganisms was assessed by statistical analyses, in which results from enumeration were converted into their decadic logarithms. Data below the limit of detection (i. e. 2 log cfu/g) were set to 1 log cfu/g. The ANOVA analyses considered the presence of *Campylobacter* as dependent factors, and number of flock (8,9,10,11), sampling date (day of fattening), total aerobic count, lactic acid bacteria, *Staphylococci*, *Enterococci*, *Enterobacteriaceae* and *E. coli* as independent factors. Discrimination between means was done with LSD test. Statistical significance was established at $p < 0.05$, unless indicated otherwise.

## Results

### Detection of *C. jejuni* in chicken fecal and cecal content

Of the four flocks three became colonized with *Campylobacter* and one remained free of thermophilic *Campylobacter* spp., respectively. Fecal samples were taken at the 6th and 7th week of fattening. Two of the positive flocks were negative for cultural detection of thermophilic *Campylobacter* spp. until the 7th week, one flock was positive in week six and seven. The 5th flock (sampled in 2013) became positive during the 7th week, samples were taken at slaughter. Slaughter was performed at the end of the 7th week. Thus, some samples were identified as positive, some as negative and with different quantitative amounts of *Campylobacter* in the ceca content.

Of these cecal samples four were defined as cultural positive for *C. jejuni*, one positive for *C. coli* and five samples defined as *C. jejuni* and *C. coli* free by cultural isolation. These ten samples were subjected to microbiome analyses using the 16S RNA genes and high throughput sequencing.

### Clusters of *C. jejuni* positive samples using PCR-SSCP microbial community analysis

We analysed the total fecal flora by amplification of the 16S RNA genes of the whole bacterial flora and analysed these single DNA strands by polyacrylamide gel electrophoresis (PCR-SSCP). Using the PCR-SSCP cluster analysis, differences were identified at flock level and an age specific pattern could be identified as well (data not shown). No seasonal cluster was identified. Despite these strong clusters seen for age and flock, a cluster of *Campylobacter* positive fecal samples was identified (Fig. 1). Figure 1 shows the cluster analysis of a PCR-SSCP gel with fecal samples of naturally *Campylobacter* colonized and non-colonized chickens. Fecal samples of *Campylobacter* colonized chickens cluster together versus not colonized, regardless of the flock and age specific pattern.

### Statistical significant lower amount of culturable microorganisms in *C. jejuni* positive samples

By cultural methods enumeration of total aerobic colony count, lactic acid bacteria, *Staphylococci*, *Enterococci*, *Enterobactericeae* and *E. coli* was performed in all fecal samples. Statistical analysis revealed that in *C. jejuni* positive samples significantly lower cfu/g fecal content were detectable than in *C. jejuni* negative samples. Figure 2 shows the difference in a logarithmical order of magnitude.

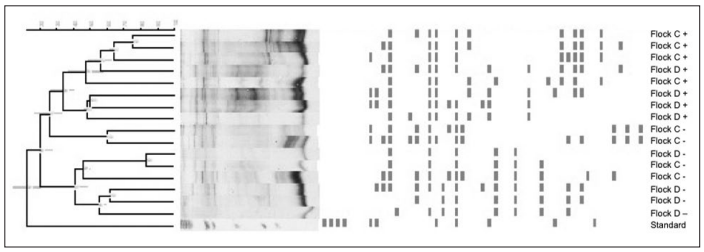


**FIGURE 1:** *PCR-SSCP microbial community cluster analysis. Campylobacter jejuni positive and negative samples of two flocks were analysed. In these samples the Campylobacter-cluster is stronger than the flock-cluster.*

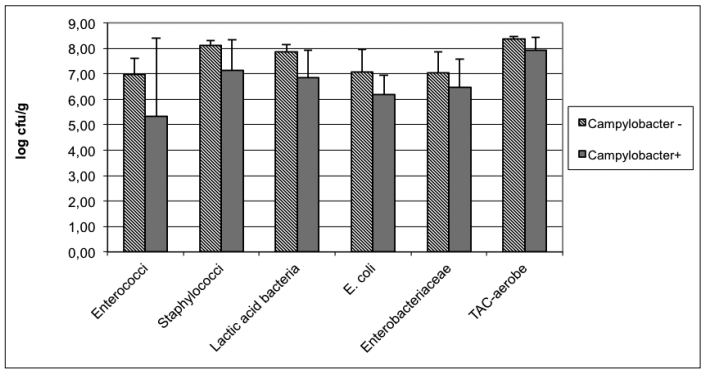


**FIGURE 2:** *Amounts of culturable microorganisms. Campylobacter jejuni positive samples are significantly lower in colony counts of Enterococci, Staphylococci, lactic acid bacteria, E. coli, Enterobactericeae and total aerobic colony count (TAC aerobe) than C. jejuni negative ones.*

### Microbiome analysis using high throughput sequencing during *C. jejuni* flock colonization

The microbial diversity in the ceca of five negative and five *Campylobacter* positive (four *C. jejuni* and one *C. coli* positive sample) was assessed. Figure 3A shows the overall proportion of the most important phyla. According to the sequence data the cecal microbiome consists of most common phyla of gut bacteria (*Firmicutes*, *Proteobacteria*, *Bacterioidetes*). The amount of these pyla in different samples analysed vary substantially: *Firmicutes* 22–81%, *Proteobacteria* 5–64% and *Bacterioidetes* 1–30% By looking at *Campylobacter* negative compared to positive samples (Fig. 3B) no statistically significant difference can be detected. On the phyla level a tendency was seen for higher amounts of *Firmicutes* in *Campylobacter* negative samples (average 62%) to *Campylobacter* positive ones (average 36.6%). Whereas, for Proteobacteria (average of 44.6% and 21.3% respectively) and Bacterioidetes (average of 15.4% and 6.5% respectively) a higher proportion in *Campylobacter* positive samples are found. A tendency, regarding higher phyla diversity, can be featured on the genus level with an average of 55 detected genera in *Campylobacter* negative samples and 47 detectable genera in positive ones. By using a 3D PCoA Plot analysis (Fig. 4) *Campylobacter* status can clearly be assessed. Interestingly the sample positive for *C. coli* clusters with

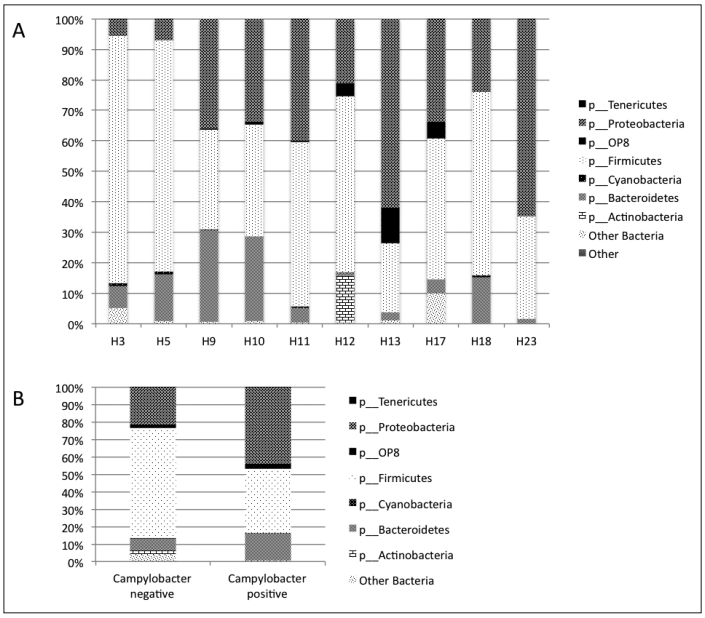


**FIGURE 3:** *Microbial diversity. A: All ten samples were analysed using high throughput sequencing. Microbial diversity is shown in graphical codes. B: Samples divided into Campylobacter positive (including the C. coli positive sample) and negative samples.*

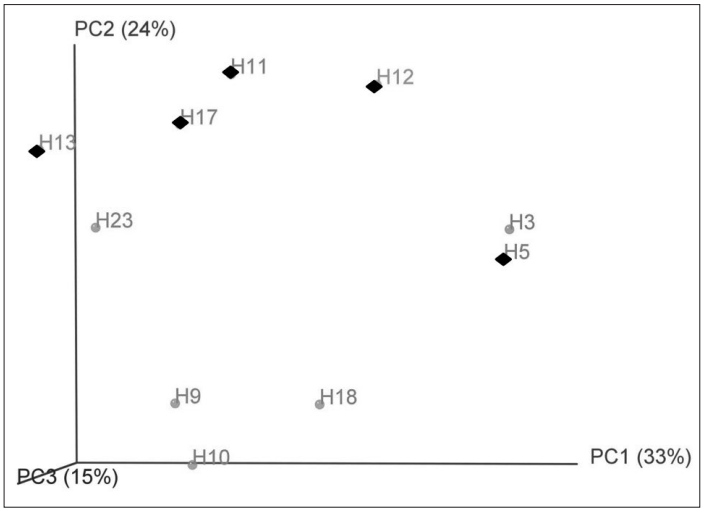


**FIGURE 4:** *3D PCoA Plot with Bray-Curtis distance. Campylobacter status. Samples with a black filled diamond are Campylobacter negative, dots are Campylobacter positive samples. Sample H23 represents the C. coli positive sample. Samples H3 and H5 harbor Helicobacter spp.*

negative *Campylobacter* samples rather than with the *C. jejuni* positive ceca microbiome. Two samples harbouring *Helicobacter* species (one of which identified as *C. jejuni* positive one as negative) cluster tightly together.

## Discussion

*Campylobacter* colonization in chickens has reached a high level in most European countries despite various efforts to reduce flock colonization. Chicken flocks are often colonized in the last weeks before slaughter and *Campylobacter* counts in the cecal and fecal content are particularly high. This leads to high contamination rates of chicken carcasses and chicken meat being processed through the automated slaughter process (Ellerbroek et al., 2010; Hue et al, 2010). Risk assessment analyses have shown that reduction in *Campylobacter* intestinal colony counts as well as a reduction in colonized chickens may serve well in a reduction of human Campylobacteriosis (Boysen et al., 2013). Recent works show that *Campylobacter* closely interacts with other microorganisms (Dicksved et al., 2014; Haag et al., 2012, Hilbert et al., 2010; Kaakoush et al., 2014). The intestinal host microbiota is important for *Campylobacter* colonization or colonization resistance in mice, humans and chickens equally well (Beereswill et al., 2011; Dicksved et al., 2014; Kaakoush et al., 2014). *Campylobacter* colonization leads to a change in the intestinal microbiota (Haag et al., 2012). Thus, strategies to enhance colonization resistance or reduce colonization in chickens may include shaping the chicken microbiota. So far most experiments using pro- or prebiotics to influence the chicken gut microbiota have not been effective (Neal-McKinney et al., 2012; Santini et al., 2010). Consequently data on the chicken microbiota are deemed necessary to provide new and effective approaches to fight *Campylobacter* colonization. By using different experimental approaches we studied the fecal and cecal microbiota of chickens in regards to the *Campylobacter* status. With all three methods used (conventional enumeration of colony counts, PCR-SSCP and high throughput sequencing) the fecal or cecal microbiota in *Campylobacter* colonized and *Campylobacter* free chickens differed. Cultural colony counts of lactic acid bacteria, *Staphylococci*, *Enterococci*, *Enterobactericeae*, *E. coli*, total aerobic colony counts were found to be significantly higher in *Campylobacter* negative samples. Recent studies showed that even though no visible signs of clinical disease are seen in chickens when colonized with *Campylobacter*, colonization can lead to epithelial lesions and an inflammatory immune response with effects on the gut mucosa and will finally result in diarrhea (Humphrey et al., 2014; Awad et al., 2014). Our results on colony counts could be a sign of diarrhea. We report here colony counts in cfu/g and thus, a higher water content in *Campylobacter* positive samples could be responsible for the significant reduction of cultural colony counts per weight. But despite the microbial quantity, the composition of the microbiota of chickens analysed by PCR-SSCP differs between *Campylobacter* positive and negative samples as seen in a cluster analysis. This has recently been shown by other authors but not in as many different flocks and not in two different age groups (Kaakoush et al., 2014). Most interesting is that these findings are consistent with new data on high throughput sequencing of this study and others (Kaakoush et al., 2014). Kaakoush and co-workers (2014) defined four groups based on enterotypes: enterotype 1 dominated by *Firmicutes*, enterotype 2 by *Firmicutes* and *Proteobacteria*, enterotype 3 *Firmicutes* and *Actinobacter* and enterotype 4 with *Firmicutes* and *Bacteroidetes*. Their results show that particularly in enterotype 3 *Campylobacter* were not found as often as in the other enterotypes. In our study we were able to find samples that were dominated by *Firmicutes* and samples could be identified as enterotype 2 and 4 but enterotype 3 was only found in one of our samples (15.4% *Acinetobacter*). This sample was culture negative

and according to sequencing data harboured no *Campylobacter* spp. From all genera associated with *C. jejuni* by Kaakoush and coworkers (2014) in our samples only the *Ruminococcaceae* were found to be linked to *Campylobacter* colonized samples. This might be due to the relatively low numbers of samples we used in our study. Haag and coworkers (2012) saw a shift due to *C. jejuni* induced Colitis in mice, regarding the microbiota composition. *E. coli, Enterobacteriaceae, Bacteroidales, Bifidobacteria, Lactobacillus* and *Clostridium* changed in quantity. In our study we found as well a change in quantity of *Firmicutes*, *Proteobacteria* and *Bacteroides* at the phyla level but we were not able to break this difference down to genus level for these bacteria. Our sample harbouring *C. coli* clustered with *Campylobacter* free samples, so we suggest that the effect of *C. coli* on the microbial composition may be much lower than the one seen for *C. jejuni*. Additionally, effects of other microorganisms like *Helicobacter* spp. as seen in our study, may have even more impact on the microbiota composition in chickens. Taken together it can be concluded that *Campylobacter* and closely related microorganisms are interacting with the intestinal microflora in their respective hosts.

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