COLONIZATION PROPERTIES OF *CAMPYLOBACTER JEJUNI* IN CHICKENS

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Campylobacter is the most common bacterial food-borne pathogen worldwide. Poultry and specifically chicken and raw chicken meat is the main source for human *Campylobacter* infection. Whilst being colonized by *Campylobacter* spp. chicken in contrast to human, do scarcely develop pathological lesions. The immune mechanisms controlling *Campylobacter* colonization and infection in chickens are still not clear. Previous studies and our investigations indicate that the ability to colonize the chicken varies significantly not only between *Campylobacter* strains but also depending on the original source of the infecting isolate.

The data provides circumstantial evidence that early immune mechanisms in the gut may play an important role in the fate of *Campylobacter* in the host.

Introduction

Campylobacter spp. are curved or spiral shaped flagellated bacteria with a size of 0.2–0.5 µm length and a width of 0.2–0.9 µm. They are gram-negative, and so far 25 species and 8 sub-species have been described [1, 2]. Among these species, some show strict host specificity such as *Campylobacter* (*C.*) *rectus* for man or *C. mucosalis* for pig. Within the family Campylobacteriaceae, some species such as *C. jejuni* subsp. *jejuni or C. coli* are linked to more than one host and have zoonotic potential in avian species. *C. jejuni* is most commonly detected specifically in poultry, but *C. coli* and *C. lari* also occur regularly in birds. Particularly, *C. coli* shows a prevalence of almost 50% in turkeys [1, 3].

Thermophilic (37–42 °C) and non-thermophilic *Campylobacter* species (< 37 °C) can be differentiated. Different from other intestinal bacteria such as Salmonella, *Campylobacter* grows only under microaerophilic conditions in an atmosphere with 10% CO_2, 5% O_2 and 85% hydrogen [4, 5].

C. jejuni is the most frequent cause for human enteritis worldwide. It appears more frequently than other bacterial pathogens such as pathogenic *Escherichia coli* or *Salmonella* spp. [6, 7]. In Europe, *C. jejuni* is the most common food-borne pathogen with an incidence of 19.4 cases/100 people/year in England for example [6]. *Campylobacter* infection causes in humans acute gastrointestinal illness and is considered to be a predisposing factor for the Guillain–Barré syndrome [8, 9]. The disease is characterized by a watery or bloody diarrhoea, fever and abdominal cramps. In most cases it is self-limiting but may also sustain for several weeks [7].

Poultry and poultry meat is considered to be one of the major sources for human campylobacteriosis [1, 10, 11]. Beside poultry and raw poultry meat other sources for *C. jejuni* have been described such as livestock, including sheep and pigs, but also cats and dogs, water, humans and vehicles, raw milk, rodents and insects are known as possible vectors [12–16]. These different sources are not only involved in the horizontal transmission of *Campylobacter* to humans but also to poultry flocks.

Due to the fact that *C. jejuni* is found ubiquitous it is difficult to control spreading and the introduction of *C. jejuni* into poultry flocks. *Campylobacter* spp. colonized flocks are common and can be found in many countries worldwide [17]. However, Scandinavian countries show a lower prevalence than other European countries [18]. This difference may be due to improved hygiene barriers [19]. Although found ubiquitous, *Campylobacter* shows a low tenacity being highly sensitive to oxygen, desiccation, low pH and high temperature [20].

Campylobacter in poultry

Birds, and especially chickens are considered as the natural host of *C. jejuni* [21]. However, *C. jejuni* is regarded as a commensal of the intestinal flora of chickens, which leads to a predominantly asymptomatic colonisation of the gut, particularly the caecum [22–25]. Beside the

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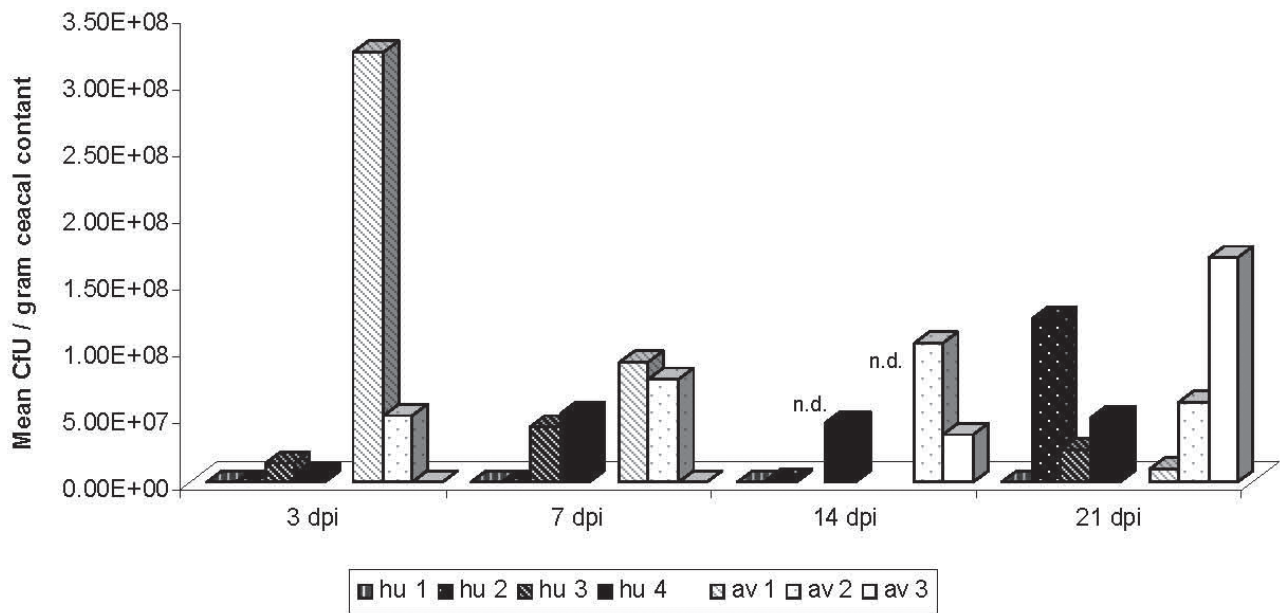
Fig. 1. Mean Colony forming Units (CfU) per gram caecal content in chickens after different days of inoculation with human (hu 1–hu 4) and avian (av 1–av 3) *C. jejnui* isolates. $n =$ six animals/day and group in each of the four experiments. For determination, caecal content was serially diluted and plated on selective agar (CCDA) for 48 h at 38 °C under microaerophilic conditions [63]. dpi = days post inoculation. n.d. = not done

lower intestine being the main reservoir, *Campylobacter* may be detected in several internal organs such as liver and spleen [26]. Both meat and laying type chickens are colonized. *C. jejuni* can be also found in other poultry species such as turkeys, Muscovy and Pekin ducks. Beside poultry, a vast variety of wild birds, such as gulls, corvids, waterfowl and passerines are also susceptible for *Campylobacter* and may act as vectors for transmission especially to poultry flocks [27–29]. The uptake of the pathogen by poultry species from the environment usually occurs between the age of two to four weeks [30, 31]. Maternal antibodies seem to have an influence on the onset of colonisation. But also birds younger then three weeks can be colonized successfully in the case of high environmental exposure [32].

Horizontal transmission within the flock occurs predominantly via shedding birds and contaminated litter. Feed and water may be sources of recurring infections. There is no vertical transmission [33]. The *C. jejuni*-prevalence within a flock is almost 100% after introduction [31, 34]. Furthermore, multi-strain colonisation is possible within one flock. A recent study concerning a field trial with nineteen poultry flocks of four avian species revealed a large genetic diversity of *Campylobacter* within individual flocks and among different flocks using amplified fragment length polymorphism (AFLP) analysis [34]. Petersen et al. [35] also showed coexistence of different *Camplyobacter* clones in broiler flocks. These results raise the question of the colonisation potential of different *Campylobacter* strains of different origin not only for human but also for poultry.

In a recent study we tested *C. jejuni* isolates of human and avian origin for their ability to colonize chicken, rep-

licate in different organs and induce lesions. So far, we compared a total of seven *C. jejuni* isolates, including four *Campylobacter* of human and three of avian origin.

Three weeks old specific-pathogen free pullets confirmed to be negative of *Campylobacter* at the day of inoculation, were inoculated orally with 10^4 colony-forming units of *C. jejuni*. Afterwards, the birds were monitored daily for clinical signs, and on selected days for weight gain and the number of colony forming units per gram caecal content and liver.

There was a clear difference in the ability of the strains to colonize the chicken gut and liver [34]. While one human isolate was not detectable by the applied cultivation method at any of the investigated time points up to two weeks post inoculation, all other strains colonized the chicken gut *(Fig. 1)*. A possible reason for different *Campylobacter* colonisation patterns may be genetic diversity of *C. jejuni* isolates [36, 37]. Only one strain of avian origin showed the ability to also invade the liver (data not shown). This observation supports previous studies demonstrating low detection rates of *Campylobacter* in liver samples, too [26, 34, 38]. Furthermore, based on the comparison of the colonisation efficiency of *C. jejuni* and of *C. coli* Korolik et al. [37] suggests that *Campylobacter* strains of human origin may lack or lost the ability to colonize the chicken intestine.

In our experiment, all inoculated birds independent of the inoculated *Campylobacter* strain were free of clinical signs, pathological or histopathological lesions such as crypt abscesses, epithelial cell ballooning, basal subnuclear vacuolation or villous tip disruption [24, 39, 40]. Their weight gain was comparable to the non-inoculated birds.

Host factors controlling *C. jejuni* in poultry

Differences in colonisation ability of *Campylobacter* may also be due to host factors. Possibly, the genetic background of the host may significantly affect *Campylobacter* colonisation and infection as it was shown for a variety of other avian pathogens [41–43].

Furthermore, an important limiting factor for bacterial diseases is the host immune response. For *Salmonella* it was demonstrated that infection induces an increase of T cell receptor (TCR) γδ, TCR αβ, CD4+ and CD8α+ T cells in chicken caecum. This increase coincides with cytokine upregulation such as IL-12, IL-18 and IL-2 indicating the stimulation of a T cell response [44].

Little is known about immune reactions involved in the control of *C. jejuni* colonisation and infection in avian species. To investigate the local immune responses of chickens after colonisation with different *C. jejuni* isolates of human and avian origin, we analyzed intraepithelial lymphocytes (IELs) of the caecum and the jejunum for CD4+ and CD8α+ T cells by flow cytometric analysis [45–48].

Interestingly, *Campylobacter* colonisation of the chicken gut hardly induced any changes in the number of CD4+ and CD8α+ T cells in the group of IELs.

Only one human strain induced a reduction in the relative number of CD4+ T cells compared to the non-inoculated control group at 3 days post inoculation (dpi) in the jejunal IELs population *(Fig. 2)*. This finding suggests that T cells may control *Campylobacter* colonisation in the early phase after inoculation because birds were negative of this *C. jejuni* strain at 3dpi and at later time points during the experiment. Further and earlier time points and further *Campylobacter* isolates

of human origin have to be investigated to confirm this observation.

The investigation of pro-inflammatory cytokines revealed a role they play in *Campylobacter* infection in mammalian species and may allow to further understand the immune system in *Campylobacter* infected chickens, especially the innate mechanisms [49, 50]. We compared the expression pattern of IL-6 and interferon (IFN)-γ after infection of chickens with seven *C. jejuni* isolates of human and avian origin.

Being both pro-inflammatoric and anti-inflammatoric, IL-6 plays an important role in steering the switch from innate to acquired immunity [51]. It is secreted from a vast variety of cells including dendritic cells, monocytes, T cells, B cells and shows a wide range of biological activities [52, 53]. Friis et al. [54] could show an increase in levels of expression and secretion of IL-6 in human intestinal epithelial cells due to infection with several *C. jejuni* isolates. In a previous study Shaughnessy et al. [55] detected an increase of IL-6 in the intestine at 48 h post infection of four weeks old chickens, which indicates that innate immune reactions may possibly be important in the control of *Campylobacter* infection also in chicken. IFN-γ being the only type II class interferon secreted by a variety of cells such as T helper, cytotoxic T cells and Natural killer cells, acts as a regulatory and effector molecule for inflammatory responses [56].

Edwards et al. [57] showed with his studies that IFN-γ plays a critical role during the early acute phase of infection in human, detecting an increase of IFN-γ in human intestinal biopsies of both ileal and colonic tissue as well as in human dendritic cells after *Campylobacter* infection.

The cytokine expression pattern of IL-6 and IFN-γ in caecum and jejunum as well as in the spleen did not sig-
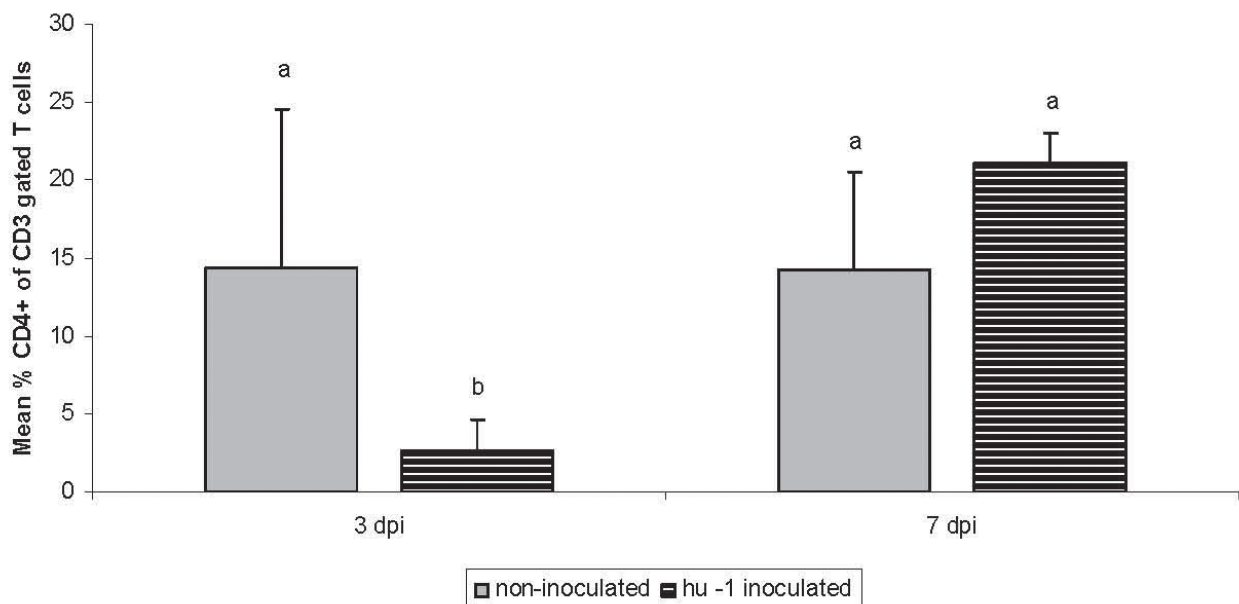
Fig. 2. Comparison of the relative percent of CD4+ T cells of intraepithelial lymphocytes (IELs) of the jejunum of a non-inoculated and a group, inoculated with a human (hu) *C. jejuni* strain at 3 and 7 days post inoculation. n = six animals/day and group representative for the four conducted experiments. IELs were isolated and processed for flow cytometric analysis using monoclonal antibodies for CD3+ and CD4+ T cells (Southern Biotechn., USA). Presented are the %CD4+ T cells within the CD3+ IEL. Different letters indicate significant differences between groups at indicated time-points ($p \leq 0.05$ Kruskal–Wallis test).

nificantly differ between *C. jejuni* inoculated and non-inoculated birds between 3 dpi to 14 dpi independent of the *Campylobacter* strain. In contrast Nyati et al. [58] could show an increase of both IFN-γ at 10 and 15 days and IL-6 at five and 10 days post inoculation with *C. jejuni* in the sciatic nerve in chickens.

Consequentially, time points earlier than 3 dpi should be investigated more carefully to elucidate the mechanisms controlling *Campylobacter* infection in chicken.

Conclusion

Our investigations clearly show that human and avian isolates may differ in their ability to colonize chickens, which is supported by previous investigations of other groups [36, 37, 59, 60].

Possible reasons may be genetic diversity between strains which may also affect the innate and eventually also the acquired immune response in the very early phase of colonisation.

Our data, as well as Shaughnessy et al. [55] provide circumstantial evidence that the immune response in the first three days may significantly affect the outcome of *Campylobacter* colonisation and infection.

Overall it may be suggested that *C. jejuni* is non pathogenic for healthy chickens.

Other predisposing factors may contribute to the systemic spread of *Campylobacter* in birds and the induction of lesions as observed under field conditions and described in the literature in the past [61, 62].

Further studies are needed to further understand important host factors responsible for the control of *Campylobacter* in chickens. This may allow the implementation of better control strategies of this important and zoonotic pathogen in poultry.

Acknowledgements

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