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Experimental infection of weaned piglets with *Campylobacter coli* – Excretion and translocation in a pig colonisation trial

Katharina Bratz [a,*], Roland Bücker [b], Greta Gölz [a], Silke S. Zakrzewski [c], Pawel Janczyk [d], Karsten Nöckler [d], Thomas Alter [a]

[a] Institute of Food Hygiene, Freie Universität Berlin, Germany
[b] Department of Gastroenterology, Infectious Diseases and Rheumatology, Division Nutritional Medicine, Charité-Universitätsmedizin Berlin, Germany
[c] Institute of Clinical Physiology, Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin, Germany
[d] Federal Institute for Risk Assessment, Berlin, Germany

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ABSTRACT

Campylobacter (*C.*) is one of the most common food-borne pathogen causing bacterial enteric infections in humans. Consumption of meat and meat products that have been contaminated with *Campylobacter* are the major source of infection. Pigs are a natural reservoir of *Campylobacter* spp. with *C. coli* as the dominant species. Even though some studies focussed on transmission of *C. coli* in pig herds and the excretion in faeces, little is known about the colonisation and excretion dynamics of *C. coli* in a complex gut microbiota present in weaned piglets and the translocation to different tissues.

Therefore, an experimental trial was conducted to evaluate the colonisation and translocation ability of the porcine strain *C. coli* 5981 in weaned pigs. Thus, ten 35 days old piglets were intragastrically inoculated with strain *C. coli* 5981 (7×10^7 CFU/animal) encoding resistances against erythromycin and neomycin. Faecal samples were taken and *C. coli* levels were enumerated over 28 days. All piglets were naturally colonised with *C. coli* before experimental inoculation, and excretion levels ranged from 10^4 to 10^7 CFU/g faeces. However, no strain showed resistances against the additional antimicrobials used. Excretion of *C. coli* 5981 was seen for all piglets seven days after inoculation and highest counts were detectable ten days after inoculation with 10^6 CFU/g faeces.

Post-mortem, translocation and subsequent invasion of luminal *C. coli* was observed for gut tissues of the small intestine and for the gut associated lymphatic tissues, such as jejunal mesenteric lymph nodes and tonsils as well as for spleen and gall bladder.

In conclusion, this pig colonisation trial offers the opportunity to study *C. coli* colonisation in weaned piglets using the porcine strain *C. coli* 5981 without the need for gnotobiotic or specific pathogen-free animals.

1. Introduction

Campylobacter (*C.*) spp. are one of the most dominant zoonotic bacterial cause of human enteritis. Infection results in clinical outcomes like diarrhoea, abdominal pain, and fever (Newell, 2001). Both species asymptomatically colonise the intestine of many farm animals but also wildlife and companion animals (Horrocks et al., 2009). Pigs are a natural reservoir of *Campylobacter* spp. (with *Campylobacter coli* predominating) with prevalence between 50% and 100%, and excretion levels ranging from 10^2 to 10^7 colony forming units (CFU)/g faeces (Alter et al., 2005; Jensen et al., 2006). Risk factors for the introduction of *C. coli* to pig herds largely remain unclear. It is assumed

* Corresponding author at: Institute of Food Hygiene, Freie Universität Berlin, Königsweg 69, 14163 Berlin, Germany. Tel.: +49 030 83 86 25 38. fax: +49 030 83 86 25 52.
E-mail address: katharina.bratz@fu-berlin.de (K. Bratz).

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that horizontal transmission via farmers, domestic animals, contaminated surface water, insects, and other environmental sources are largely responsible for introduction into herds (Guerin et al., 2007; Wassenaar, 2011). Vertical transmission from mothers to offspring may occur via the faecal–oral route (Alter et al., 2005). Although *C. coli* are responsible for only 10% of human *Campylobacter* infections the impact on human health is still substantial (Wilson et al., 2008). *C. coli* can be transmitted from pigs to humans through the consumption of contaminated pork. The consumption of raw minced meat was identified as specific risk factor for *C. coli* infection (Gillespie et al., 2002).

The colonisation and excretion dynamics as well as the translocation ability into deep tissues of *C. coli* in pigs have not been thoroughly evaluated yet. A reason for this could be the high prevalence of *C. coli* in pigs. Subsequently high efforts would have to be made using gnotobiotic or specific pathogen-free (SPF) animals. However, such data are a prerequisite for assessing the risk of human *C. coli* infections derived from pork.

The objectives of this study were to investigate the (i) colonisation dynamics and (ii) excretion pattern and (iii) to describe *C. coli* translocation into different tissues by using a *C. coli* colonisation trial in weaned piglets.

2. Material and methods

2.1. Bacterial strains and inoculum preparation

The *C. coli* 5981 strain used in the trial was originally isolated from pig faeces in 2007. *C. coli* 5981 belongs to the ST-828 clonal complex and is a typical representative of porcine *C. coli* based on MLST analysis (Sheppard et al., 2010). In preliminary in vitro experiments we found that its pathogenic potential is comparable to *C. coli* ATCC 33559, as in infected human intestinal HT-29/B6 cells the epithelial integrity was disturbed by the induction of apoptosis. The strain *C. coli* 5981 was chosen due to its two additional antimicrobial resistances against erythromycin and neomycin. Preliminary tests on antimicrobial resistance patterns based on 91 porcine *C. coli* strains revealed a very low distribution (3.3%) for this particular combination. Hence, this strain is largely distinguishable from the natural *C. coli* population present in the gastrointestinal (GI) tract by its specific antimicrobial resistance pattern.

C. coli 5981 was recovered from stocks kept at −80 °C by plating cryobeads (Cryobank System, Mast Diagnostica, Reinfeld, Germany) on Mueller-Hinton agar with 5% sheep blood (MHB; OXOID, Wesel, Germany) for 48 h at 37 °C under microaerobic conditions (6% O_2, 7% CO_2, 80% N_2, 7% H_2) generated by the Mart Anoxomat^{TM} system (Drachten, Netherlands). Liquid cultures were obtained by inoculation of colonies in *Brucella* broth (BB) (BD, Heidelberg, Germany) and cultivation under the same conditions for 24 h.

For pig inoculation, colonies of *C. coli* 5981 were cultivated in BB and incubated for 16 h under microaerobic conditions. 0.5 ml of this overnight (o/n) culture with an optical density of approx. 0.3 at 600 nm were inoculated in 20 ml BB and cultivated for 4 h in order to obtain a solution of approx. 7×10^7 CFU/ml. Cell numbers were determined by performing standard plate counts according to ISO 10272-2.

2.2. Animals and experimental design of the trial

All animals ($n = 10$) were housed and treated in accordance with the regulation of the local authority (LandesamtfürGesundheit und Soziales, Berlin; approval no. G0349/09). German Landrace piglets were received from the Institute of Animal Nutrition (Freie Universität, Berlin, Germany). Piglets were weaned for 28 days and transferred to the experimental facility where they were allocated to pens based on litter origin, gender, and weight. Animals were kept pairwise. After one week of adaption all animals were inoculated with a single dosage of approx.7×10^7 CFU of *C. coli* 5981 by intragastric application using a stomach feeding tube (B. Braun, Melsungen, Germany) under azaperone (1.5 mg/kg; Stresnil, Janssen-Cilag, Germany) sedation. All piglets were weighed twice a week for 28 days. 28 days post inoculation (p.i.) the piglets were sacrificed for collecting gut contents and various tissue samples. Sedation was performed by intramuscular administration of azaperone (2 mg/kg, Stresnil) with a following induction of deep anaesthesia with ketamine (25 mg/kg, Ursotamin, SerumwerkeBernburg, Bernburg, Germany), and a final overdose achieved by intravenous application of pentobarbital (200 mg/kg, Narcoren, Merial, Hallbogmoos, Germany).

2.3. Sampling of faeces and post-mortem sample preparation

Faecal samples were collected in intervals over the whole experimental period in order to monitor *C. coli* excretion and the faecal consistency. Faecal consistency was assessed using a subjective score on a five-point scale ranging from 1 to 5 (1: watery diarrhoea to 5: hard dry stool), representing one major parameter of the health status in weaned piglets. Faecal samples from all piglets were collected directly from the rectum daily for the first week and subsequently twice a week until the end of the trial. Prior experimental inoculation with *C. coli* 5981, faecal samples from mother sows (14 and 28 days postpartum) and piglets were examined for *Campylobacter* presence and tested for absence of strains exhibiting antimicrobial resistances against both erythromycin and neomycin. The use of antimicrobials as supplements to the selective media enabled the differentiation of the inoculation strain from naturally colonised *C. coli* population present in all piglets. To study the colonisation ability of *C. coli* in different gut sections along the intestine and their translocation towards selected organs, tissue of the small intestine and several organs were dissected at 28 days p.i.

After euthanasia, the abdominal cavity was opened along the *lineaalba*. Jejunal mesenteric lymph nodes (MLNs), spleen, and gall bladder were dissected prior to the collection of gut tissues of jejunum, ileum, and caecum as well as gut contents of stomach, ileum, caecum and colon. Additionally, tonsils were dissected after ventral cut through the neck tissue. Sterile instruments for sample