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SEROPREVALENCE OF *CAMPYLOBACTER*-SPECIFIC ANTIBODIES IN TWO GERMAN DUCK FARMS – A PROSPECTIVE FOLLOW-UP STUDY

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Several studies have shown that about 60–100% of farmed ducks are colonized by *Campylobacter* species. Because of this, a higher risk of campylobacteriosis among duck farm workers can be assumed.

To estimate the risk of *Campylobacter* infections in duck farm workers, we investigated the prevalence of *Campylobacter* spp. in ducks of two duck farms and the seroprevalence of anti-*Campylobacter* antibodies (IgA and IgG) in two cohorts of workers. The first cohort consisted of high-exposed stable workers and slaughterers, which was compared to a second cohort of non-/low-exposed persons. Duck caecal swabs and serum samples were collected in 2004, 2007, and 2010.

The colonization rate in the examined ducks was found to be 80–90%. The seroprevalence of anti-*Campylobacter* IgA and IgG antibodies among the non-exposed cohort was found to be 0.00% in all 3 years. In contrast, the exposed cohort demonstrated an IgA seroprevalence of 4.17% in 2004, 5.71% in 2007, and 0.00% in 2010 and an IgG seroprevalence of 8.33% in 2004, 0.00% in 2007, and 4.29% in 2010.

In conclusion, in 2004, we observed a significantly higher anti-*Campylobacter* antibody seroprevalence in the exposed cohort followed by a steady reduction in 2007 and 2010 under occupational health and safety measures.

Keywords: campylobacteriosis, *Campylobacter,* seroprevalence, duck farm workers, duck slaughterhouse workers, occupational health and safety protective measures

Abbreviations: AP, alkaline phosphatase; BAuA, Federal Institute for Occupational Safety and Health (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin); HRP, horseradish peroxidase; LB, lysogeny broth; NBT/BCIP, nitroblue-tetrazolium-chloride/5-brome-4-chlor-3-indolylphosphate; NCTC, National Collection of Type Cultures; OHS, occupational health and safety; NP40, Nonidet® P 40 substitute/4-nonylphenyl-polyethylene glycol; PBS, phosphate buffered saline; PVDF, polyvinylidene difluoride; TRBA, Technical Rule for Biological Agents

Introduction

Campylobacteriosis continues to be the most prevalent bacterial enteral infection worldwide [1]. The majority of campylobacteriosis cases are caused by *Campylobacter jejuni* while a smaller but significant portion is caused by other *Campylobacter* spp. Watery diarrhea and general weakness are the main symptoms of the disease. The disease is self-limiting, but in severe cases or in immunocompromised patients, antibiotic treatment with erythromycin or quinolones is recommended. In some cases, post-infectious sequelae, namely, Guillain Barré Syndrome, reactive arthritis, and inflammatory bowel disease, can arise [2]. The underlying mechanisms that govern the establishment of post-infectious sequelae from acute campylobacteriosis have not been fully understood. Although investigations into the global health burden are inconclusive, it has been estimated that the case-fatality rates range from <0.01% to 8.8% with post-infectious sequelae making the highest contribution [3].

Campylobacter spp. are mainly transmitted to humans through close association with livestock or feeding on

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contaminated livestock meat from chicken, turkey, swine, cattle, sheep, and ducks [4]. Birds are the natural reservoir of *Campylobacter* spp. The bacterium colonizes the gut, small intestines, crop, and gizzard of these animals. In addition, contaminated environment with animal feces has been shown to play a role in transmission, but studies on human to human transmission have generated ambiguous findings [5].

Poultry, in particular chicken, is the major source of *Campylobacter* spp. to humans [6]; hence, studies on campylobacteriosis have mainly focused on chicken. However, due to increase in consumption of turkey, ducks, and geese, their contribution to the ever high prevalence of campylobacteriosis is worth investigating [7].

In the recent past, *Campylobacter* outbreaks associated with domesticated ducks have been reported raising a speculation that ducks could be another major *Campylobacter* spp. reservoir [8, 9]. Similarly, studies on the prevalence of *Campylobacter* spp. in domesticated ducks have been generating interesting results. Wei and coworkers recorded a *Campylobacter* spp. prevalence of 96.6% (*C. jejuni:* 82.1%, *Campylobacter coli:* 16.1%) [10], Weber and coworkers recorded a *Campylobacter* spp. colonization rate of 59.6% in Pekin duck flocks (*C. jejuni:* 59.3%; *C. coli:* 40.7%) and of 68.2% in Muscovy duck flocks (*C. jejuni:* 83.5%; *C. coli:* 16.5%) [11], Adzitey and coworkers recorded a *Campylobacter* spp. colonization rate of 85.0% (*C. jejuni:* 86.0%, *C. coli:* 7.0%, *Campylobacter lari:* 7.0%) [12], and Colles and coworkers found a *Campylobacter* spp. prevalence of 93.3% to 100% (*C. jejuni:* ≈74.6%; *C. coli:* ≈25.4%) in farmed mallard ducks of different ages [13]. Therefore, it is likely that duck farm workers are at a greater risk of contracting campylobacteriosis due to their close contact with ducks and their droppings. According to Kasrazadeh et al., all ducklings are colonized by *Campylobacter* spp. after the 11th day of age [14].

Detection of antibodies in human sera is one of the best established methods for diagnosing infections. During a *Campylobacter* infection, the human immune system responds by releasing IgA, IgG, and IgM antibodies against *Campylobacter* antigens. The decline period of IgG and IgA after infection to baseline levels has been found to be 4.5 months and 2.5 months, respectively, making them suitable markers for serological investigation of campylobacteriosis [15]. We recently developed a highly sensitive and specific *Campylobacter* serological assay, which utilizes *C. jejuni* protein P39 (CJ0017c) as antigen to detect *Campylobacter*-specific IgA and IgG antibodies [16, 17].

In this study, we have used this assay to investigate the seroprevalence of anti-*Campylobacter* antibodies in duck handling employees in two duck farms and two duck slaughterhouses in Germany.

Materials and methods

Sera, study design, and ethical approval

Sera were collected from workers of two duck farms in Germany and workers of two slaughterhouses in Germany. As control group, we included office workers without contact to ducks, personnel working in the management offices, cereal suppliers, craftsmen, drivers, and technicians in the companies who are not or only scarcely in contact with ducks or duck meat. For simplification, we formed two cohorts of subjects. The first cohort consists of individuals with very high and high exposure to ducks and duck meat subsumed under "exposure". The second cohort includes subjects with no or low/rare contact with ducks or duck meat subsumed under "no exposure".

Table 1. Overview of subjects/sera used in this study

Group of subjects	Description	Category	No. of workers/sera		
			2010	2007	2004
Management	No exposure	No exposure	13	6	10
Management (local)	Low/no exposure*	No exposure	11	3	6
General services†	Low/mean exposure	No exposure	12	8	8
Cereal service staff	Low/no exposure	No exposure	0	1	0
Stable workers	High exposure	Exposure	45	19	31
Hatchery workers	High exposure to ducklings	Exposure	7	11	9
Bird receipt	Very high exposure	Exposure	10	2	5
Slaughterers	High exposure	Exposure	8	3	3
Sum no exposure			36	18	24
Sum exposure			70	35	48
Sum all			106	53	72

*An occasional exposure to ducks/duck products may occur.

†The group of general services includes subjects as craftsmen, drivers, and technicians, which are not exposed to ducks/duck products during ca. 90% of their work time. Only occasionally (ca. 10% of their work time), these people are exposed to ducks/duck components.

Occupational health and safety (OHS) protective measures were realized according to the technical rule TRBA 230 [18], which includes technical recommendations of the German Ordinance on Biological Working Agents (BioStoffV; 90/679/EWG). Briefly, the employer shall reduce the exposure of employees to a minimum by suitable physical, technical, and organizational measures at first. If these measures are not sufficient, additional personal protective measures (respirator masks or powered air purifying respirators) should be provided to reduce risk for employees. This study was designed as prospective follow-up study including subjects/sera drawn in 2004, 2007, and 2010 and conducted by the Federal Institute for Occupational Safety and Health. All participants gave their written consent. The study was approved by the ethics commission of the medical association of Berlin (Eth-013/07). Blood samples were drawn during a period from September 28th to November 14th of each year. During the 3 years, a sum of 229 sera of 94 male and 67 female participants were collected and analyzed; 72 drawn in 2004, 53 drawn in 2007, and 106 drawn in 2010. The mean age of all participants was 39 (±11) and the median age 40 in 2004. Details are shown in *Table 1*.

Isolation, culture, and identification of Campylobacter *spp.*

In order to get an impression of the *Campylobacter* spp. prevalence in the duck flocks during each year, caeca of 30 adult ducks from each duck farm were swabbed and examined for the presence of *Campylobacter* spp. The swabbing was done concurrently with drawing of serum samples from participants during the period of September 28th to November 14th of each year.

Isolation of *Campylobacter* spp. was carried out using enrichment culture and direct agar plate culture. Briefly, duck caeca were swabbed, and the swabs were streaked directly onto modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid, Wesel, Germany). To increase *Campylobacter* spp. recovery chances, samples were simultaneously inoculated in 10 ml Bolton broth (Oxoid, Wesel, Germany) supplemented with Bolton broth selective supplement (Oxoid, Wesel, Germany) and laked horse blood (Oxoid, Wesel, Germany). The broth cultures were incubated at 42 °C for 48 h under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2). After 48 h incubation, one standardized inoculation loop (10 µl) was streaked onto mCCDA agar. Both directly inoculated agar plates and agar plates inoculated with enrichment material were likewise incubated at 42 °C for 48 h under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2). Presumptive *Campylobacter* spp. colonies were subcultured on Columbia agar supplemented with sheep blood (Oxoid, Wesel, Germany) under conditions stated above. In order to identify the genus *Campylobacter,* Gram-stain, catalase, oxidase reaction, and Oxoid DrySpot *Campylobacter* latex agglutination test (Oxoid, Wesel, Germany) were performed on each morphologically different colony that was identified on Columbia agar culture plates. DrySpot *Campylobacter* test kit responds positively to *C. jejuni, C. coli, C. lari,* and *Campylobacter upsaliensis*. Hence, there was no need for detecting the specific *Campylobacter* spp., which was recovered; there was no further species identification.

Recombinant expression and purification of protein P39

Gene *cj0017c,* which encodes protein P39, was amplified by PCR from *C. jejuni* NCTC 11168 genomic DNA using primers: FB7 5′-GG-GATCC-GCCTGTAAGATTT AGTTTAAA-3′ and FB8 5′-CG-GGATCC-GTTAGTT-TAAAGTATAAAGCTTG-3′ [16]. The PCR conditions were initial melting temperature of 94 °C for 1 min, followed by 35 cycles of 94 °C for 120 s, 50 °C for 60 s, 72 °C for 60 s, and a final elongation step at 72 °C for 5 min. The PCR products were confirmed by gel electrophoresis and ligated into the *Bam*HI site of the dephosphorylated pASK-IBA 16 expression vector (IBA BioTAGnology, Göttingen, Germany). The ligated vector was transformed into competent *Escherichia coli* DH5α, grown on LB agar supplement with 100 mg/ml ampicillin and incubated at 37 °C overnight. Colonies were randomly selected and transferred into a vessel that was containing 50 ml LB broth supplemented with 100 mg/ml ampicillin and incubated while shaking at 37 °C till OD_{550} was 1.0. At this stage, a) a control sample of 1 ml was harvested and stored and b) expression of protein P39 was induced by addition of 5 µl of 200 ng/ml anhydrotetracycline (IBA BioTAGnology, Göttingen, Germany) into the culture. The culture was incubated at 37 °C for 4 h while shaking at 200 rpm. Cells were harvested by centrifugation at 10,000 *g* for 15 min at 4 °C. The cell pellet was lysed at 37 °C for 1 h by suspension in lysis buffer containing 100 µl EDTA-free proteinase inhibitor cocktail set III (Calbiochem-Merck Chemicals Ltd., Nottingham, UK), 10 Units Benzonase (Novagen-Merck Chemicals Ltd., Nottingham, UK), and lysozyme (Sigma-Aldrich, Taufkirchen, Germany). The lysate was centrifuged for 15 min at 4600 *g*, and the supernatant containing expressed proteins was harvested. Inclusion bodies present in the harvested protein supernatant were dissolved by the addition of 3 ml of 8 M urea solution containing 5 mM imidazole and incubation at 57 °C for 15 min. The solution was centrifuged at 10,000 *g* for 15 min at 4 °C; the supernatant containing proteins was harvested. The P39 protein was purified by Ni-NTA agarose (QIAGEN, Hilden, Germany) under denaturing conditions using an imidazole gradient and stored at −20 °C for further usage.

Preparation of recombinant P39 immunoblot strips

An amount of 10 µl of the purified protein P39 was diluted in 4× sample buffer and electrophoresed on 15% sodium

dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels at 200 V for 35 min. After electrophoresis, P39 was transferred to polyvinylidene difluoride (PVDF) membranes by semi-dry blotting as follows: the gels were sandwiched between PVDF membranes soaked in 100% methanol and filter papers soaked in 25 mM Tris–HCl and 20% methanol. The blot was run at 0.8 mA/cm^2 for 1 h. Successful transfer of P39 to the blots was tested using primary antibody mouse anti-hexa-histidine-tag (BD Pharmingen, San Jose, US) and secondary antibody HRP-conjugated goat anti-mouse IgG (Jackson Immuno Research Inc., West Grove, US) as described before [19]. The blot membranes were developed using nitroblue-tetrazoliumchloride/5-brome-4-chlor-3-indolylphosphate (NBT/BCIP) substrate (Roche, Germany). Upon confirmation of successful transfer of P39 to the membranes, the rest of the membranes were cut into strips for the stage below.

Serological evaluation of C. jejuni *IgA and IgG antigens in sera*

The strips were covered with 20 µl human serum diluted 1:100 with blocking buffer (3% milk powder in phosphate buffered saline (PBS) with 0.1% NP40 (Nonidet® P 40 substitute/4-nonylphenyl-polyethylene glycol; Sigma-Aldrich, Taufkirchen, Germany)). For positive control, some strips were covered with purified mouse anti-hexa-histidine-tag antibody (BD Pharmingen, San Jose, USA). Both test and control strips were incubated at 4 °C for 60 min followed by three washings using PBS with 0.1% NP40. Washing was followed by antibody conjugation; strips were covered with 1 ml of both alkaline phosphatase (AP)-conjugate *AffiniPure* rabbit anti-human IgA-F_α and alkaline phosphatase (AP)-conjugate *AffiniPure* rabbit anti-human IgG-Fc_γ (Jackson Immuno Research Inc., West Grove, USA) that had been diluted 1:4000 in PBS with 0.1% NP40 and incubated for 1 h at room temperature. The strips were then washed thrice using PBS with 0.1% NP40. Antibody binding was visualized with NBT/BCIP substrate (Roche, Freiburg i. Br., Germany) in accordance to manufacturer's instructions. Presence of a band on a strip indicated positive presence of antibodies IgA or IgG in the serum and, hence, positive for campylobacteriosis. In a previous study, the P39-based assay demonstrated a specificity of 90.9% and a sensitivity of 40.7% for the detection of anti-*Campylobacter*-specific IgA antibodies as well as a specificity of 90.5% and a sensitivity of 57.4% for the detection of anti-*Campylobacter*-specific IgG antibodies [17]. Thus, it is currently the most sensitive and most specific test antigen for the detection of anti-*Campylobacter*-specific antibodies, reported so far [17]. As already mentioned, the assay is based on P39 from *C. jejuni* NCTC 11168, but homologues to P39 can also be found in other *Campylobacter* spp., e.g., *C. coli* or *C. lari*. Therefore, the P39-based assay is not *C. jejuni* specific. It has been shown that *C. coli* caused by campylobacteriosis triggers antibodies that can be detected using P39/CJ0017c [17].

Statistical analysis

The χ^2 test was used to test for significant differences. p values of <0.05 are interpreted as significant.

Results

Results from cloacal-swab cultures revealed that 80% (48/60), 90% (54/60), and 85% (51/60) of sampled ducks during the years 2004, 2007, and 2010, respectively, were colonized by *Campylobacter* spp. These findings support our hypothesis that workers, mainly stable workers, hatchery workers, bird receipt workers, and slaughterers, are at a greater risk of occupational associated campylobacteriosis.

Anti-P39-specific antibody seroprevalence of IgA, IgG, and a combination of both IgA and IgG for 2010, 2007, and 2004 is shown in *Table 2*. In the year 2010, no worker in the category no-exposure was tested positive for IgA, IgG, and a combination of IgA and IgG antibodies. In the category exposure, no worker was positive for anti-*Campylobacter* IgA antibodies but three workers were IgG seropositive. Accordingly, three workers were tested positive for a combination of IgA and IgG antibodies, but there

Table 2. Seroprevalence of *Campylobacter*-specific (P39) antibodies in duck workers in the years 2010, 2007, and 2004

Year	2010		2007		2004	
Exposure	No exposure	Exposure	No exposure	Exposure	No exposure	Exposure
IgA	0.00% (0/36)	0.00% (0/70)	0.00% (0/18)	5.71% (2/35)	0.00% (0/24)	4.17% (2/48)
IgG	0.00% (0/36)	4.29% (3/70)	0.00% (0/18)	0.00% (0/35)	0.00% (0/24)	8.33% (4/48)
IgA + IgG	0.00% (0/36)	4.29% (3/70)	0.00% (0/18)	5.71% (2/35)	0.00% (0/24)	12.5% (6/48)
p value IgA	1.000		0.160		0.159	
p value IgG	0.083		1.000		0.044	
p value IgA + IgA	0.083		0.160		0.013	

The first three lines of *Table 2* list sera that tested positive as percentages, and in parentheses is the absolute number of sera that tested positive in relation to the total number of sera tested in the specific subgroup in a particular year. The last three lines list the p values for the comparison of the exposed and the non-exposed group. p values of <0.05 are interpreted as significant.

was no statistically significant ($p = 0.083$) difference for IgA, IgG, and a combination of IgA and IgG in both no-exposure and exposure groups.

In 2007, the obtained results were as follows: no worker in the category no-exposure was tested positive for anti-*Campylobacter*-specific antibodies, neither for IgA nor for IgG. Only in two workers in the category exposure, anti-*Campylobacter*-specific IgA antibodies were detected, but no worker was tested positive for IgG; hence, two exposed workers were positive for a combination of both IgA and IgG. Statistically, there were no significant differences ($p < 0.05$) between IgA, IgG, and a combination of IgA and IgG in both groups.

In the year 2004, no worker in the category no-exposure was positive for anti-*Campylobacter*-specific IgA antibodies and no worker was tested positive for IgG. Therefore, no non-exposed worker was positive for a combination of both IgA and IgG. Anti-*Campylobacter*-specific IgA antibodies were detected in two exposed workers, and anti-*Campylobacter*-specific IgG antibodies were found in four exposed workers. As a result, six exposed workers were tested positive for a combination of IgA and IgG. There was no statistically significant ($p = 0.159$) difference between the no-exposure and exposure group for IgA. Conversely, the difference in anti-*Campylobacter*-specific IgG antibody seroprevalence and in a combination of IgA and IgG between the no-exposure and exposure groups was shown to be statistically significant ($p = 0.044$ and $p < 0.013$, respectively).

All workers who were tested positive had been in close contact with ducks or duck meat. They handled the ducks or duck meat at different units in the slaughterhouses and farms as described below: six of the seven workers who were positive for IgG worked in the slaughterhouses; five at the bird receipt point, and one at the dissection unit. The seventh *Campylobacter*-specific IgG antibody positive worker was a stable worker who was responsible for duck vaccination. On the other hand, two of the four workers who were tested positive for anti-*Campylobacter*-specific IgA antibodies worked in duck stables, and the remaining two worked as a veterinarian responsible for vaccination and a bird receiver, respectively.

The seroprevalence of anti-*Campylobacter*-specific antibodies, following a combination of IgA and IgG antibodies, decreased gradually from 2004 to 2010 ($p_{2004–2007} = 0.250$ and $p_{2004–2010} = 0.133$; *Table 2*). The specific IgG and IgA antibody seroprevalence did not depend on the period of employment of exposed workers *(Table 3)*.

Discussion

Our findings on the prevalence of *Campylobacter* spp. in duck guts (80% to 90%) agree with a previous study that has been carried out to investigate the prevalence of *Campylobacter* spp. in duck guts, duck meat at a farm, and the associated processing plant, which found its prevalence to be low in duck meat (6% to 20%) compared to the high gut colonization rates of 80% to 100% [10, 12–14, 20]. Consequently, the occupational *Campylobacter* exposure for stable and bird receipt workers was higher compared to slaughterhouse workers and of course to management, general service, and cereal service staff.

Seroprevalence is an important tool, which is widely used to determine the prevalence of a given infectious disease or its history in a patient or in a community. In this study, the seroprevalence of anti-*Campylobacter*-specific IgA and IgG antibodies among workers in duck farms and slaughterhouses was investigated using a P39-based assay with an aim to establish if ducks play a significant role in the transmission of *Campylobacter* spp. to humans.

The overall prevalence of anti-*Campylobacter*-specific IgA and IgG antibodies in both the farm workers and slaughterhouse workers was low during all the years under investigation: 2004 = 6 of 48 sera (12.50%), 2007 = 2 of 35 sera (5.71%), and 2010 = 3 of 70 sera (4.29%). One reason for this could be due to the seasonality of campylobacteriosis, which has been reported to have high incidence in the summer months and low incidence during winter periods [1]. In this study, blood samples were drawn during the autumn period (from September 28 to November 14) of each year probably explaining the low anti-*Campylobacter*-specific IgA and IgG prevalence.

Other reasons include the following. First, it could be a result of scientifically approved OHS protective measures which are religiously promoted by various European agencies to eliminate zoonotic transmitted infections and which were improved in particular between 2004 and 2010 [3, 21]. Second, it could be a result of advanced building standards which have ensured zero interactions between ducks and the external environment which hosts reservoirs of *Campylobacter* spp. such as wild birds, wild rats, and pond waters [22]. Third, the management of the slaughterhouses has been continuously investing in modern technologies of slaughter and packaging techniques which could be reducing transmission of *Campylobacter* spp. from ducks to slaughterhouse environment and its workers [23]. These reasons are further supported by the results that were ob-

Table 3. Period of employment of exposed duck workers, tested positive for IgA and IgG ($(IgA + IgG)^+$) or negative for IgA and IgG ($(IgA + IgG)^-$) in the years 2010, 2007, and 2004

Year	2010			2007			2004		
Exposure period	*n*	mean (month)	±SD (month)	*n*	mean (month)	±SD (month)	*n*	mean (month)	±SD (month)
$(IgA + IgG)^-$	67	69.1	47.3	33	39.8	22.2	42	16.7	11.9
$(IgA + IgG)^+$	3	76.7	33.5	2	34.5	19.1	6	25.7	15.4

tained in the no-exposure category; zero seropositive sera in the 3 years of investigation show null transmission of *Campylobacter* spp. from duck houses to workers situated in other buildings.

In summary, although the consumption of duck meat is on the rise and colonization of duck gut by *Campylobacter* spp. is persistently high, this study has shown that ducks are not a major source of campylobacteriosis to duck farm workers. This could be due to success of campaigns/trainings in good farming and slaughter practices. However, surveillance studies and programs monitoring the prevalence of *Campylobacter* spp. in domesticated ducks, duck farms, duck farm workers, and duck slaughterhouses should be encouraged in order to keep duck-associated campylobacteriosis in check.

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Conflict of interest

The authors declare that they have no competing interests.

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