

Passive and active immunity of broiler chickens against *Campylobacter jejuni* and ways of disease transmission

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Abstract

The study deals with passive and active immunity of fifty-three broiler chickens after infection with culture of *Campylobacter jejuni*. Potential transfer of infection by faecal-oral and aerogenic routes was also investigated. Cloacal swabs and ceecal content were analyzed microbiologically. Identification of *C. jejuni* was carried out by polymerase chain reaction. Observation of passive immunity of broilers from 3 days of age showed that no transfer of *C. jejuni* infection occurred up to 12 day post-infection (p.i.). Observations of active immunity in fourteen 21 days old chickens infected with *C. jejuni* showed that 6 chickens were positive on day 3 p.i. and all infected chickens were positive on day 5 p.i. Investigations of the transfer of *C. jejuni* by faecal-oral route revealed positivity in two broilers on day 3 p.i. and in all tested chickens on day 5 p.i. Aerogenic transfer of infection was not recorded. This was one of the first studies in our country dealing with passive and active immunity of broiler chickens against *C. jejuni* and spreading of this zoonotic disease.

Campylobacteriosis, PCR, microbiological examination, disease transfer

The food chain is considered one of the most important ways of transfer of alimentary infections among animals and humans. The spreading of infections in poultry production is related to insufficient hygiene of rearing which is stressful to housed birds and results in increased numbers of heterophils (Bedáňová et al. 2007).

Campylobacter jejuni is world-wide considered the principal cause of human diarrhoeal diseases and gastroenteritis transmissible in food. *Campylobacteriosis* is a zoonosis; many animals serve as reservoirs of this infectious disease. *Campylobacter* sp. enters the environment also via drinking water contaminated with excrements of animals, birds or infected people (Nogva et al. 2000).

The aim of the study was to investigate passive and active immunity of broiler chickens against *C. jejuni* and potential transfer of this disease by faecal-oral and aerogenic routes.

Materials and Methods

Experimental animals

Fifty-three broiler chickens of the Ross 308 hybrid were obtained from an approved hatchery Častá (No. SK-LH-PK-01-MACH poultry, Budmerice s.r.o., Slovak Republic). The chickens were kept in the experimental facilities of the Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy (UVMP) in Košice.

Before the experiment, cloacal swabs were taken from all chickens included in the study and all were negative for *Campylobacter* sp. Then, after 2 days of acclimatization, they were assigned to four experimental groups: Group 1 (n = 13), Group 2 (n = 20), Groups 3 (n = 10) and Group 4 (n = 10).

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Infection

To infect the chickens we administered bacterial culture of reference strain of *C. jejuni* CCM 6207 (Masaryk University Brno, Czech Republic) *per os* at a dose of 0.2 ml per experimental chick. The colony-forming unit (CFU) counts at testing of passive and active immunity were 10^3-ml^{-1} and 10^4-ml^{-1} , respectively.

Seven chicks from Group 1 were infected with *C. jejuni* at the age of 3 days, to evaluate their passive immunity. For evaluation of active immunity we infected 14 chickens from Group 2 at the age of 21 days with the same culture of *C. jejuni*. The remaining six chickens of Groups 1 and 2 were not infected and were housed together with the infected ones to observe potential transfer by the faecal-oral route. Group 3 was kept in a separate cage in the same experimental room to observe the possibility of aerogenic transfer. Group 4 was housed in a separate room and served as control.

In the first week of age the chickens were exposed to a temperature of 25–30 °C which was then decreased to 22–27 °C. Standard mixed feed BR1, BR2 and BR3 was supplied to the chickens according to the age. They were fed *ad libitum* from a feeder and had free access to drinking water.

Sampling and microbiological diagnosis

Using sterile swabs, two cloacal samples were taken from all broilers on predetermined days (Tables 1 and 2). One swab was transferred to a tube with Bolton liquid multiplication medium and incubated under micro-aerobic conditions for 4 to 6 h at 37 °C and then for 44 h \pm 4 h at 41.5 °C. After incubation, we inoculated a loopful of the culture onto two selective modified media, mCCDA and Skirrow agar medium.

The second cloacal swab was used to inoculate directly the mCCDA and Skirrow agar media. The inoculated media were incubated at 41.5 °C under micro-aerobic conditions and the colonies were counted after 44 h \pm 4 h. The procedures used for confirmation of the genus *Campylobacter* and for differential diagnostics complied with the Slovak standard STN EN ISO 10272-1.

The broilers were killed by cervical dislocation on predetermined days (Tables 1 and 2). Determination of *Campylobacter* was carried out on 1 g of the caecal sac content using decimal dilutions (ISO/TS 10272-2, Slovak standard STN EN ISO 10272-1).

PCR diagnosis

The DNA was isolated from *C. jejuni* colonies grown on the above described selective solid media by re-suspending them in 500 μ l sterile distilled water and incubation at 100 °C for 5–20 min. The sample was then centrifuged at 12 000 g for 5 min (twice) and the supernatant was used for PCR. Concentration of the DNA was determined by a spectrophotometer Spectronic Genesys 5. For PCR we used 100 ng of DNA of the examined sample, 5 μ l of 10 \times concentrated buffer solution according to manufacturer's recommendation, 0.2 mM dNTPs, 1 μ M primers, 3.5 mM MgCl₂, 1.0 U AmpliTaq DNA Polymerase (Perkin Elmer, USA). The sample volume was made up to 50 μ l with distilled water. The primers for genus *Campylobacter* were C412F and C1228R, resulting

in an amplicon 816 bp and the primers for species *C. jejuni* were Jej1F and Jej2R, resulting in an amplicon 793 bp (Brown et al. 2004). To identify PCR products we used system GelLogic 100.

The results obtained were evaluated statistically using one-way ANOVA, Friedman test, Dunn's post-test and Mann-Whitney test.

Table 1. Testing of passive immunity and potential faecal-oral infection transfer in broilers from Group 1.

Number of birds	Microbiological examination of cloacal swabs and caecal sacs		
	Day 3, 5, 7 p.i.	Day 9 p.i.	Day 12 p.i.
Infected with <i>C. jejuni</i> (passive immunity)			
1	-	- *	
2	-	- *	
3	-	- *	
4	-	-	- *
5	-	-	- *
6	-	-	- *
7	-	-	- *
Non infected (faecal-oral transfer)			
8	-	- *	
9	-	- *	
10	-	- *	
11	-	-	- *
12	-	-	- *
13	-	-	- *

*collection of samples from caecal sacs after slaughtering the chickens

Results

The tests of passive immunity of 3-day-old chickens infected with *C. jejuni* (Group 1) carried out on days 3, 5, 7, 9 and 12 post-infection (p.i.), provided negative results for *Campylobacter* (Table 1). Throughout the experiment we observed no health changes in the experimental chickens.

The testing of active

Table 2. Testing of active immunity and potential faecal-oral infection transfer in broilers from Group 2

Number of birds	Microbiological and PCR examination of cloacal swabs and caecal sacs			
	Day 3 p.i.	Day 5, 7 p.i.	Day 9 p.i.	Day 12 p.i.
Infected with <i>C. jejuni</i> (active immunity)				
1	-	+	+	*
2	-	+	+	*
3	-	+	+	*
4	-	+	+	*
5	-	+	+	*
6	-	+	+	*
7	+	+	+	*
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
11	+	+	+	+
12	+	+	+	+
13	-	+	+	+
14	-	+	+	+
Non infected (faecal-oral transfer)				
15	-	+	+	*
16	-	+	+	*
17	+	+	+	*
18	-	+	+	+
19	+	+	+	+
20	-	+	+	+

*sampling of caecal sacs after slaughter

immunity of 21-day-old chickens infected with *C. jejuni* (Group 2), carried out on day 3 p.i., showed positivity in 6 infected chickens and 2 chickens were infected by the faecal-oral route. In this period the chickens showed no marked health changes. On day 5, 7, 9 and 12 p.i., the microbiological and PCR methods proved the positivity to *C. jejuni* in all infected birds but, at the same time, also in all 6 chickens infected by the faecal-oral route (Table 2).

Clinical observations of the chickens from Group 2 showed that on day 7 p.i. two infected chickens suffered from watery diarrhoea. On day 9 p.i. we slaughtered 7 infected chickens and 3 chickens from the group observed for the faecal-oral transfer, collected their caecal sacs and carried out pathological-anatomical

examination. Two slaughtered chickens infected with *C. jejuni* showed dilatation of jejunum. On day 12 p.i. additional three chickens from this group, infected with *C. jejuni*, were affected with diarrhoea. All remaining chickens were slaughtered on this day. *Post mortem* examination showed dilatation of jejunum in four infected chickens and disseminated haemorrhagic enteritis in one of them.

The plate counts of *C. jejuni* in 1g of caecal content of infected chickens (Group 2) ranged from 3.6×10^5 to 5.1×10^6 CFU and in chickens infected by the faecal-oral route from 7.4×10^4 to 4.3×10^6 CFU.

Their body mass in Group 2 ranged between 0.9 kg and 1.2 kg (mean 0.98 kg).

Statistical evaluation of the day of colonization of intestinal tract with *C. jejuni* in Group 2 by means of transformation 0 (negative) and 1 (positive) by Friedman test and Dunn's post-test showed significant difference between days 1 and 5 p.i. at the level of significance $\alpha = 0.01$. Evaluation of findings by Mann-Whitney test on day 3 p.i. in Group 2 comparing the spreading of infection by direct and faecal-oral route showed no significant differences ($\alpha = 0.05$) meaning that the infection spread at the same rate.

The cloacal swabs taken from all chickens from Groups 3 and 4 on days 3, 7, 17 and 33 of the experiment were negative for *C. jejuni*. The health of these chickens was good throughout the experiment. The body mass of chickens from Group 3 ranged between 1.3 and 1.7 kg and that of chickens from Group 4 between 1.3 and 1.9 kg but the mean body mass was the same in both groups (1.5 kg).

Discussion

PCR methods have been frequently used to diagnose agents of human (Jautová et al. 2001) and animal diseases (Holoda et al. 2005).

Microbiological and PCR methods used in our study showed that all investigated broiler chickens from Group 1 were negative for *C. jejuni* up to slaughter (12 and 15 days of age). This could be ascribed to the high concentration of circulating maternal specific antibodies that confer passive immunity and start to decrease only after 14 days of age (Sahin et al. 2001).

All broiler chickens infected in our study with *C. jejuni* at the age of 21 days (Group 2) were positive for *C. jejuni*. The faecal-oral route of infection transfer was confirmed. The infection spread at the same rate by both direct and faecal-oral route by day 3 p.i., as witnessed by Mann-Whitney test ($\alpha = 0.05$). No aerogenic transfer of infection was observed.

Previous observations (Mead et al. 1995) showed that colonization of 1 g of caecal content with *Campylobacter* during fattening of broiler chickens reached 10^7 CFU. The *C. jejuni* counts determined in our experiment (Group 2) were lower.

The body mass of chickens of the Ross 308 hybrid reaches approximately 1.8 kg at the age of 32 days (Havenstein et al. 2003). The weight gain of chickens involved in the present study of active immunity of chickens (Group 2) infected with *C. jejuni* was lower.

References

- Bedáňová I, Voslářová E, Večerek V, Pištěková V, Chloupek P 2007: Haematological profile of broiler chickens under acute stress dues to shackling. *Acta Vet Brno* **76**: 129-135
- Brown PE, Christensen OF, Clough HE, Diggle PJ, Hart, CA, Hazel S, Kemp R, Leatherbarrow AJH, Moore A, Sutherst J, Turner, J, Williams NJ, Wriht EJ, French, NP 2004: Frequency and spatial distribution of environmental *Campylobacter* spp. *Appl Environ Microbiol* **70**: 6501-6511
- Havenstein GB, Ferket PR, Qureshi MA 2003: Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poultry Sci* **82**: 1509-1518
- Holoda E, Vu-Khac, H, Andrašková S, Chomová Z, Wantrubová A, Krajňák M, Pilipčinec E 2005: PCR assay for detection and differentiation of K88ab1, K88ab2, K88ac and K88ad fimbrial adhesins in *E.coli* strains isolated from diarrheic piglets. *Folia Microbiol* **50**: 107-112
- ISO/TS 10272:2006 Microbiology of food and feed. Horizontal method for confirmation and determination of plate counts of bacteria *Campylobacter*. Part 2: Determination of plate counts (Mikrobiológia potravín a krmív. Horizontálna metóda na dôkaz a stanovenie počtu baktérií *Campylobacter*. Časť 2: Stanovenie počtu) (In Slovak)
- Jautová J, Virágová S, Ondrašovič M, Holoda E 2001: Incidence of *Candida* species isolated from human skin and nails: a survey. *Folia Microbiol* **46**: 333-337
- Mead GC, Hutson WR, Hinton MH 1995: Effect of changes in processing to improve hygiene control on contamination of poultry carcasses with *Campylobacter*. *Epidemiol Infect* **115**: 495-500
- Nogva HK, Bergh A, Holck A, Rudi K 2000: Application of the 5'-nuclease PCR assay in evaluation and development of methods for quantitative detection of *Campylobacter jejuni*. *Appl Environ Microbiol* **66**: 4029-4039
- Sahin O, Zhang Q, Meitzler JC, Harr BS, Morishita TY, Mohan R 2001: Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. *Appl Environ Microbiol* **67**: 3951-3957
- STN EN ISO 10272:2006 Microbiology of food and feed. Horizontal method for confirmation and determination of plate counts of bacteria *Campylobacter*. Part 1: Confirmation method (Mikrobiológia potravín a krmív. Horizontálna metóda na dôkaz a stanovenie počtu baktérií *Campylobacter*. Časť 1: Metóda dôkazu) (In Slovak). SÚTN, Bratislava