CRIMSON PUBLISHERS
Wings to the Research

Approaches in Poultry, Dairy & Veterinary Sciences

ISSN: 2576-9162

Research Article

Detection of *Campylobacter* spp. in Cloacal Swab of Hens Reared in Different Breeding Conditions in Slovakia

Martin Levkut Jn[1], Viera Revajová[1]*, Katarína Bobíková[1], Mária Levkutová[1], Zuzana Ševčíková and Mikuláš Levkut[1,2]

[1]*Department of Pathological Anatomy and Pathological Physiology, Slovak Republic*

[2]*Institute of Neuroimmunology, Slovak Academy of Science, Slovak Republic*

***Corresponding author:** Viera Revajová, Department of Pathological Anatomy and Pathological Physiology, Institute of Pathological Anatomy, Komenského 73, Košice, Post code: 041 81, Slovak Republic

Submission: November 19, 2018; **Published:** November 26, 2018

Abstract

The study evaluated the detection of the *Campylobacter* spp. in cloacal swabs of hens at six poultry farms: one battery cage, three indoor and two free range. Enzyme-linked immunosorbent assay (ELISA) used for detection of Campylobacter spp. determined positivity in the range from 3.4% to 10%. The lowest values of *Campylobacter* spp. were recorded in free range poultry farms. Our results demonstrated low number of *Campylobacter* spp. in cloacal swabs of hens with no evident prevalence of positive birds under the various breeding conditions of present in the evaluated farms. Obtained results also indicate a self-limitation nature of the infection in majority of birds and would be helpful in the future studies. Finally, our study shows that detection of *Campylobacter* spp. in hens is not influenced by breeding of poultry under different farm conditions.

Keywords: Campylobacter; Positivity; ELISA; Poultry farms

Introduction

Poultry meat is reported as the most common transmission factor of alimentary disease – campylobacteriosis [1]. *Campylobacter* spp., especially *Campylobacter jejuni* and *C. coli,* are the main cause of human bacterial gastroenteritis in the developed world [2,3]. Epidemiological investigations of commercial flocks indicate that naturally acquired flock colonization is age-dependent. Newly hatched chicks appear to be free of campylobacter. In Europe, this negativity persists until at least 10 days of age (the so-called lag phase), and most flocks become infected only 2 to 3 weeks after the placement of chicks into a broiler house [4]. The duration of colonization and shedding of campylobacter in poultry has not been fully determined. It is generally accepted that colonization in chickens persists at least for the life span of a broiler, without a clinical form of infection [5]. Moreover, after 8 weeks, colonization may gradually reduce in terms of both the number of organisms recoverable from cecal contents and the number of colonized birds [6]. Elderly hens can be antibody positive without colonization, suggesting that an antibody response may be associated with elimination of infection [7]. Several changes in stock management may occur during altered feed composition even in organically produced birds. The prevalence of flock positivity is also dependent on flock size, type of production system [8], and age of birds [9]. There is generally a higher rate of infection in summer than in winter, and the timing of this peak also appears to vary with latitude. Some studies, on the other hand, have failed to demonstrate seasonality in the prevalence of *C. jejuni* in poultry [7,9]. The reason for seasonal variation is unknown but may reflect levels of environmental contamination. Certainly, poultry houses have more ventilation in the summer, increasing the contact of the birds with the outside environment. Individually cages hens also have a seasonal variation in excretion rates [10].

Due to the fact that the beginning of the chain of *Campylobacter* spp. infection in poultry relates with their breeding conditions and age of flocks the aim of this study was to detect enteropathogenic bacteria *Campylobacter* spp. in cloacal swab of hens reared under different breeding conditions.

Materials and Methods

In this study were used 180 hens of laying breeds placed on six farms in range from 6 to 12 month of age. For the identification of *Campylobacter* spp. in cloacal swab one battery-cage farm, three indoor farms, and two free range farms were chosen. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Samples were collected in the spring period (May-June). For collection of samples 30 hens were randomly chosen from each defined farm. Cloacal swab from individual hen was collected by swab stick and ELISA (enzyme-linked immuno sorbent assay) method was used for analysis - commercial ProspecT™ *Campylobacter* kit (Oxoid, USA) - to identify enteropathogenic *Campylobacte*r spp. Diluted samples were added into pre-designated wells of microplate strips at a dose of 200µl. Ninety six well-microtiter plates were coated with rabbit polyclonal anti-*Campylobacter* SA (sialic acid) antibody. After the incubation of microtiter plates (20-25 °C, 60min), the content of the wells was aspirated and washed three times with wash solution (component of ELISA kit). Then 200µl of diluted enzyme-antibody conjugate binding with horseradish peroxidase in stabilizing buffer was applied into the plate wells, and incubated at 20-25 °C 30 minutes. After incubation, the plate was 3 times washed and 200µl of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added into each well. The reaction was stopped with 50µl stop solution and absorbance was measured spectrophotometrically at 450nm on microplate reader (Revelation Quicklink, Opsys MR, Dynex Technologies, USA). Interpretation was made using a calibration curve prepared according to the manufacturer's protocol.

One-way ANOVA with Tukey post-test by Minitab 16 software was used (SC&C Partner, Brno, Czech Republic) for statistical analysis.

Results

The results demonstrated low *Campylobacter* spp. positivity in hens reared in different types of examined poultry farms (Table 1). The presence of *Campylobacter* spp. was confirmed in one indoor poultry farm in 1 of 30 analyzed samples, representing 3.40%. Another two indoor poultry farms demonstrated the presence of *Campylobacter* spp. in 2 respectively in 3 of 30 analyzed samples, representing 6.70% and 10%. Battery-cage farm showed the presence of *Campylobacter* spp. in 2 of 30 samples, representing 6.70%. The presence of bacterial antigen was confirmed in both free range farms in 1 of 30 analyzed samples, representing 3.40%. However, differences between mean values for various rearing hence were not statistically significant.

Table 1: Seropositivity of poultry farms to *Campylobacter spp.*

	Positive Hens	Negative Hens	Number of Samples	Total Number of Farm Hens	Percentage of Positive Hens	Percentage of Negative Hens
Indoor farm 1	1	29	30	6000	3,40%	96,60%
Indoor farm 2	3	27	30	4500	10%	90%
Indoor farm 3	2	28	30	4500	6,70%	93,30%
Battery-cage	2	28	30	50000	6,70%	93,30%
Free range farm 1	1	29	30	70	3,40%	96,60%
Free range farm 2	1	29	30	50	3,40%	96,60%

Discussion

Percentage of *Campylobacter* spp. positive hens was low in all monitored flocks. No notable difference in positivity of hens was found among farms with difference flock sizes. Barrios et al. [11] and Rushton et al. [12] demonstrated that environmental occurrence of *Campylobacter* spp. is related to its seasonal character with the peak in the flock prevalence during the warm summer months (July-August). In our study, the percentage of bacterial positivity in examined hens in spring months was low. On the other hand, the comparative monitoring during winter months was not done. However, low percentage of positive hens in spring months suggests no evident seasonality [2] in monitored flocks. Similarly, no significant differences in terms of hens positive to *Campylobacter* spp. indicate that contact of hens with contaminated water and soil from the environment did not play important role [13] in the campylobacter infection. In our experiment, the lowest values were demonstrated in free range farms. This finding could be related to the time of sampling - the occurrence of pathogenic bacteria *Campylobacter* spp. in the environment in the spring months is low. The diversified feed intake during the growing season could be also the possible impact on the lower frequency of positivity to *Campylobacter* spp. It would be useful to examine a higher number of poultry farms to obtain more extensive database of results.

How to cite this article: Martin L J, Viera R, Katarína B, Mária L, Zuzana Š. Detection of Campylobacter spp. in Cloacal Swab of Hens Reared in Different Breeding Conditions in Slovakia. Appro Poult Dairy & Vet Sci. 5(3). APDV.000611. 2018. DOI: 10.31031/APDV.2018.05.000611

The duration of colonization and shedding in poultry has not been fully determined. It is generally accepted that colonization in chickens persists at least for the life span of a broiler. However, there is evidence that colonization may gradually reduce when the birds get older and individual birds excrete campylobacters intermittently [6]. In retail chickens, prevalence of *Campylobacter* spp. was found to be at 75% [14]. Finally, more recently obtained results in our laboratory show that experimental infection of chickens with *C. jejuni* induces IgA mucosal antibody response [15] and increase of IgM+ cells in the intestinal mucosa [16], which demonstrates immune response development in *Campylobacter* spp.-infected chickens. Moreover, elderly hens can be antibody positive without colonization, suggesting that the antibody response may be associated with elimination of infection [7]. Self-limitation of infection has been reported in other naturally colonized birds, for example, gulls can become negative for *Campylobacter* spp. within a period of 4 weeks [17]. Comparing to high positivity to followed bacteria in chickens [14,18] our results suggest self-limitation of infection in majority of examined birds.

In conclusion, our results demonstrate positivity to *Campylobacter* spp. in low number of monitored hens without evident effect of rearing in different farming modes on prevalence of positive hens. Self-limitation may be one of the reasons of low campylobacterial numbers. The factors involved in the self-limitation of colonization are unclear but may include acquired immunity.

Acknowledgement

The authors are grateful to E. Husáková for assistance and samples analyses. This work was supported by the Grant Agency for Science of Slovak Republik VEGA 1/0562/16, Slovak Research and Developmental Agency APVV 15-0165.

Referances

1. Corry JEL, Atabay HI (2001) Poultry as a source of Campylobacter and related organisms. J Appl Microbiol (30): 96S-114S.
2. Jorgensen F, Ellis Iversen J, Rushton S, Bull SA, Harris SA, et al. (2011) Influence of season and geography on Campylobacter jejuni and C. coli subtypes in housed broiler flocks reared in Great Britain. Appl Environ Microbiol 77: 3741-3748.
3. Wieczorek K, Osek J (2011) Molecular characterization of Campylobacter spp. isolated from faeces and carcasses in Poland. Acta Vet Brno 80: 19-27.
4. Evans SJ, Sayers AR (2000) A longitudinal study of Campylobacter infection of broiler flocks in Great Britain. Prev Vet Med 46(3): 209-223.
5. Ondrašovičová S, Pipová M, Dvořák P, Hričínová M, Hromada, R, et al. (2012) Passive and active immunity of broiler chickens against Campylobacter jejuni and ways of disease transmission. Acta Vet Brno 81: 103-106.
6. Achen M, Morishita TY, Ley EC (1998) Shedding and colonization of Campylobacter jejuni in broilers from day-of-hatch to slaughter age. Avian Dis 42(4): 732-737.
7. Newell DG, Fearnley C (2003) Sources of Campylobacter colonization in broiler chickens. Appl Environ Microbiol 69(8): 4343-4351.
8. Berndtson E, Danielsson Tham ML, Engvall A (1996) Campylobacter incidence on a chicken farm and the spread of Campylobacter during the slaughter process. Int J Food Microbiol 32(1-2): 35-47.
9. Humphrey TJ, Henley A, Lanning DG (1993) The colonization of broiler chickens with Campylobacter jejuni: Some epidemiological investigations. Epidemiol Infect 110(3): 601-607.
10. Evans SJ (1997) Epidemiological studies of Salmonella and Campylobacter in poultry. Dissertation, University of London, UK.
11. Barrios PR, Reiersen J, Lowman R, Bisaillon JR, Michel P, et al. (2006) Risk factors for Campylobacter spp. colonization in broiler flocks in Iceland. Prev Vet Med 74(4): 264-278.
12. Rushton SP, Humphrey TJ, Shirley MD, Bull S, Jørgensen F (2009) Campylobacter in housed broiler chickens: A longitudinal study of risk factors. Epidemiol Infect 137(8): 1099-1110.
13. Vandeplas S, Marcq C, Dauphin RD, Beckers Y, Thonart P, et al. (2008) Contamination of poultry flocks by the human pathogen Campylobacter spp. and strategies to reduce its prevalence at the farm level. Biotechnol Agron Soc Environ 12: 317-334.
14. European Food Safety Authority (2010) Analysis of the baseline survey on the prevalence of Campylobacter in broiler batches and of Campylobacter and Sallmonella on broiler carcasses in the EU. EFSA J 8: 1522.
15. Karaffová V, Marcinková E, Bobíková K, Herich R, Revajová V, et al. (2017) TLR4 and TLR21 expression, MIF, IFN-beta, MD-2, CD14 activation, and sIgA production in chickens administered with EFAL41 strain challenged with Campylobacter jejuni. Folia Microbiol 62(2): 89-97.
16. Revajova V, Bobikova K, Karaffova V, Levkutova M, Levkut M (2017) Evaluation of IgA gene expression, sIgA and IgA+ lymphocytes in chickens administered with Enterococcus faecium and Campylobacter spp. CEEPC 2017, Košice, Slovakia, p. 89.
17. Glunder G, Neumann U, Braune S (1992) Occurrence of Campylobacter spp. in young gulls, duration of Campylobacter infection and reinfection by contact. Zentralbl Veterinarmed B 39(2): 119-122.
18. Meldrum RJ, Wilson IG (2007) Salmonella and Campylobacter in United Kingdom retail raw chicken in 2005. J Food Prot 70(8): 1937-1939.