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GENETIC DIVERSITY OF CAMPYLOBACTERJEJUNI IN LAYING HEN FLOCKS INCREASES WITH BIRD'S AGE

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ABSTRACT

Campylobacter jejuni (C. jejuni) is frequently found in laying hen flocks. Little is known about the genetic diversity of these strains. In 11 commercial laying hen flocks kept in aviary systems on 11 different farms, cloacal swab samples were taken from 30 randomly selected birds per flock (330 in total) between February and September of one year, 6 samplings took place during the cooler season (February to April) and 5 under summer conditions (July to September). Grown colonies of all samples were analysed by microscopy, biochemical tests and PCR. The identified isolates were typed by *flaA* typing using *Ddel* restriction enzymes. C. *jejuni* was found in all examined flocks. It was found that 172 out of the 330 sampled hens were positive from cloacal swabs. In the summer period 90 % of the samples were positive whereas in the winter period 27 % were positive only. The number of positive samples and the number *of flaA* types increased with the age of the hens. In the 20 weeks old flock one *flaA* type was found out of 8 isolates while 9 *flaA* types out of 27 isolates were detected in the 52 weeks old flock. 61 different patterns were found in the 172 isolates. The *flaA* types seem to be flock specific because no identical types were found in different flocks. The reason for the observed diversity and distribution of *flaA* types remains unclear but may be caused either by mutation or by horizontal transmission from sources outside the barn. This also demonstrates that an aging laying hen flock represents an important reservoir of various C. *jejuni* genotypes. This diversity *offlaA* types could also be used in epidemiological studies for identifying sources from which C. *jejuni* isolates were emitted.

Key words: *Campylobacter jejuni,* laying hens,/7oA types diversity

INTRODUCTION

Campylobacter jejuni (C. *jejuni)* is a microaerophilic, thermotolerant Gram negative bacterium which can colonize the digestive tract of humans and most warm blooded animals (13), preferentially of poultry (18), known to be one of the main causes of food-borne human infections mainly -transmitted by broiler meat (8, 17). It is well known that *Campylobacter* is wide spread in broiler flocks and most birds presented for slaughter are carriers (31) displaying a wide range of different *flaA* types which is probably caused by a considerable genetic instability (20, 27). F/oA-typing by restriction fragment length polymorphism (RFLP) is reported to be a rapid, reliable, sensitive and cost-effective method for typing *Campylobacter* isolates, which requires only minute amounts (<50 ng) of DNA (5, 23) and *Ddel* appears to provide the best discrimination for veterinary isolates (2). Unlike broilers, occurrence of C. *jejuni* types in commercial laying hens has not been extensively studied probably due to the rather low risk of vertical transmission of *Campylobacter* from breeder flocks to the progeny (6, 19) and because of the low rate of *Campylobacter* outbreaks associated with eggs (10). However, it is well known that hens and roosters can carry *Campylobacter* in feces, internal organs and in their reproductive tract (4, 12, 29). A recent study from South Africa reports about a prevalence of 94 % of *Canpylobacter* spp. in laying hen farms (3) This means that laying hens represent quite well a possible reservoir for C. *jejuni* which can be horizontally transmitted to humans or other animals as assumed by Jacobs-Reitsma (16). However, little is known about the genetic diversity of C *jejuni* in laying hen flocks and which influence the age of the birds which live much longer than broilers has on the diversity and distribution of C. *jejuni* types in this environment. This paper reports about the occurrence of C. *jejuni* in 11 commercial laying hen flocks in the Northern part of Germany and different types of C. *jejuni* at farm level using *fla* typing with *Ddel* restriction which provides the best discrimination for veterinary isolates (Ayling et al., 1996). The results may give an insight in the genetic diversity of C. *jejuni* and may help to raise our understanding of the epidemiology of *Campylobacter* in the laying hen industry.

MATERIAL AND METHODS

Farms

Samples were taken from 11 laying hen flocks (A to K) on 11 farms located in different geographical regions in Germany. The flocks A to F were sampled in February and April (winter season) while, flocks G to K were sampled in July and September 2010 (summer season). The flock size ranged from 1300 to 20,000 birds. The hens were about 20 to 52 weeks old and were kept in aviary systems.

Sample collection

In each flock, 30 randomly selected birds were captured from different parts of the hen house and investigated by cloacal swabs (EUROTUBO®, DELTALAB, Spain). A swab obtained from one bird was streaked directly onto modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA, Oxoid, Germany) and Brilliance CampyCount agar (Oxoid, Germany). Thereafter, the swab was placed in a tube with 9 ml Bolton Broth (Oxoid, Germany). Samples were transported to the laboratory under cooled conditions together with air samples. Isolation and identification of C. *jejuni* from swab samples was carried out following the method described by Ahmed et al. (1). A sample size of 30 swabs allows detection of a 10 % prevalence at a level of confidence 95 % (Win Episcope 2.0) within a flock.

Genomic DNA from the C. *jejuni* isolates as well as from the reference strains were extracted from an overnight cell cultures using peq GOLD bacterial DNA Kit (PeQlab Biotechnologie, Germany) following the manufacturer's instructions. The DNA concentration was determined by measuring the optical density at 260 nm. The isolated DNA was used for the *C. jejuni* specific *MapA* PCR and *FlagellineA* typing PCR-RFLP as described by Ahmed et al. (1).

RESULTS

In total, 61 C. *jejuni* types were isolated from 172 positive cloacal swabs of 11 laying hen flocks (Table 1). All types could be detected after streaking directly on mCCDA and Brilliance CampyCount Agar without enrichment. All presumptive C. *jejuni* types were identified by phenotyping, biochemical reactions and PCR. Prevalence of C. *jejuni* are given in Table (1) ranging from 27 % (flock A, youngest hens) to 90 % (flock K, oldest hens). It seems to be due to influences of both seasonal and age of the flock. The prevalence of *C. jejuni* was in each single flock clearly higher during summer season than in winter The *flaA* types seem to be flock specific because no identical types were found in different flocks and the number of genotypes seems progressively to increase with the age of the birds (Table 2). In some flocks there are dominating genotypes such as *Gz,* He, l2, Ji and K3 which were found 6, 5, 6, 8, and 7 times, representing 37.5, 26.3, 30, 30.8 and 25.9% of all isolates, respectively (Table 2). In other flocks (B to F) no dominating genotypes could be found. In the youngest flock only one genotype (Al) was detected out of 8 isolates.

Table 1: Sampling time, hen's age in weeks, C. *jejuni* positive samples of 30 cloacal swabs of 11 laying hen flocks and prevalence percent

Time sampling	of Flock	Hens' age (week)	Positive samples (n)	Prevalence (%)
February	A	20	8	26,67
February	B	36	9	30,00
March	C	36	10	33,33
March	D	37	11	36,67
April	E	38	12	40,0
April	F	44	14	46,67
Juli	G	43	16	53,33
Juli	H	47	19	63,33
August	I	47	20	66,67
August	J	49	26	86,67
September	K	52	27	90,0

Table 2: Results *otflaA* typing of positive samples per laying hen flock

Hock	Positive samples	No.yZoA types	*flaA* type detected (no. of isolates)
A	8	1	Al(8)
B	9	4	B1(3),B2(2),B3(1),B4(3)
C	10	4	Cl(3), C2 (3), C3 (2), C4 (2)
D	11	4	D1(4),D2(2),D3(3),D4(2)
E	12	5	E1(3),E2(1),E3(4),E4(1),E5(3)
F	14	7	Fl(3), F2 (3), F3 (1), F4 (1), F5 (2), F6 (1), F7 (3)
G	16	5	Gl(2), G2 (6), G3 (4), G4 (1), G5 (3)
H	19	7	Hl(3), H2 (1), H3 (1), H4 (3), H5 (3), H6 (5), H7 (2)
I	20	8	11(3), 12 (6), 13 (2), 14 (1), 15 (3), 16 (2), 17 (1). 18 (2)
J	26	7	Jl(8), J2 (2), J3 (4), J4 (3), J5 (2), J6 (4), J7 (3)
K	27	9	Kl(5), K2 (4), K3 (7), K4 (3), K5 (2), K6 (4), K7 (3), K8 (2), K9 (1)

DISCUSSION

The presented data show that all investigated laying hen flocks were contaminated with C. *jejuni* displaying a large variety of different *flaA* -typing (61 genotypes in 172 positive samples) using cloacal swabs directly plated on mCCDA and Brilliance CampyCount Agar . The prevalence within flock ranges from about 27 to 90 % which is lower than in broilers at slaughter age (24, 27) but clearly higher than reported by Stojanov et al (28) who isolated C. *jejuni* from cloacal swabs and cecum of laying hens with prevalences of 38 % and 71 %, respectively. However, Sulonen et al., (2007) found a prevalence within flock ranging from 98 to 100 % in organic laying hens. The remarkable wide diversity of genotypes found in laying hen flocks in our study is not reported elsewhere to our knowledge.. This result may have been influenced by the high number of C. *jejuni* isolates which were typed (172 positive samples out of 330 cloacal swabs) and the use of the sensitive *flaA* typing technique (23). Another reason could be the known genetic instability of *Campylobacter* spp. (11, 26) caused e.g. by hypervariable regions within the/7oA-gene of C. *jejuni* strains (21) which may have generated subtypes during the laying period increasing the variety of types with increasing flock age.

The increasing diversity of isolates with birds' age may also reflect the persistence of *Campylobacter* in poultry flocks (26, 30) or the influx of C. *jejuni* types from neighbouring farms or similar sources in the environment. Transmission can occur airborne or by living vectors such as beetles, flies and wild birds.

The observation that no identical types could be found on different farms supports the hypothesis of an in-house production of the various strains (15, 24). A direct exchange of C. *jejuni* types between the investigated flocks is also unlikely because the flocks are located in different geographical regions without the possibility of contact or exchange. The high prevalence of *C. jejuni* observed in summer season in our study is consistent with other reports, which also indicate a higher prevalence of C. *jejuni* in poultry flocks in summer and autumn than in winter (9, 22, 25). Elevated temperatures and high humidity seem to increase *Campylobacter* transmission and persistence within the farm environment and increase *Campylobacter* carriage in laying hens (7) supported by migratory birds, beetles, or rodents, whose activities are highly temperature dependent (14). Overall our findings confirm the role of laying hens as an important reservoir of *Campylobacter* spp. The findings in this study demonstrate that *Campylobacter* is indeed present in the laying hens with high prevalence and a high genetic diversity. The considerable variation in *flaA* typing may be caused by the geographical spread of the farms and more probably by the age of the birds.

REFERENCES

Ahmed, M, Schuiz, J Hartung J. Laying hens as a source of *Campylobacter jejuni.* In:K6fer, J.;Schobesberger, H. (eds.): XVth International Congress of the International Society for Animal Hygiene. Animal hygiene and sustainable livestock production. Proc. Vol. 1'Vienna, Austria, 3-7 July 2011,173-175. 2011

Ayling, R. D., M. J. Woodward, S. Evans, and D. G. Newell. Restriction fragment length polymorphism of polymerase chain reaction products applied to the differentiation of poultry campylobacters for epidemiological investigations. Res. Vet. Sci. 60:168-172.1996.

Bester, L. A., and S. Y. Essack. Observational study of the prevalence and antibiotic resistance of *Campylobacter spp.* from different poultry production systems in KwaZulu-Natal, South Africa. J. Food Prot., 75:154-159. 2012.

Buhr, R. J., N. A. Cox, N. J. Stern, M. T. Musgrove, J. L.Wilson, and K. L. Hiett. Recovery of *Campylobacter* from segments of the reproductive tract of broiler breeder hens. Avian Dis. 46:919-924.2002.

Camarda, A., D. G. Newell, R. Nasti, and G. D. Modugnoa. Genotyping *Campylobacter jejuni* strains isolated from the gut and oviduct of laying hens. Avian Dis. 44: 907-912. 2000.

Cox, N. A., L. J. Richardson, R. J. Buhr, and P. J. Fedorka-Cray. *Campylobacter* species occurrence within internal organs and tissues of commercial caged Leghorn laying hens. Poult. Sci. 88:2449-2456. 2009.

Doyle, M. P. Association of *Campylobacter jejuni* with laying hens and eggs. Appl. Environ. Microbiol. 47:533-536.1984.

European Food Safety Authority (EFSA). The community summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA J. 8(1):1496. pp 111-136. 2010a.

European Food Safety Authority (EFSA). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of Campylobacter and Salmonella on broiler carcasses in the EU, 2008. Part B. Analysis of factors associated with Campylobacter colonisation of broiler batches and with

Campylobacter contamination of broiler carcasses; and investigation of the culture method diagnostic characteristics used to analyse broiler carcass samples. EFSA J. 8(8): 1522. pp 1-132. 2010b.

Greig, J. D., and A. Ravel. Analysis of foodborne outbreak data reported internationally for source attribution. Int. J. Food Microbiol. 130:77-87. 2009.

Hanel, E. B., J. Muller, W. Muller, B. Pauly, E. M. Liebler-Tenorio, and F. Schulze. Genomic and phenotypic changes of *Campylobacter jejuni* strains after passage of the chicken gut. Vet. Microbiol. 136:121-129. 2009.

Hiett, K. L., G. R. Siragusa, N. A. Cox, R. J. Buhr, M. T. Musgrove, N. J. Stern, and J. L. Wilson. Genotype analyses of *Campylobacter* isolated from gastrointestinal tracts and the reproductive tracts of broiler breeder roosters. Avian Dis. 47:406-414. 2003.

Humphrey, T., S. O'Brien, and M. Madsen. *Campy lobacters* as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. 117:237-257. 2007.

Jacobs-Reitsma, W. F., N. M. Bolder and R. W. Mulder. Cecal carriage of *Campylobacter* and Salmonella in Dutch broiler flocks at slaughter; a one year study. Poult. Sci. 73:1260-1266.1994.

Jacobs-Reitsma, W. F., A. W. Van De Giessen, N. M. Bolder, and R. W. Mulder. Epidemiology *of Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114:413-421.1995.

Jacobs-Reitsma, W. F. Aspects of epidemiology of Campylobacter in poultry. Vet. Q. 19:113-117.1997.

Keener, K. M., M. P. Bashor, P. A. Curtis, B. W. Sheldon, and S. Katharious. Comprehensive review of *Campylobacter* and poultry processing. Comp. Rev. Food Sci. Food Saf. 3:105-116.2004.

Moore, J. E., D. Corcoran, J. S. Dooley, S. Fanning, B. Lucey, M. Matsuda, D. A. McDowell, F. Megraud, B. C. Millar, R. O'Mahony, L. O'Riorday, M. O'Rourke, J. R. Rao, P. J. Rooney, A. Sails, and P. Whyte. *Campylobacter.* Vet. Res. 36:351-382. 2005.

Nachamkin, I., X. H. Yang, and N. J. Stem. Role of *Campylobacter jejuni* flagella as colonization factors for three-day-old chicks: analysis with flagellar mutants. Appl. Environ. Microbiol. 59:1269-1273.1993.

Nadeau, E., S. Messier, and S. Quessy. Prevalence and comparison of genetic profiles of *Campylobacter* strains isolated from poultry and sporadic cases of campylobacteriosis in humans. J. Food Prot. 65:73-78. 2002.

Parkhill, J., B. W. Wren, K. Mungall, J. M. Ketley, C. Churcher, D. Basham, T. Chillingworth, R. M. Davies, T. Feltwell, S. Holroyd, K. Jagels, A.V. Karlyshev, S. Moule, M. J. Fallen, C. W. Penn, M. A. Quail, M. A. Rajandream K. M. Rutherford, A. H. van Vliet, S. Whitehead, and B. G. Barrell. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature 403:665-668. 2000.

Patrick, M. E., L.E. Christiansen, M. Waino, S. Ethelberg, and H. Madsen. Effects of climate on incidence of *Campylobacter* spp. in humans and prevalence in broiler flocks in Denmark. Appl. Environ. Microbiol. 70:7474-7480. 2004.

Petersen, L., and D. G. Newell. The ability of Ratyping schemes to discriminate between strains *of Campylobacter jejuni. J.* Appl. Microbiol. 91:217-224. 2001.

Petersen, L., E. M. Nielsen, and S. L. On. Serotype and genotype diversity and hatchery transmission of *Campylobacter jejuni* in commercial poultry flocks. Vet. Microbiol. 82:141-154. 2001.

Refregier-Petton, J., N. Rose, M. Denis, and G. Salvat. Risk factors for *Campylobacter* spp. contamination in French broiler chicken flocks at the end of the rearing period. Prevent. Vet. Med. 50:89-100. 2001.

Ridley A. M., M. J. Toszeghy, S. A. Cawthraw, T. M. Wassenaar, and D. G. Newell. Genetic instability is associated with changes in the colonization potential of *Campylobacter jejuni* in the avian intestine. J. Appl. Microbiol. 105:95-104. 2008.

Rivoal, K., C. Ragimbeau, G. Salvat, P. Colin, and G. Ermel. Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from free range broiler farms and comparison with isolates of various origins. Appl. Environ. Microbiol. 71:6216—6227. 2005.

Stojanov, I., D. Orlic, D. Stojanovic, R. Ratajac. Importance of *Campylobacter spp.* In laying hens. LUCR. §T. MED. VET. TIMI§OARA, XL. 177-181. 2007.

Sulonen, J., R. Karenlampi, U. Holma, and M. L. Hanninen. *Campylobacter* in Finnish organic laying hens in autum 2003 and spring 2004. Poult. Sci. 86:1223-1228. 2007.

Wilson, D. L., V. A. Rathinam, W. Qi, L. M .Wick, and J. Landgraf. Genetic diversity in *Campylobacter jejuni* is associated with differential colonization of broiler chickens and C57BL/6J ILlO-deficient mice. Microbiol.l56:2046-2057. 2010.

Wingstrand, A., J. Neimann, J. Engberg, E. M. Nielsen, P. Gemer-Smidt, H. C. Wegener, and K. Molbak. Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg. Infect. Dis., 12(2):280-284. 2006.

الملخص العربي

لقد وجد ميكروب الكامبيلوباكتر في وضع قطعان الدجاج . ولكن لا يعرف إلا القليل عن التنوع الوراثي لهذه السلالات .تم معزل ميكروب الكامبيلوباكتر في ١١ قطيع من الدجاج البياض في ١١مزرعه مختلفة ، وتم أخذ مسحات من ٣٠ الطيور التى تم اختيارها عشوائيا (٣٣٠ في المجموع) بين فبراير وسبتمبر من نفس العام ،٦ قطعان تم اخذ العينات خلال موسم الشتاء (فبرايرإلى أبريل) و٥ في ظل ظروف الصيف (يوليو إلى سبتمبر) . وقد تم فحص جميع العينات بواسطة المجهر والاختبارات البيوكيميائية وPCR . ثم بواسطة flaA باستخدام Ddel . تم العثور ميكروب الكامبيلولاكتر فى جميع قطعان فحصها. تبين أن ١٧٢ من أصل٣٣٠عينات الدجاج كانت إيجابية من المسحات . فى فترة الصيف كانت ٩٠٪ من العينات إيجابية في حين أنه في فترة الشتاء ٢٧٪ كانت إيجايبة فقط . زيادة عدد العينات الإيجابية وعدد من أنواع flaA مع عمر الدجاج . في قطيع ٢٠ أسابيع من العمر وقد وجدت نوع واحد flaA من ٨عزلات بينما تم الكشف عن ٩ أنواع من أصل ٢٧ flaA العزلات في قطيع عمره ٥٢ أسبوع . تم العثور على ٦١ نمط مختلف في ١٧٢ عزله . يبدو أن أنواع flaA أن يتدفق محددة لأن لا توجد أنواع متطابقة في قطعان مختلفة . سبب تنوع وحظ وتوزيع flaA أنواع يزال من غير الواضح لكن قد يكون سبب إما عن طريق الطفرات أو عن طريق انتقال الأفقي من مصادر خارج الحظيرة . هذا يوضح أيضا أن وضع قطيع دجاجة يمثل خزان مهم من مختلف مورثات لميكروب الكامبيلوباكتر ويمكن أيضا أن تستخدم هذا التنوع في أنواع flaA في دراسات وبانية لتحديد اله ممادر التي كانت تنبعث العزلات ميكروب الكامبيلوباكنر.