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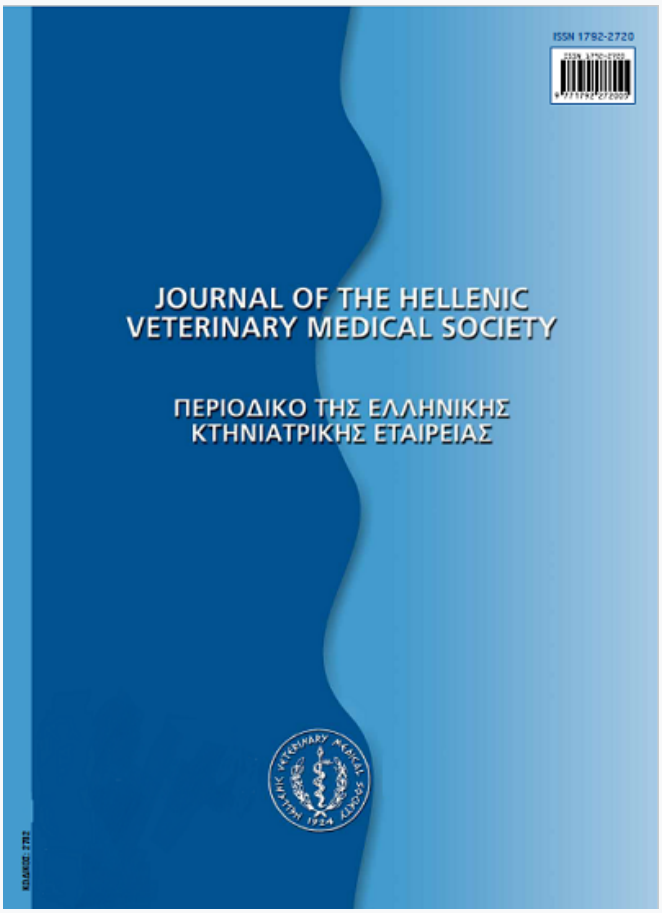

Campylobacter spp. infection in humans and poultry

G. NATSOS, K. C. KOUTOULIS, E. SOSSIDOU, M. CHEMALY, N. K. MOUTTOTOU

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Review article
Ανασκόπηση

Campylobacter spp. infection in humans and poultry

Natsos G.[1], Koutoulis K.C.[1], Sossidou E.[2], Chemaly M.[3,4], Mouttotou N.K.[5]

[1]Department of Poultry Diseases, Faculty of Veterinary Science, University of Thessaly, Karditsa

[2]Hellenic Agricultural Organization - Demeter, Veterinary Research Institute, Thessaloniki

[3]Anses, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, UHQPAP, Unité Hygiène et Qualité des produits avicoles et porcins BP 53, 22440 Ploufragan, France

[4]UEB: Université Européenne de Bretagne

[5]Ministry of Rural Development and Foods, National Reference Laboratory of Salmonella and Antimicrobial Resistance, Chalkida

Μόλυνση με Campylobacter spp. στους ανθρώπους και τα πτηνά

Νάτσος Γ.[1], Κουτουλής Κ.Χ.[1], Σωσσίδου Ε.[2], Chemaly M.[3,4], Μουττωτού Ν.Κ.[5]

[1]Κλινική Παθολογίας Πτηνών, Τμήμα Κτηνιατρικής, Σχολή Επιστημών Υγείας, Πανεπιστήμιο Θεσσαλίας

[2]Ελληνικός Γεωργικός Οργανισμός – Δήμητρα, Ινστιτούτο Κτηνιατρικών Ερευνών, Θεσσαλονίκη

[3]Anses, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, UHQPAP, Unité Hygiène et Qualité des produits avicoles et porcins BP 53, 22440 Ploufragan, France.

[4]UEB: Université Européenne de Bretagne

[5]Υπουργείο Αγροτικής Ανάπτυξης & Τροφίμων, Εθνικό Εργαστήριο Αναφοράς Σαλμονελλώσεων και Μικροβιοαντοχής, Χαλκίδα

ABSTRACT. Campylobacter is well recognized as the leading cause of bacterial foodborne diarrheal disease worldwide. The infection may be subclinical or cause disease of variable severity. The eating and handling of improperly cooked or raw broiler meat has been shown to be one of the most important sources of human campylobacteriosis. Birds carrying Campylobacter are asymptomatic colonizers without any clinical signs. Broilers are considered Campylobacter free after hatching and become colonized by exposure to viable bacteria from the environment. Several risk factors can result in the introduction of Campylobacter into the flocks making it difficult to keep chicken flocks free of Campylobacter throughout the rearing period. Lack of biosecurity measures, season, age, partial depopulation practices, flock size, type of production system, presence of other animals on farm, water quality, presence of rodents and mechanical transmission via insects are considered to be some of the risk factors associated with horizontal transmission. The control of

Corresponding author: Konstantinos C. Koutoulis,
Department of Avian Pathology, Faculty of Veterinary Science, University of Thessaly, Trikalon 224, 43100, Karditsa, kkoutoulis@vet.uth.gr

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Campylobacter in poultry seems crucial for the reduction of human campylobacteriosis cases. In Greece, there has been a dearth of information on prevalence and risk factors of Campylobacter in broiler flocks. Therefore, it is essential to initially investigate the prevalence of Campylobacter infection on farms and in poultry carcasses and subsequently the risk factors at all production stages of broiler meat and plan intervention studies to help reducing the disease in humans. This paper review the most recent data reported worldwide on Campylobacter infection in humans and poultry in order to provide an overview of trends, risks, possible causes and mechanisms of transmission routes.

Keywords: *broilers, Campylobacter, campylobacteriosis, foodborne pathogens, Greece, poultry, prevalence, risk factors*

ΠΕΡΙΛΗΨΗ. Το Campylobacter είναι παγκοσμίως αναγνωρισμένο ως η κύρια αιτία της διαρροϊκής, βακτηριακής αιτιολογίας, τροφοδηλητηρίασης. Η μόλυνση μπορεί αν είναι υποκλινικής μορφής ή να προκαλεί ασθένεια διαφορετικής σοβαρότητας. Η κατανάλωση και ο χειρισμός του πλημμελώς μαγειρεμένου ή ωμού ορνίθιου κρέατος έχει αποδειχθεί ως μία από τις πιο σημαντικές πηγές της ανθρώπινης καμπυλοβακτηρίωσης. Τα πτηνά που μεταφέρουν το Campylobacter είναι ασυμπτωματικοί φορείς, χωρίς κλινικά συμπτώματα. Τα ορνίθια κρεοπαραγωγής θεωρούνται ελεύθερα από Campylobacter μετά την εκκόλαψη και μολύνονται με την έκθεση τους στα βακτήρια από το περιβάλλον. Αρκετοί παράγοντες κινδύνου μπορούν να οδηγήσουν στην μόλυνση των σμηνών από Campylobacter, γεγονός που καθιστά πολύ δύσκολο να μείνουν τα σμήνη των πτηνών απαλλαγμένα καθ 'όλη τη διάρκεια της εκτροφής. Η έλλειψη μέτρων βιοασφάλειας, η εποχή, η ηλικία, πρακτικές αραίωσης του πληθυσμού, το μέγεθος του σμήνους, το είδος του συστήματος παραγωγής, η παρουσία άλλων ζώων στην εκμετάλλευση, η ποιότητα των υδάτων, η παρουσία τρωκτικών και η μηχανική μετάδοση μέσω εντόμων θεωρούνται μερικοί από τους παράγοντες κινδύνου που συνδέονται με την οριζόντια μετάδοση. Ο έλεγχος του Campylobacter στα πτηνά είναι πολύ σημαντικός για τη μείωση των περιστατικών της καμπυλοβακτηρίωσης στους ανθρώπους. Στην Ελλάδα, υπάρχει έλλειψη δεδομένων σχετικά με τον επιπολασμό και τους παράγοντες κινδύνου του Campylobacter στα σμήνη ορνιθίων κρεοπαραγωγής. Έτσι, είναι πολύ σημαντικό να διερευνηθεί αρχικά, ο επιπολασμός του Campylobacter στις πτηνοτροφικές εκμεταλλεύσεις και στα σφάγια πουλερικών και στη συνέχεια, οι παράγοντες κινδύνου σε όλα τα στάδια της παραγωγής του ορνίθιου κρέατος, έτσι ώστε να σχεδιαστούν μελέτες παρέμβασης που θα βοηθήσουν να μειωθούν τα περιστατικά της ασθένειας στον άνθρωπο. Η εργασία αυτή κάνει μια ανασκόπηση των πιο σύγχρονων δεδομένων που έχουν αναφερθεί παγκοσμίως για τη μόλυνση με Campylobacter στους ανθρώπους και τα πτηνά προκειμένου να προσφέρει μια επισκόπηση των τάσεων, των παραγόντων κινδύνου, τις πιθανές αιτίες και των μηχανισμών των οδών μετάδοσης.

Λέξεις ευρετηρίασης: *ορνίθια κρεοπαραγωγής, Campylobacter, καμπυλοβακτηρίωση, τροφιμογενή παθογόνα, Ελλάδα, πτηνά, επιπολασμός, παράγοντες κινδύνου*

INTRODUCTION

Human campylobacteriosis is considered an important public health problem and poultry has been identified as a significant source for human infections with *Campylobacter* species. Although thermophilic *Campylobacter* spp. are not significant pathogens for poultry, they are of importance to food safety and public health, with *C. jejuni* being responsible for the majority of human campylobacteriosis, followed by *C. coli*, and rarely by *C. lari* (Zhang and Sahin, 2013). Other *Campylobacter* species, such as *C. upsaliensis* and *C. fetus*, may also be associated with human diarrhea. Although the detection of non-*C. jejuni/coli* is uncommon in human cases in the industrialized world, it is more common in the developing world (Lastovica and Allos, 2008). This paper review the most recent data reported worldwide on *Campylobacter* infection in humans and poultry in order to provide an overview of trends, risks, possible causes and mechanisms of transmission routes.

CAMPYLOBACTERIOSIS AND PUBLIC HEALTH

Incidence, severity and costs

Since 1990's the incidence of human campylobacteriosis has been steadily rising worldwide (Baker et al., 2007; WHO, 2011; EFSA, 2014). This is in accordance with the Community Zoonoses Reports of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). In the EU, campylobacteriosis has been the most commonly reported zoonosis since 2005, followed by salmonellosis (EFSA, 2006; EFSA, 2014). Information submitted by 27 European Union Member States (EU MS) on the occurrence of zoonoses and food-borne outbreaks in 2012, showed that there were 214,268 confirmed human cases of campylobacteriosis (EFSA, 2014). Thus, the overall notification rate of human campylobacteriosis was 55.49 per 100,000 population (range: 0.39 - 174.08 per 100,000 population). There was a wide variation in incidences between countries which probably reflects differences in the healthcare and reporting systems, and in microbiological methods for the detection of *Campylobacter* (Olson et al., 2008; Vally et al., 2009; EFSA, 2014). Even though clinical cases of campylobacteriosis tended to be under-reported, "there may be not less than 2 million and possibly as high as 20 million cases of clinical campylobacteriosis per year in the 27 EU MS" (EFSA, 2010c). The number of confirmed cases of campylobacteriosis in the European Union has followed a statistically significant ($p < 0.001$) increasing trend in the last five years (2008-2012), along with a clear seasonal trend (summer months) (EFSA, 2014). Considering the high number of human campylobacteriosis cases, the severity in terms of reported fatalities was low (0.03%) (EFSA, 2014).

According to Scallan et al (2011) *Campylobacter* is the third-leading cause of bacterial foodborne illness in the United States. Information provided by the Foodborne Diseases Active Surveillance Network (FoodNet) of the Centers for Disease Control and Prevention (CDC), from 10 State Health Departments in the USA, indicated campylobacteriosis as the second most common infection (35%), following salmonellosis (40%). CDC also estimated that in 2012, the number of reported infections and incidence per 100,000 population by *Campylobacter* was 6,793 and 14.30, respectively (CDC, 2013). In the same report the estimated incidence of infection for *Campylobacter* showed a 14% increase in 2012, compared with 2006–2008. Also, in the USA, it is estimated that *Campylobacter* causes 2.5 million illnesses, 13,000 hospitalizations, and over 100 deaths each year (Patrick, 2007).

In Australia, *Campylobacter* is currently the most common cause of acute bacterial diarrhea among all the notified enteric pathogens with more than 15,000 cases each year (Stafford, 2010). The incidence of notified campylobacteriosis has steadily increased during the past 15 years from 67.0/100,000 population in 1991 to 121.4/100,000 in 2005 (Stafford, 2010). According to the same researcher, adjusting for under-reporting, there may be an estimation of 225,000 infections occurring each year in Australia, but most of which are sporadic in nature.

In many developing areas of the world, human campylobacteriosis is hyperendemic and the disease differs from campylobacteriosis in developed countries (Coker et al., 2002). In developing areas, campylobacteriosis is predominantly a pediatric problem affecting children under the age of five while adults are generally less prone to the disease (Oberhelman and Taylor, 2000; Coker et al., 2002). Generally, developing countries do not have national surveillance programs for campylobacteriosis; therefore, incidence values in terms of number of cases for a population do not exist (Coker et al., 2002). Most estimates of incidence came from laboratory-based surveillance of pathogens responsible for diarrhea. Oberhelman & Taylor (2000) estimated that *Campylobacter* isolation rates in developing countries ranged from 5 to 20%. In Asiatic countries like Thailand for example, the overall isolation rate of *Campylobacter* from diarrheal children under year 5 was 6.8% (Yang et al., 2008). This rate was 12.1% in Laos, with *C. jejuni* and *C. coli* occurring in 7.1% and 4% of enteric infection in children aged < 1 year and 1–5 years, respectively (Yamashiro et al., 1998).

There are no sufficient data on campylobacteriosis in Greece, because the disease is not under surveillance through Mandatory Notification System. According to Hellenic Center for Disease Control & Protection (HCDCP) factsheet of 2013, although there are few hospitals with laboratory ability of *Campylobacter* isolation, the number of positive cultures for this pathogen was high (623 positive cultures) in 2012, even

greater than the frequency of salmonellosis (HCDCP, 2013). These data indicate the need of integration of campylobacteriosis on the Mandatory Notification System in order to achieve full illustration of the morbidity caused by the microorganism in question and the need of setting a specialized reference laboratory. Nevertheless, there have been several references about *Campylobacter* spp. and its contribution on acute gastroenteritis among patient in Greek hospitals, especially children (Kafetzis et al., 2001; Maltezou et al., 2001; Chatzipanagiotou et al., 2002; Chatzipanagiotou et al., 2003a; Maraki et al., 2003; Ioannidis et al., 2006; Papavasileiou et al., 2007; Ioannidis et al., 2009; Maragkoudakis et al., 2010; Mellou et al., 2010; Mammas et al., 2012; Maraki et al., 2012; Ioannidis et al., 2013). Moreover, the first diagnosed *C. jejuni*-associated Guillain-Barré Syndrome case from Greece in 2003 reported by Chatzipanagiotou et al. (2003b).

Human infections with *Campylobacter* pathogenic strains are characterized by nausea, vomiting, stomachache, malaise, profuse watery diarrhea, blood in feces and high fever (Blaser et al., 2008). The infective dose of campylobacteriosis can be as low as few hundred cells (Black et al., 1988). In most cases the illness is self-limiting, but it may be severe and life threatening in susceptible people such as young children, the elderly, or people with immunosuppressive diseases, such as AIDS and cancer (EFSA, 2011). In cases where antibiotic treatment is needed, fluoroquinolones and erythromycin are considered the drugs of choice, but attention should be paid since a rapidly increasing proportion of *Campylobacter* strains all over the world have been found to be resistant to these antibiotics (Allos, 2001; EFSA 2013b). The incubation period is up to 10 days with typical symptoms related to enteritis, with diarrhea, cramps, abdominal pain and fever. In susceptible humans, *C. jejuni/coli* infection is associated with acute enteritis and abdominal pain lasting for up to seven days or longer (Allos, 2001). Infection is sometimes complicated by the development of serious post infection complications, such as bacteraemia, Guillain–Barré syndrome (GBS), reactive arthritis, inflammatory bowel disease, irritable bowel syndrome (Allos, 2001; Helms et al., 2003; Havelaar et al., 2005; Mangen et al., 2005; Smith and Bayles, 2007; Gradel et al., 2009; Haagsma et al., 2010) and even death (Havelaar et al., 2005; Gradel et al., 2008). GBS is an acute demyelinating disease of the peripheral nervous system resulting in temporary ascending flaccid paralysis (Allos, 2001). There are enough data on the incidence of GBS in Europe and North America (McGrogan et al., 2009; Sejvar et al., 2011). The disease has also been well studied in China, where it may implicate in outbreaks, and in Japan, whereas seasonal patterns of GBS have been described in Mexico, China, Argentina, Curacao, South Africa and other countries (Coker et al., 2002; WHO, 2013).

The socioeconomic costs of the disease in humans can be very high (Samuel et al., 2004) and this is expected, if one takes under consideration that there may be approximately nine million cases of human campylobacteriosis per year in the 27 EU MS (EFSA, 2011). The public health impact of campylobacteriosis and its sequelae is 0.35 million disability-adjusted life years (DALYs) per year and total annual costs are 2.4 billion euros (EFSA, 2011). These costs reflect to medical expenses, lost wages, product recalls, legal costs, and other indirect expenses (CAST, 1994). Havelaar et al. (2005), estimated that in the Netherlands (with approximately 80,000 cases of gastroenteritis per year), the costs of illness caused by campylobacteriosis are about 21 million euros / year.

Outbreaks of *Campylobacter* spp. - Sources and transmission of infection

Most campylobacteriosis cases are sporadic or small-scale family outbreaks (Olson et al. 2008). Even though outbreaks of *Campylobacter* infections are rarely reported, they might be more common than previously suspected (Gillespie et al., 2003; Miller et al., 2004; Fussing et al., 2007; Isohanni, 2013). Because the incubation period before the onset of symptoms can be long, it might be difficult to determine the source of infection. Numerous epidemiological studies have been conducted to identify potential sources for human campylobacteriosis. Most cases of outbreaks in the literature were associated with handling raw poultry, eating raw or undercooked poultry meat or cross-contamination of raw to cooked foods (Tauxe et al., 1997; Studahl and Andersson, 2000; Corry and Atabay, 2001; Nadeau et al., 2002; Kapperud et al., 2003; Neimann et al., 2003; Nielsen et al., 2006; Stafford et al., 2007; Doorduyn et al., 2010; EFSA, 2014). The consumption of chicken and chicken by-products has been increased due to their low price, special taste, and the short

time required for preparation and consequently they have been implicated over the recent years in a large number of outbreaks of acute campylobacteriosis in human populations worldwide, in both industrialized and developing countries, and especially in children, the elderly and immuno-suppressed patients (Skirrow, 1998; Corry and Atabay,2001). In particular, the handling, preparation and consumption of broiler meat accounted for 20% to 30% of campylobacteriosis cases, while 50% to 80% attributed to the chicken reservoir as a whole (EFSA, 2010c). Furthermore, broiler meat was the most commonly implicated food vehicle, accounting for 11of the 25 strong-evidence outbreaks (44.0%) (EFSA, 2014).

Other possible sources of campylobacteriosis include other contaminated food, contaminated water, direct contact with farm animals, environmental sources and foreign travel. According to EFSA's report for 2012, among 19 EU MSs a total of 501 foodborne *Campylobacter* outbreaks were reported and this counted for 9.3 of the total reported foodborne outbreaks in the EU (EFSA, 2014).

Besides broiler meat, contaminated livers constitute a notable source of human campylobacteriosis. Outbreaks of *Campylobacter* infections linked to chicken and duck liver pâté have been reported in the United Kingdom (O'Leary et al., 2009), Australia (Parry et al., 2012), Europe (EFSA, 2013a) and USA (Tompkins et al., 2013). In addition, since 2007, England and Wales have mentioned a significant increase in the proportion of *Campylobacter* outbreaks linked to the consumption of chicken livers used in pâté (Little et al., 2010). These outbreaks did not come as a surprise, given that previous studies had shown that 77% of retail chicken livers were contaminated with *Campylobacter* (Little et al., 2010).

Some researchers point out eggs as a possible route of transmission since fecal contamination of the shell may take place and the survival of *Campylobacter* on eggshell is being promoted by the shell's moisture (Cox et al., 2012). In a study conducted by Messelhäusser et al. (2011) viable bacteria of *Campylobacter* spp. were found in 4.1% of the eggshell samples, whereas Jones and Musgrove (2007) found 0.5% of the restricted shell eggs investigated positive for thermotolerant *Campylobacter* spp. In Japan, Sato and Sashihara (2010) found that between 27.9 and 36% of unpasteurized liquid egg samples were positive for *Campylobacter*. Therefore, a contaminated eggshell always creates the risk of cross-contaminating the egg yolk with pathogens and of initiating food-borne infections by producing ready-to-eat food with raw or undercooked egg content. The other possibility is cross-contamination from the eggshell to other ready-to-eat products which do not contain the egg content itself (Cox et al., 2012).

In addition to risks from food, contact with animals, either domestic pets or farm animals, presents another exposure pathway for human infection (Saeed et al., 1993; Schorr et al., 1994; Studahl and Andersson, 2000; Moore et al., 2005). Other foods (such as pork, beef and unpasteurized milk), or direct contact with these animals were mentioned in the literature as pathways to acquire Campylobacter infection (Moore et al., 2005; Jacobs-Reitsma et al., 2008). The digestive tract of healthy cattle can be a significant reservoir for a number of Campylobacter species, with a prevalence of the enteropathogen in cattle ranging from 0–80% (Atabay and Corry, 1998) whereas the prevalence of Campylobacter spp.in sheep was about 20% (Zweifel and Stephan, 2004). Pig carcasses have been shown to be more frequently contaminated than either beef or sheep (Nesbakken et al., 2003). This is most likely attributable to the fact that pig carcasses undergo a communal scalding stage early in the slaughtering process combined with the fact that the skin remains on the carcass following all of the dressing procedures (Moore et al., 2005).

Raw milk has also been identified as a vehicle of human gastroenteritis caused by ***Campylobacter*** spp. (Weltman et al., 2013; EFSA, 2014). Especially, ***C. jejuni was found to be*** present in milk due to faecal cross-contamination during milking or as a result of udder infection (Orr et al., 1995).

Waterborne outbreaks of *Campylobacter* have been reported in many developed countries (Allos, 2001; Martin et al., 2006; Jakopanec et al., 2008; EFSA, 2013a).

In Greece, a waterborne Campylobacter jejuni outbreak occurred in Crete in 2009. Most cases originated from rural areas, served by a different water-supply system from that of the adjacent town and there was strong epidemiological evidence that tap water was the vehicle of the outbreak (Karagiannis et al., 2010a, Karagiannis et al., 2010b). Consumption of untreated

water (Schorr et al., 1994) or rainwater (Eberhart-Phillips et al., 1997) was associated with campylobacteriosis in other studies. In an ecological study in Sweden, positive associations were found between the incidence of Campylobacter spp. and the average volume of water consumed per person. These observations suggested that drinking water and contamination from livestock might also be important factors in explaining at least a proportion of human sporadic campylobacteriosis cases (Nygard et al., 2004).

Contaminated shellfish have also been implicated as a vehicle in the dissemination of campylobacteriosis. Harvesting shellfish from *Campylobacter*-contaminated waters would appear to be the most likely cause of infection (Wilson & Moore, 1996).

Travel to a developing country is a risk factor for acquiring *Campylobacter*-associated diarrhea, which is more severe, and strains are more likely to be associated with antibiotic resistance (Coker et al., 2002). Campylobacteriosis acquired abroad contributes to the number of cases reported in developed countries and, as a result, represents an important subset of all cases. In the USA, 13% of *Campylobacter* infections are associated with international travel, and *Campylobacter* is the most frequently reported travel-associated infection (Kendall et al., 2012). In Scandinavia, the proportion of travel-related cases is higher, and systematic reporting of such infections has provided proxy surveillance information for parts of the world where diagnostic testing or reporting of the infection is less frequent (Ekdahl and Andersson, 2004).

CAMPYLOBACTER IN BROILER PRODUCTION

Broiler farms

Broiler intestines are a particularly favorable environment for the proliferation of thermophilic *Campylobacters*, such as *C. jejuni* and *C. coli*. Birds carrying *Campylobacter* are asymptomatic colonizers without any clinical signs (Lee & Newell, 2006). Broilers are considered *Campylobacter* free after hatching, since most evidence suggest that vertical transmission plays a minor role, if any (Jacobs-Reitsma et al., 1995; Pearson et al., 1996; Petersen & Wedderkopp, 2001; Sahin et al., 2003; Callicott et al., 2006) and in general, broiler flocks remain *Campylobacter* free for the first two weeks (Annan-Prah & Janc, 1988; Stern, 1992). Nevertheless, Cox et al. (2012) referred to trans-ovarian transmission since fecal bacteria, including *Campylobacter*, can contaminate the shell, shell membranes, and albumen of freshly laid eggs and the chick can become colonized after ingestion of the pathogen when it emerges from the egg. After the first colonization (usually at two to three weeks of age), following exposure to viable bacteria from the environment, *Campylobacter* spread quickly within the flock. The presence of *Campylobacter* in the caeca can be at a detectable level few hours after the exposure (Bull et al, 2006), while birds remain highly colonized until slaughter (Berndtson et al. 1996a, van Gerwe et al. 2009), representing an important public health risk.

The prevalence of *Campylobacter* in broiler flocks varies among different countries. A harmonized baseline survey was conducted in the EU in 2008, generating representative data regarding national production, in order to estimate the prevalence of *Campylobacter* in broilers and on broiler meat (EFSA, 2010a). Approximately 71.2% of broiler batches were estimated to be colonized by *Campylobacter* at the slaughterhouse. The prevalence of *Campylobacter*-colonized broiler batches among the EU member states varied widely, ranging from as low as 2.0% up to 96.8% (EFSA 2010a). The results of the EU baseline survey were consistent with several other studies (Rasschaert et al., 2007; Allen et al., 2008; Kuana et al., 2008; Hue et al., 2010; Hue et al., 2011; Lawes et al., 2012; Powell et al., 2012). In 2012, the overall proportion of *Campylobacter*-positive broiler flocks was 33.56 % (range: 0 % - 83.6 %) among the five MSs (e.g. Denmark, Germany, Hungary, Slovenia, Sweden) which reported flock-based data (EFSA, 2014). Several other flock-based studies have showed a prevalence from 15% up to 76% (Barrios et al., 2006; Arseunault et al., 2007a; Guerin et al., 2007; McDowell et al., 2008; Sasaki et al., 2010; Ansari-Lari et al., 2010)

Campylobacter jejuni is the predominant species isolated from poultry samples, followed by *C. coli*, with other *Campylobacter* species such as *C. lari* being less detected. In the southern EU MSs the presence of *C. coli* was more abundant, whereas *C. jejuni* was the only species isolated in the northern countries (EFSA, 2010a). Climatic conditions, environmental reservoirs, broiler housing and age of slaughter that vary significantly from northern to southern Europe

could partly explain the observed variation of the species distribution (EFSA, 2010a). In addition, *C. coli* is more frequently identified in older animals and particularly from organic systems (El-Shibiny et al., 2005). Some studies mention that *C. coli* is more commonly isolated from poultry in the developing world. Specifically, *C. coli* was the dominant *Campylobacter* species isolated from poultry in Nigeria and Thailand (Aboaba and Smith, 2005; Padungtod and Kaneene, 2005). Poultry flocks and individual chickens might be infected with different *Campylobacter* strains at the same time (Jacobs-Reitsma et al., 1995; Rivoal et al., 1999). Furthermore, mixed infections can result in new strains through the exchange of genetic material (Jacobs-Reitsma et al., 1995; De Boer et al., 2002; Hook et al., 2005).

There is a paucity of data about the prevalence of *Campylobacter* spp. in broiler flocks in our country since Greece did not participated in the European union-wide baseline survey carried out in 2008. The isolation, identification, and antimicrobial resistance of *Campylobacter* spp. from poultry farms and slaughter houses has been investigated and reported for the first time in Greece by Marinou et al (2013). The results of this study showed a low prevalence (16/830 (1.9%) fecal samples) of *Campylobacter* spp. in five poultry farms in a geographical region around Athens, with the predominance of *C. coli*. However, the need for a surveillance and monitoring system for the prevalence, risk factors and antimicrobial resistance of *Campylobacter* in poultry and other food animals is a requisite and more studies about this topic should be carried out.

The incidence and prevalence of *Campylobacter* in positive broiler flocks varies depending on geographical, farming and environmental conditions. Seasonality effects have been observed with a marked peak during summer months, much more noticeable in Northern Europe (Bouwknegt et al., 2004; Patrick et al., 2004; Hofshagen and Kruse, 2005; Hansson et al., 2007; van Asselt et al., 2008; Jore et al. 2010; Zoonosis Centre, 2012;) than in Southern Europe (Nylen et al., 2002). In contrast, some studies in the United Kingdom, USA, and Canada have reported no seasonal influence on *Campylobacter* prevalence (Humphrey et al., 1993; Gregory et al., 1997; Nadeau et al., 2002). Seasonality effects could be explained by environmental factors, which require further investigation, such as humidity, temperature and sunlight (Wallace et al., 1997; Arsenault et al., 2007a; Guerin et al., 2008). For instance, a warmer mean temperature and the moister climate during summertime provide conditions favoring environmental *Campylobacter* survival, as well as increase the amount of insects, wild birds and rodents, which act as mechanical vectors for the pathogen, around the broiler house (Hald et al. 2004, Rushton et al. 2009, Jore et al. 2010). Except of the abundance of flies, the increased ventilation because of higher temperatures during the summer has also been related to the seasonal variation (Hald et al., 2008). It has been also claimed, that in the Nordic countries, the cold winters contribute to the decrease of the *Campylobacter* environmental load.

Remarkably, the increase in human cases can sometimes occur previous to infections in chickens, suggesting that there might be a common risk factor responsible for the increase in *Campylobacter* cases. Flies can transmit *Campylobacter* to chickens and humans and they could partly explain the seasonality of human cases (Hald et al., 2004; Nichols, 2005; Ekdahl et al., 2005; Nelson et al., 2006; Guerin et al., 2008; Hald et al., 2008; Nichols, 2010).

Broiler slaughterhouses - Carcasses

The intestinal colonization of broilers with *Campylobacter* during rearing is responsible for the contamination of the carcasses and equipment with *Campylobacter* during slaughtering (Rosenquist et al. 2006, Reich et al. 2008; Silva et al., 2011). Food processing areas that constitute critical control points in poultry processing plants are usually scalding, defeathering and evisceration, since the carcass contamination occurs there by leakage of the contaminated faeces from the cloaca and visceral rupture of the ceca carrying a high *Campylobacter* load (Berrang et al., 2001; Stern & Robach, 2003; Takahashi et al., 2006; Boysen & Rosenquist, 2009; Silva et al., 2011). Automated defeathering represents a high risk practice since cloacal contents can cause contamination of the carcasses (Berrang et al., 2001). *Campylobacter* spp. remain in a liquid film on the skin and become entrapped in its cervices and channels which provides a favourable environment for cross contamination (Chantarapanont et al., 2003). Cross-contamination of *Campylobacter* strains between slaughtered flocks may also occure via contacts with contaminated surfaces of the slaughter facilities, processing water and air (Peyrat et al. 2008,

Perko-Mäkelä et al. 2009; Isohanni, 2013). Furthermore, the persistence and survival of *Campylobacter* spp. are fostered by a suitable microenvironment of the skin (Chantarapanont et al., 2003) and even under frozen conditions or storage at 4°C, *Campylobacter* spp. are able to persist in the carcass (Maziero and de Oliveira, 2010). Previous studies reported that growth on skin stored at room temperature in a controlled atmosphere package is possible, increasing the risk for consumers if contaminated chicken is not adequately stored or handled (Lee et al.,1998; Scherer et al.,2006). It has been found that carcasses from batches with *Campylobacter*-positive caeca have significantly higher quantitative loads than those from batches with negative ceca, which is in accordance with other studies, indicates that reduction in intestinal contamination could be a possible way to reduce the amount of bacteria on carcasses (EFSA, 2010a; Hue et al. 2011).

The average prevalence of *Campylobacter* contamination on broiler carcasses worldwide is reported to be in the range of 60-80% (Suzuki & Yamamoto, 2009; Isohanni, 2013). According to EFSA (2010a), the prevalence in the EU of *Campylobacter*-contaminated broiler carcasses, in 2008, was reported as 75.8% and varied from 4.9% to 100.0% among the EU MSs. That prevalence is higher than the respective prevalence for broiler batches, which come into accordance with the results of other studies (Hue et al., 2011; Powell et al., 2012; Chokboonmongkol et al., 2013), assuming that cross-contamination from positive batches to negative batches does occur during the slaughtering process and associated carcass preparation (Jørgensen et al. 2002; Johannessen et al. 2007; EFSA, 2010a; Hue O. et al, 2011) through contamination of the slaughterhouse environment (Johnsen et al 2006). The counts of *Campylobacter* bacteria on broiler carcasses varied widely also between countries, which might be due to differences in slaughterhouse hygiene and processing practices (Habib et al., 2008; Sampers et al., 2008; EFSA, 2010a). In general there was a tendency for high counts in countries with high *Campylobacter* prevalence. Low *Campylobacter* numbers on broiler carcasses may reflect effective pre-harvest production procedures, good slaughter hygiene, low within-flock prevalence or low cross-contamination of carcasses of a *Campylobacter*-negative batch from a previous positive batch (Johannessen et al. 2007). The elevated levels of *Campylobacter* can be recovered from the broiler carcasses and transmitted in the food chain during further processing (EFSA 2010a).

The distribution of *Campylobacter* species isolated from broiler carcasses varies among different countries. *Campylobacter jejuni* proved to be the predominant species at EU level, with about two-thirds of the total isolates being identified as *C. jejuni*, while approximately one-third was *C. coli*. Other *Campylobacter* species are less frequently identified (EFSA, 2010a). Still, the reverse situation was observed in some MSs reporting dominance of *C. coli* isolates. Moreover, a high proportion of *C. coli* in poultry meat has been reported from some other parts of the world (Meeyam et al., 2004; Padungtod et al., 2005; van Nierop et al., 2005; Suzuki & Yamamoto, 2009). In Greece, no information is available, since there is no surveillance and monitoring system. According with the study performed by Marinou et al. (2013), no *Campylobacter* was isolated from the cecal samples of the chicken carcasses.

Retail broiler meat products

Broiler meat is considered to be the main foodborne source of human campylobacteriosis. According to EFSA (2014), a large share of retail broiler meat remains contaminated with *Campylobacter*. In 2012, approximately 30% of the samples of poultry meat in retail were found to be positive in the 9 EU MSs reporting data on testing of single broiler samples, (range: 0 % - 80.6 %). The reported levels of *Campylobacter* in fresh broiler meat products at retail vary between log 1 to log 4 cfu/100 g (or a fillet) of meat, depending on the different studies and methodologies used (Jacobs-Reitsma et al., 2008). Studies report that *C. jejuni* was usually the dominant *Campylobacter* species isolated from retail broiler meat products worldwide, but the ratio of *C. coli* to *C. jejuni* varied between countries (Suzuki and Yamamoto, 2009). Limited studies have been published on the prevalence of *Campylobacter* in broiler meat at the Greek retail level. The presence of *Campylobacter* spp. in poultry meat, along with isolation, identification at species level and determination of the antibiotic resistance of the isolates has been investigated by Petridou and Zdragas (2009) in Northern Greece. The results of Petridou & Zdragas study showed that 73% of the samples were *Campylobacter* positive, while *Campylobacter jejuni* seemed to be the predominant species. Moreover, the prevalence

of *Campylobacter* spp. in raw broiler meat was investigated by Zisidis (2011) during the period from 2005 to 2010. The samples were collected from several slaughterhouses, poultry meat selling points and restaurants of Western Greece. The results showed that 28.7% of the samples were *Campylobacter* positive, with *C. jejuni* as predominant species and a remarkable decline of positive results was observed through the study from 50% in 2005 to 18.5% in 2010. However, there is still a need of more investigation in order to determine the true prevalence of *Campylobacter* spp. in our country.

Risk factors associated with *Campylobacter* spp. colonization in broiler flocks and broiler carcasses contamination

Several risk factors can result in the introduction of *Campylobacter* into the flocks making it difficult to keep chicken flocks free of *Campylobacter* throughout the rearing period. The possible sources and transmission routes of *Campylobacter* for poultry flocks have been investigated extensively, focusing on different parts of the production processes and practices. Most epidemiological studies have focused on the outcome being the flock becoming infected, not considering the within flock prevalence nor the amount of *Campylobacter* in the infected chickens. The outside environment has been suggested as the ultimate source of colonization for broiler flocks. In addition, many factors - such as adjacent broiler units or other animals, farm workers, drinking water, rodents, wild birds, flies and other insects - may have a role in transmitting *Campylobacter* to broiler flocks (Hald et al. 2004, Bull et al. 2006, Rushton et al. 2009).

The most important risk factors associated with horizontal transmission of *Campylobacter* spp. to broiler flocks and broiler carcass contamination during the slaughtering process are shown in *Table 1* and *Table 2* respectively.

Controlling of *Campylobacter* spp. *infection through active surveillance*

Burden of disease studies provide evidence that there is a need for control measures across all outcomes of campylobacteriosis while taking into consideration its underestimation (WHO, 2013). Nowadays, the implementation of effective controls to reduce the burden of disease in humans is considered a priority in many areas of the world. Consequently, the control of *Campylobacter* in poultry seems crucial for the reduction of human campylobacteriosis cases.

European Food Safety Authority has emphasized the importance and recommended the establishment of an active surveillance of campylobacteriosis in all MS, including efforts to determine the uncertain and unreported campylobacteriosis cases. In addition, storage and genotyping of human and putative reservoirs of isolates in all MS have also been recommended (EFSA, 2011). Thereafter, it would be important to identify the *Campylobacter* properties of virulence, survival characteristics and ecology (EFSA, 2011).

CONCLUDING REMARKS

In conclusion, the necessity to study the prevalence of the disease in the poultry population and identify the risk factors associated with this in Greece should be stressed. The cross sectional study which is currently being carried out in Greece, will give important information on prevalence of *Campylobacter* infection in poultry production and will be the foundation in understanding the epidemiology of the microorganism countrywide.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

Table 1. Risk factors with an increased association with Campylobacter spp. colonization in broiler flocks along with the corresponding references.

RISK FACTOR	REFERENCES
Season (summer months)	Bouwknegt et al., 2004; Barrios et al., 2006; Huneau-Salaün et al., 2007; Zweifel et al., 2008; McDowell et al., 2008; Ellis-Iversen et al., 2009; Jore et al., 2010; EFSA, 2010b; Lawes et al., 2012; Chowdhury et al., 2012a
Age of broilers	Berndtson et al., 1996b; Evans & Sayers, 2000; Bouwknegt et al., 2004; Barrios et al., 2006; McDowell et al., 2008; EFSA, 2010b; Ansari- Lari et al., 2011; Chowdhury et al., 2012a; Lawes et al., 2012; Sommer et al., 2013
Partial depopulation practices	Hald et al., 2000; Hald et al., 2001; Slader et al., 2002; Ellis-Iversen et al., 2009; Hannson et al., 2010; EFSA, 2010b; Lawes et al., 2012
Lack of biosecurity measures	Humphrey et al., 1993; Van de Giessen et al. 1996; Gibbens et al., 2001; Herman et al., 2003; Cardinale et al., 2004
Flock size	Berndtson et al., 1996b; Barrios et al., 2006; Guerin et al., 2007a; Nather et al., 2009
Human traffic and farm equipment	Berndtson et al., 1996b; Evans & Sayers, 2000; Hald et al., 2000; Cardinale et al., 2004; Ramabu et al., 2004; Hofshagen & Kruse, 2005
Other animals on the farm or very close to the farm	van de Giessen et al., 1996; Bouwknegt et al., 2004; Cardinale et al., 2004; Lyngstad et al., 2008; Ellis-Iversen et al., 2009; Hannson et al., 2010; Sommer et al., 2013
General farm hygiene	Hald et al., 2000; Evans & Sayers, 2000; McDowell et al., 2008; Hannson et al., 2010
Type of drinking system	Näther et al., 2009
Contaminated water	Pearson et al., 1993; Zimmer et al., 2003
Contaminated air from adjacent poultry houses	Berndtson et al., 1996a
Mechanical transmission via insects	Berndtson et al., 1996a; Refregier-Petton et al., 2001
Infected wild birds	Chuma et al., 2000; Craven et al., 2000
Health and welfare status	Bull et al., 2008
Presence of rodents	Gregory et al., 1997; Huneau-Salaün et al., 2007; McDowell et al., 2008; Sommer et al., 2013
Free-range & organic flocks	Näther et al., 2009

Table 2. Risk factors with an increased association with broiler carcass contamination along with the corresponding references.

RISK FACTOR	REFERENCES
Slaughter in summer months	EFSA, 2010b; Powell et al., 2012
Age of broilers	EFSA, 2010b
Previous thinning of the flock	Hue et al., 2010
Batch was not slaughtered first in the slaughter program	Hue et al., 2010
Temperature in evisceration room (oC)	Hue et al., 2010
Presence of dirty marks on eviscerated carcasses	Hue et al., 2010
Time (hour) of sampling during day	EFSA, 2010b
Campylobacter-colonization in the broiler batch	Arsenault et al., 2007b; EFSA, 2010b
Batches with higher standard deviation of carcass weight	Mahler et al., 2011

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