Universiteit Utrecht

MASTER THESIS

‘Envrironmental transmission routes of *Campylobacter*: filling the gap of unexplained human campylobacteriosis.’

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INTRODUCTION

Campylobacter Bacteria

The genus *Campylobacter* was first described in 1880 by Theodore Escherich [1]. Its name derived from the Greek word ‘campylos’, which means curved. These bacteria are Gram negative, slender, spiral curved rods having dimensions from 0.2μm to 0.8μm wide and from 0.5μm to 5μm long. Extremely rapid, darting, reciprocating motility can be seen with a phase contrast microscope, with comma-shaped, S, or gull wing–shaped cells. Motile, with either uni- or bi-polar fragella, the organisms have a characteristic appearance (see photo) and are oxidase-positive [2]. As *Campylobacter* cells begin to age, they become coccoid in shape [3]. Several investigations have shown an association between the transition from the spiral to coccoid morphology with a nonculturable state [3] [4] [5] [6]. However, recent studies, suggest no correlation between culturability and cell morphology [7] [8] [9].

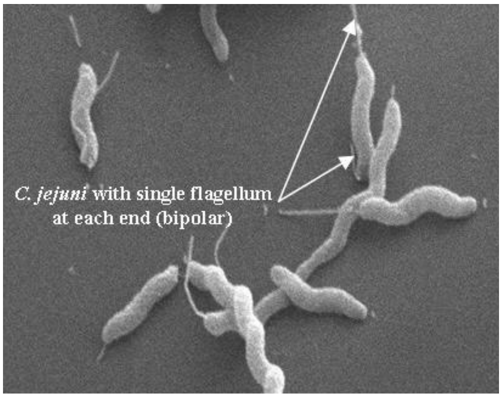

Figure 1: Campylobacter jejuni shape. [10]

There are many species and subspecies assigned to the genus *Campylobacter*, of which the most frequently reported are *C. jejuni* (subspecies jejuni) and *C. coli*. Most species prefer a micro-aerobic (containing 3-10% oxygen) atmosphere for growth. A few species tend to favour an anaerobic environment, although they will grow under micro-aerobic conditions also.

The genomes of several *Campylobacter* species have been sequenced, providing insights into their mechanisms of pathogenesis [11]. The first *Campylobacter* genome to be sequenced was *C. jejuni*, in 2000 [12]. *Campylobacter* species contain two flagellin genes in tandem for motility, *flaA and flaB*. These genes undergo intergenic recombination, further contributing to their virulence [13]. Non-motile mutants do not colonize.

Campylobacter is a fastidious organism which is capable to survive in various environments. It is a commensal organism routinely observed in cattle, sheep, swine, and poultry species and it has been found in rivers, estuarine, and coastal waters, at populations ranging from 10 to 230 colony-forming units (CFU)/100 mL [14] [15] . Poultry species are the most common host for *Campylobacter*, probably due to their high body temperature [16]. A study by Bolton and others [14] [15] investigated the effect of environmental temperatures over different seasons and nutrients on the survival of *Campylobacter jejuni* and they found a peak isolation during the late fall and winter months. Moreover, Willis and Murray [17] found *Campylobacter* to be at their highest populations on poultry during the warmer months (May through October). During these months, 87% to 97% of the samples tested were positive for *C. jejuni*. They also reported substantial variability in the intestinal colonization of *C. jejuni* across different broiler flocks at different ages in the production cycle.

Campylobacter jejuni and *Campylobacter coli* are commonly referred to as “thermophilic” campylobacters, being able to grow from 37 °C to 42 °C with an optimum growing temperature of 42 °C and a 60% to 62% relative humidity [18] . Additionally, it needs a pH range of 4.9 to 9.0 in order to survive, and it grows optimally at pH 6.5 to 7.5 [19] .*Campylobacter jejuni* is unusually sensitive to oxygen and dehydration, requiring a special atmosphere for survival, which usually consists of 5% oxygen, 10% carbon dioxide, and 85% nitrogen for growth in or on laboratory media [20] . Furthermore, scientists have demonstrated that several factors can influence the rate of inactivation of *Campylobacter*, including bacterial strain, temperature, humidity, and the suspension medium [21] and their results [22] suggest that *C. jejuni* is quite sensitive to drying and storage at room temperature, but at refrigeration temperatures and appropriate humidity, large numbers may survive and remain viable for several weeks.

Campylobacter Infection

Campylobacteriosis is an infectious disease caused by *Campylobacter* bacteria. First clinical symptoms including fever, headache, and myalgias last as long as 24 hours. The actual latent period is 2-5 days (sometimes 1-6 days), and it typically takes 1-2 days until actual symptoms develop, which are diarrhoea or dysentery, cramps, abdominal pain and fever as high as 40°C. In most people, the illness lasts for 2–10 days, symptoms are self-limiting and no use of antibiotics is required. *Campylobacter* organisms can be detected on gram stain of stool with high specificity and a sensitivity of ~60%, but are most often diagnosed by stool culture. Faecal leukocytes are present and indicate inflammatory diarrhoea [84].

Campylobacters are widely distributed and occur in most warm-blooded domestic, production and wild animals. They are prevalent in food animals such as poultry, cattle, pigs, sheep, ostriches and shellfish; and in pets, including cats and dogs. The main route of transmission is generally believed to be foodborne, via undercooked meats and meat products, as well as raw or contaminated milk [39]. The ingestion of contaminated water or ice is also a recognized source of infection. *Campylobacter jejuni* and *Campylobacter coli* account for the majority of human infections [23]. Public health risks related to the important pathogenic microorganisms *Campylobacter* are associated with the consumption of poultry meat.

Public Health Impact

In most industrialized countries, the reported incidence of campylobacteriosis has increased during the last decade. In 2004, a total of 183,961 cases of confirmed campylobacteriosis were recorded in the EU. The overall incidence was 47.6 per 100,000 populations, which is slightly higher than for *Salmonella* and this makes *Campylobacter* the most commonly reported gastrointestinal bacterial pathogen in humans in the EU [24]. In 1984, the sentinel laboratories network recorded only just 1,703 cases of infection. During the '90s, campylobacteriosis incidence has continually increased to reach 7,473 cases in 2000, although the increase in the number of *Campylobacter* infections cases until 1996 could mainly be attributed to problems at the surveillance level [25]. From 2000 to 2003, the illness incidence was reduced. It is usually estimated that 90% of *Campylobacter* contamination are due to meat consumption and 80% specifically come from poultry meat [85]. Nevertheless, the rise of *Campylobacter* incidence observed for more than 20 years may also be partly due to an increase of the poultry meat consumption during this period, rather than only an increase in the proportion of contaminated poultry [26]. The high incidence of *Campylobacter,* its duration and possible sequelae, make campylobacteriosis important from a socio-economic perspective.

Economic and Social Importance

Campylobacter affects each year a significant number of humans worldwide. Besides the discomfort felt by sick people, this infection has major economic repercussions by direct illness costs, such as laboratory diagnosis, consultations, medical cares, hospitalization and indirect costs, such as work inefficacy, days lost work [26] [27]. In The Netherlands, the economic costs of campylobacteriosis are estimated at 21 million euros per year for a population of 16 million [28].

Aim of the study

The illness caused by *Campylobacter* contamination is clearly a major issue in the food system worldwide. Several epidemiological studies, regardless of methodology, indicate the consumption of poultry meat the major source of campylobacteriosis. Because such a large majority of contamination is associated with poultry, approximately 50% to 70%, it is important to focus on this vehicle.

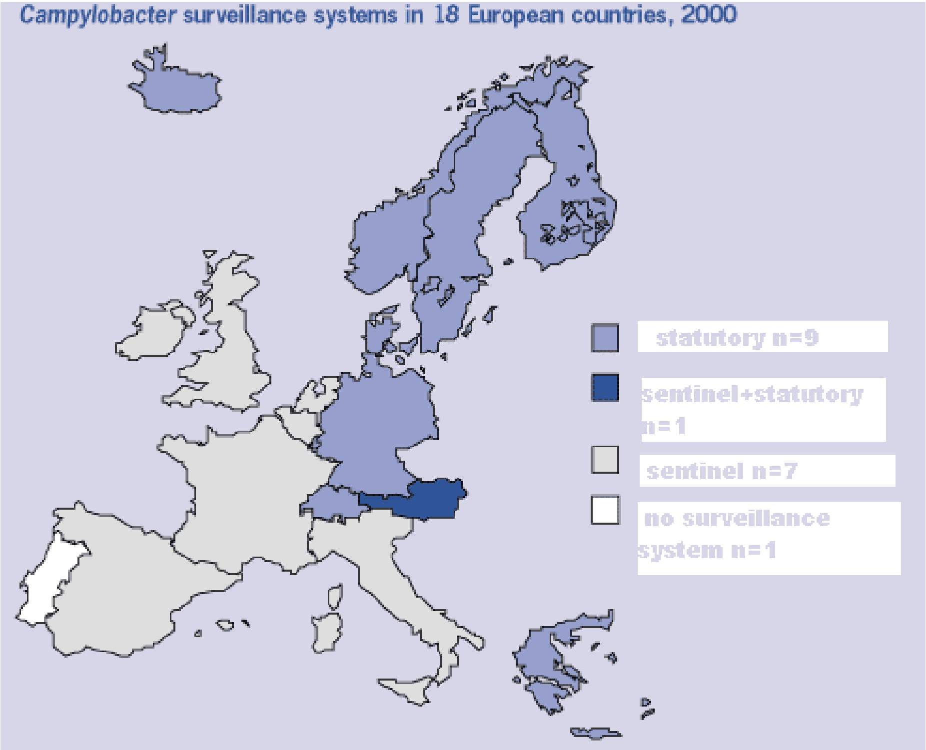

Figure 2: Camplylobacter surveillance systems in 18 European Countries in 2000.

LITERATURE REVIEW

Epidemiological Studies

Many studies have shown that *Campylobacter* bacteria and its transmission in the environment are strongly related to broiler houses and current trends indicate that there are many factors contribute to *Campylobacter* contamination in poultry processing plants. Significant *Campylobacter* populations can be found on a majority of birds entering the processing plant, and since the bacteria enters the plant at such high levels, it can easily be spread. All the existing data suggests that environmental contamination from poultry production at the farm level, even from closed houses, can be widespread. However, contamination constitutes not only a potential risk for farm staff and visitors, but may also constitute a risk to the general public entering the environment.

The problem of *Campylobacter* contamination of food must be addressed. According to a Newell and Wagenaar (2000) [29] study, no practical or effective control measures have been available. At the consumer level, accidental ingestion of 1 drop of raw chicken juice can easily constitute an infectious dose, which is as little as 500 organisms [1] [29] . Furthermore, differences in the survival of *Campylobacter* in the various environments, strain virulence and colonisation potential and the susceptibility of humans will also contribute to variations in the risk of disease.

Contamination in the processing plant

The available data on *Campylobacter* populations in poultry production is mainly from studies on chickens. In a typical broiler processing operation, freshly laid fertile eggs are collected and incubated at a hatchery. After they hatch, the chicks are delivered to farms where they are reared until they are ready for slaughter, and then transported to a processing plant. Various strategies have been advanced in order to reduce the incidence rates of poultry contamination, such as the introduction of competing microbial populations into newly hatched chicks or chlorination of poultry drinking water.

To analyze it further, at the processing plant (Figure 2), birds are unloaded, shackled, killed, scalded, defeathered, eviscerated, washed, cooled, and packaged.

Figure 3: Poultry processing plant.

Scalding

During the scalding procedure, feather follicles are open in order to facilitate the removal of feathers. Chicken skin has been shown to harbour and support the survival of *Campylobacter*. [30] The potential risk for *Campylobacter* contamination during scalding is well recognized. [31] A study by Cason and others (1999) [32] reported the microbiological effect of removing feathers from the carcasses while they are out of the scald water and data showed no reduction in *Campylobacter* population on carcasses during scalding and defeathering. Moreover, Berrang and Dickens (2000) [33] studied the presence and level of *Campylobacter* on broiler carcasses throughout the processing plant and they resulted the highest to be when carcasses were sampled pre-scald.

Defeathering

According to Berrang and Dickens (2000) [33] found that the *Campylobacter* counts increased significantly after defeathering procedure. A previous study by Wempe and others (1983) [34] had showed that the water used in rinsing the birds in the feather picker physically removed the *Campylobacter* bacteria and thus reduced the number of organisms on the edible parts. They recovered the bacteria from all recycled water samples tested. The use of recycled water to clean the gutters may further contaminate the receiving room with *Campylobacter* while further distribution of these bacteria may also occur through movement of plant personnel from the one area to other areas of the plant.

Evisceration

As it was mentioned above, chicken skin has been shown to harbour and support the survival of *Campylobacter.* [30] Many studies have reported a high incidence of contaminated neck flaps and breast tissue, which suggest that the crop contents might be an important source of *Campylobacter* contamination during processing.

Carcass washing

Carcass washing has been allowed for poultry since 1978 as an alternative to knife trimming because studies have shown it to be equally effective in removing faecal contamination. [35]. Limited studies have been conducted on evaluating the performance and effectiveness of poultry washers and sanitizing treatments within the processing plant.

There are numerous parameters which can affect the overall effectiveness of the carcass washing system, including number of washers and types and operating parameters such as wash water temperature, water pressure, flow rate, line speed ect. However, further design modifications of carcass washers are needed to reduce water consumption, provide scrubbing action, and introduce surfactants during the washing process that can lower water surface tension and aid in bacteria and faecal removal.

Carcass chilling

As the final part of processing plant, poultry carcasses are required to be cooled rapidly to prevent bacterial growth. [36] In many plants, water chillers are used for rapid cooling of carcasses. Recent studies on *Campylobacter* corroborate its potential for cross-contamination in water chillers. [37] [38] Additionally, Sanchez and others (2002) [37] showed that *Campylobacter* levels on chilled carcasses were significantly higher in immersion chilling than air chilling.

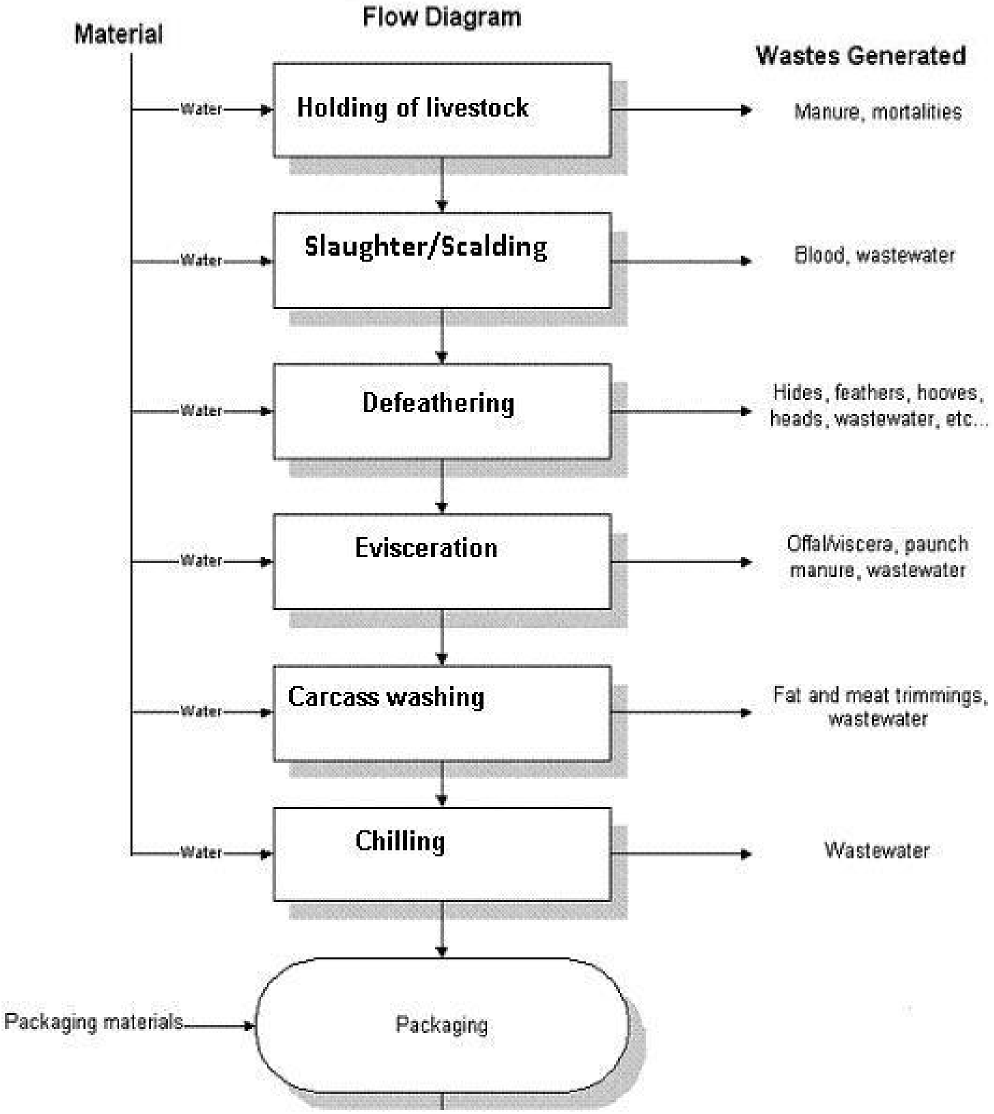

Figure 4: Flow diagram.

Potential Transmission Routes

Transmission Vectors

Campylobacter, as *Salmonella*, may be carried asymptomatically, as commensal organism, in the alimentary tract of all warm-blood animals. *Campylobacter* is considered as zoonotic bacteria due to its transmission, from animals to man [39]. Human can be infected by direct contact with contaminated animals or animal carcasses. In regard with domesticated animals as cattle, sheep, goats, pigs and especially poultry, pathogens can spread via the slaughter process to raw and finished products. *Campylobacter* may also be transferred to humans by consumption of undercooked or recontaminated meat, or the handling of raw products [40]. However, meat does not represent the only food vehicle for *Campylobacter* and large campylobacteriosis outbreaks are usually associated with contaminated drinking water or raw or contaminated milk [41]. Birds, especially breeding poultry, appear to be the main reservoir for these pathogens, because their internal temperature, 41-42°C, is favourable for thermophilic *Campylobacter* proliferation [42] and foods of poultry origin have also been identified as significant sources.

Namely, in Belgium, more than the 40% of infection cases would be associated to poultry meat consumption [43]. In 1999, the Belgian authorities, in the face of dioxin-contaminated meat, withdraw all poultry meat and eggs from the market. The estimated reduction in *Campylobacter* infection cases during the following months was 40% without any other explicative reason. Besides, the Belgian poultry reintroduction 4 weeks later on the market lead back to the previous campylobacteriosis incidence situation. Moreover, another factor that could link together chicken consumption and human pathogen acquisition is the important similarity between human and poultry serotypes [44]. Multiple studies have shown that some *Campylobacter* strains colonizing chicken are not human pathogens while some human isolated strains are unable to colonize poultry [45].

Poultry Colonisation

Colonized chickens usually show no observable clinical symptoms of infection even when, under experimental conditions, young animals are exposed to high doses [46]. Many experiments have shown that the dose of viable *Campylobacter jejuni* required to colonize chicks and chickens can be as low as 40 CFU (Colony Forming Unit) even if numbers from 10^4 to 10^7 CFU can be frequently found in literature [47] [48] . Infection pattern in poultry is also age-dependent. More specific, *Campylobacter* is rarely detected in chicks less than 2 to 3 weeks of age under commercial broiler production conditions, and that may be related to high levels of circulating *Campylobacter*-specific maternal antibodies in young chickens, which gradually decrease to undetectable levels at 2 to 3 weeks of age [49]. In chickens, *C. jejuni* colonizes the mucus overlying the epithelial cells primarily in the cæca and the small intestine but may also be recovered from elsewhere in the gut and from spleen and liver [50] [51] . The microorganism remains in the intestinal lumen at the crypts level, without adhesion. Once colonization is established, *Campylobacter* can rapidly reach extremely high numbers in the cæcal contents, from 10^5 to 10^9 CFU/g of content [51] [52] [53].

Survival in Transmission Routes

Flock-to-flock transmission

Campylobacter is sensitive to detergents and disinfectants .Dry and aerobic conditions of clean fresh litter are considered harmful to *C. jejuni* as reported by Newell et al. (2003) [46] and Hutchinson et al. (2005) [54]. However, litter can be contaminated by broiler fæcal droppings which favour the bacteria's transmission through the flock. Dirty contaminated litter spread over the land can scatter the microorganism in the environment. Contaminated sewage is attractive for wild birds and insects that can be infected and then become *Campylobacter* vectors. [55] [56]

Surrounding Environment

The influence of the open-air range on the contamination is not yet fully understood and described. However, *Campylobacter* is able to survive in the house surroundings soil and farmers can therefore act as pathogen vectors for *Campylobacter* entrance in the broiler house. [46] [57] The open-air range to which broilers have access in free range poultry production could also be a major environmental source for flock contamination. When *Campylobacter* is isolated from the open-air range soil or from stagnant water, before the birds go out, the precedent flock may be responsible for the contamination.

Survival in Faeces and Slurry

The understanding of survival of *Campylobacter* in faeces is crucial, since this is the link between the reservoirs and the transmission routes. Faeces indicate the ultimate source of this organism, whether it reaches humans via food, water or any other route. However, little information is known in regard with this aspect of the organism's survival. It has been reported that *Campylobacter* were present in sheep faeces at levels between from 35,000 to 56,000/g and could be isolated from the faeces for 3-4 days when stored outside at ambient temperatures. [58] Furthermore, experiments with *Campylobacter*-positive human faeces stored at 4°C, shown that 10 from 20 samples were positive for *Campylobacter* after 24 hours, 8 were positive after 2-7 days storage, and 2 were positive after 12 to 20 days storage. [59]

Water

Concerning the survival of *Campylobacter,* water can easily be contaminated by fæcal droppings during the rearing period and can serve as transmission route. [57] Moreover, a study by Shanker et al. (1990) [60], shown that water can be a real contamination vector for broiler chickens. *Campylobacter*'s transmission in water will be further analyzed in a separate passage below.

Air

Campylobacter can be isolated from air, either from the broiler house or from the surrounding houses. [57] The bacteria are entrapped in aerosols or dust. [61] Nevertheless, there is an assumption that *C. jejuni* cannot survive for long period within the dehydrating conditions of dust and according to Newell et al. (2003) [46] , the location of ventilation fans can affect the risk of flock positivity, and the use of air conditioning increased this risk.

Sediment

It has been reported in a study by Obiri-Danso and Jones (1999) [62] that the *Campylobacter* present in sediments did not follow a seasonal trend, as the bacteria could be isolated from the sediments at all times of the year. This suggests either that there is a continuous input from agricultural or other sources, or that *Campylobacter* survive for longer periods in sediments compared to water.

Campylobacter in water

As it was mentioned above, water can easily be defiled and hence it can be a real contamination vector. Speaking of water, it is necessary to segregate the different sources through which campylobacter can carry out a threat.

Fresh Water

Streams, rivers, groundwater, ponds, lakes, canals and reservoirs are among the possible fresh water routes. Canals and groundwater are widely considered to be microbiologically clean. Canals contain low number of *Campylobacter* and their density peaks in the winter period [63] whereas the first culturable evidence of Campylobacter occurrence in groundwater was provided by Stanley et al [64] though it was not considered as a rich source of bacteria.

On the contrary, streams, ponds, rivers and lakes are deemed to be the richest *Campylobacter* origins [55]. The presence of bacteria in streams varies with location, season and agricultural practice. Many studies of streams have shown that *Campylobacter* are absent from streams running through upland moors but present in the same streams running through lowland [63] [65] while the composition of the bacteria population dependents on the path of the stream [62]. Additionally, C*ampylobacter* are omnipresent in rivers, ponds and lakes, mostly in those which are exposed to agricultural run-off and effluent from water treatment plants [15] [62] [65] [66] [67] .A study of rivers and lakes in the Warsaw, by Popowski et al [67], showed that 70% of water samples were positive for *Campylobacter* with C. jejuni making up 65% of the isolates and municipal sewage was defined to be the main source, with minor inputs from the droppings of wild animals. Regarding the reservoirs, there are few studies from The Netherlands and the UK which have shown that *Campylobacter,* originating from birds, were detected in large numbers in pristine reservoirs only in winter months [7] [63] .

Marine

Coastal and bathing waters, as well as estuaries have been studied referring to *Campylobacter*'s transmission. Many studies have shown that such bacteria are prevalent in coastal and bathing water [63] [68]. Namely, the bacteria are widespread in coastal waters, and they are more numerous in winter compared to summer months, while in bathing period the coastal and bathing water show a diurnal variation with the highest amount of *Campylobacter* in early morning [68] .Furthermore, Jones et al. [63] have shown that the presence of *Campylobacter* in bathing and coastal waters was due to sewage effluent.

Regarding the embouchure of the rivers and the *Campylobacter* presence and transmission via them, estuaries follow the same seasonal template as the coastal and bathing waters. Moreover, it had been assumed that the main possible sources of the bacteria for the estuaries were various streams and contaminated overflows, the fresh water section of rivers, Waste Water Treatment (WWT) or sheep and cattle grazing stocks [69].

Sewage

Many studies have shown that *Campylobacter* are ubiquitous in sewage. The major sources were considered to be the wastes from humans and animals treatment plants [63] [70] 71] [72] .In these studies, samples from different sewage treatment plants were analyzed and it was resulted that *Campylobacter* numbers were related to the presence of animal effluent from abattoirs and poultry processing [63] .Furthermore, *Campylobacter* in sewage showed a seasonal variation, scilicet a large peak in the end of May and June and a small one in September and October [73]. These results led to the presumption that annual peaks in human infections are strongly associated with increased amounts of the bacteria in the environment, which are in turn determined by changes in the numbers of Campylobacter within livestock, poultry and wild animals [73].

To sum up, these observations confirm the generally held view that agricultural slurries, manure and municipal sewage following land disposal, or unintentional leakage from sewers, farm stockpiles or slurry holding pits, are the major sources of water contamination.

Control Strategies

The control of *Campylobacter* bacteria in the food chain is efficacious when the poultry colonisation can be prevented. By reducing the prevalence of *Campylobacter* infection in the first step, a high number of the bacteria cab be decreased in the following steps. It is necessary to identify the risk factors for the *Campylobacter* import, thus specific and effective strategies must be applied.

Generally, an all-in, all-out policy regarding the movement of stock into and out of houses and preferably, into and out of individual sites, should be practiced. In all cases, the poultry houses should be sound in structure and capable of being cleaned and disinfected and all entrances into poultry houses should have a well drained, concreted surround. Furthermore, all animal waste, dead birds and unused feed and leftovers should be removed from houses and be disposed of hygienically. Finally, in cases where the flock has been found to have been infected with *Campylobacter*, the houses should be examined using several means, in order to assess the efficacy of the cleaning and disinfection procedures followed. However, the action plan recommends the development of the control measures. Below, the most important strategies are briefly described.

Hygiene

Practical biosecurity measures at the farm level have been determined as the primary strategy to prevent colonisation of flocks with *Campylobacter* entering the processing plant and consequently the food chain. [26] [74] [75] [76] However, many studies have shown that biosecurity measures are partly effective in controlling *Campylobacter* bacteria contamination. [77] [78] [79] Strategies that are important to protect the poultry, include the house cleaning, the washing of hands, the wearing of protective clothing and footwear and generally the respect of disinfection protocols. This hygiene measures may be difficult to be applied, when broiler houses are confronted with environmental factors that are barely controllable such as open-air range and domesticated animals' faeces. Nevertheless, even if biosecurity measures can not guarantee infection prevention, they can help to delay or to reduce the *Campylobacter* colonization.

Antibiotics use

The use of antibiotics is a modern strategy nowadays. However, it could not be a solution for prevention and reduction of *Campylobacter* incidence. Many studies have shown association between the veterinary use of antibiotics and the emergence of resistant strains of *Campylobacter* related to human enteritis. [80] [81] [82]

Vaccination

To date, there is no commercially available vaccination against *Campylobacter* in poultry. Moreover, the development of such vaccines is blocked up by serious problems such as the lack of knowledge of antigens which induce a protective immune response.

Acidification

It is generally acknowledged that *Campylobacter* is sensitive to acid conditions. [83] Several strategies developed to reduce *Campylobacter* populations are based on the acidification of the pathogen environment.

DISCUSSION

Campylobacter bacteria, first identified as a human diarrhoeal pathogen in 1973, are one of the main recognized causes of human acute gastroenteritis throughout the world. The serious effects on humans causing campylobacteriosis, in relation to the economic cost, lead these bacteria to be considered as one of a high public health concern. The consumption of poultry meat and cross-contamination of other foods with drippings from raw poultry are leading risk factors for human campylobacteriosis.

In order to reduce the exposure of humans to *Campylobacter*, an integrated approach, including control measures implemented altogether the poultry production chain, has been investigated. At the primary production level, bio-security measures are only partly effective and are banned in the E.U. since 2006. Furthermore, different control strategies have been improved in recent years including farming techniques and several methods during poultry processing. However, these strategies still require significant improvements to completely remove or significantly reduce the threat of *Campylobacter* contamination.

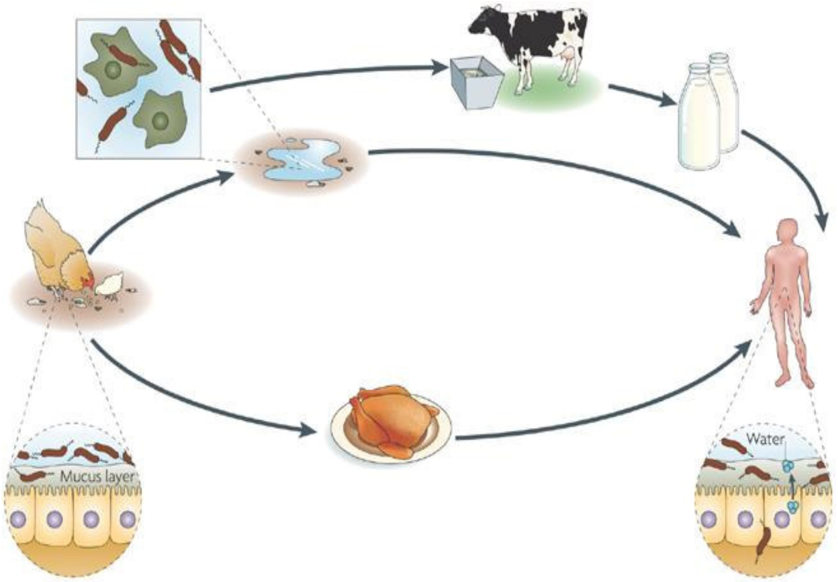

Figure 5 : *Campylobacter* cycle.

To date, a majority of poultry processing plants does not measure Campylobacter levels regularly and bacteria can be found on a generality of chickens entering the processing plant. This contamination is easily spread from carcass to carcass during processing. The key to prevention is the identification of the aetiological fraction for each of the known sources. Further effort is needed to design more efficient and effective systems and to reduce the levels of contamination below the threat to public health since as few as 500 organisms can make a person ill.

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