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Phytotherapy and Prevention of Campylobacter

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Abstract

Since 2005, campylobacteriosis become the most important gastrointestinal infectious disease in Europe. The disease affects especially infants under 4 years of age, causing primarily gastroenteric symptoms but also responsible of different extra intestinal pathologies. The most frequent way of infection is considered to be related to contaminated poultry meat consumption. Prevention relies on general hygienic measures. Of outmost importance is the reduction of bacterial burden in raw meat, to ensure a radical decrease of clinical forms. Phytotherapy might represent a sustainable prevention strategy for the achievement of such objective.

Keywords: *Campylobacter*; Infectious disease; Phytotherapy; Prevention

In Europe the infection caused by thermo tolerant *Campylobacter* spp. in man is in constant increase. Since 2005, the disease represents the most common reported infectious gastrointestinal pathology, with more cases than those caused by *Salmonella* spp. [1]. According to the European Center for Disease Prevention & Control (ECDC) surveillance report 2011 based on data from the European Surveillance System (TESSy), campylobacteriosis in Europe accounted for 178,000 cases in 2006 and 202,000 cases (53.07 per 100,000) in 2009. In 2012, 212,000 confirmed cases have been notified in Europe. More than 60,000 cases have been reported in Germany, Hungary and United Kingdom. In Switzerland campylobacteriosis is recognized the most frequent bacterial zoonosis. In 2009, notified cases were above 8,000 (100.1 per 100,000) [2]. This induced the authorities to undertake an active monitoring plan, named *Campylobacter* platform. Also in the United States campylobacteriosis in humans is among the most common causes of food borne illness. Active surveillance through the Food borne Diseases Active Surveillance Network (Food Net) indicates that about 14 cases are diagnosed each year for each 100,000 persons in the population (6.033 notified cases in 2009). Many more cases go undiagnosed or unreported to public health authorities, and campylobacteriosis is estimated to affect over 1.3 million persons every year [3]. The disease shows a seasonal distribution, with the majority of the cases during summer months. *Campylobacter jejuni* and *C. coli* are the species mainly isolated in man. Most frequently reported *Campylobacter* species in 2009 was *C. jejuni* (36.4%), *C. coli* (2.5%), *C. lari* (0.19%) and *C. upsaliensis* (0.01%). The other confirmed cases (51%) could not be characterized at species level or the species were unknown. Many domestic and wild animal species, primarily avian species, are natural reservoirs. Transmission to humans occurs through contact with animals and their products, such as avian meat and raw milk, consumption of contaminated meat not sufficiently cooked. Often, contaminations occur indirectly in the kitchen through stoves or other kitchen ware utilized first for raw meat and after for other food. Symptoms in man are primarily gastroenteric. A limited number of bacteria are sufficient to cause violent abdominal pain and diarrhea. Both *C. jejuni* and *C. coli* may provoque diarrhea in any category of age. However, the disease affects especially infants under 4 years of age (144.34 per 100,000 in 2009) [1]. In particular *C. jejuni* can cause also extra intestinal forms: bacteremia, meningitis, peritonitis, pancreatitis, cholecystitis, urinary infections, neonatal sepsis, abortion, endocarditis, osteomyelitis, septic trombophlebitis, septic arthritis, immunomediate chronic forms and nodous eritema. *C. jejuni* is also suspected in the etiopathogenesis of the post infective neurological Guillain Barré syndrome and of the rare variant Miller-Fisher syndrome. The similarity between bacterial lipopolysaccarids and gangliosides might be at the origin of an auto-immune reaction [4-6]. The case-fatality rate for *Campylobacter* infection is generally 0.05 per 1000 infections [4]. However, infection related mortality may also be not negligible. In the Netherlands, in 2008, out of 3,340 confirmed cases, 45 patients died, and in 2010 deaths were 58 out of 4,322 cases [7].

Generally, animals are asymptomatic carriers of *Campylobacter* spp. In the framework of the Swiss *Campylobacter* platform, poultry samples showed 44% positivity, with mainly *C. jejuni* strains. In pigs the 67% was positive. Almost the totalities of the isolated strains were *C. coli*. In calves only the 1% resulted positive to both *C. jejuni* and *C. coli* [2]. *Campylobacter* is often detected in poultry meat [8]. High percentages of contamination have been reported in the United Kingdom (71%) [9] and in Italy (81.3%) [10]. In wild animals *C. jejuni* have been associated also to different pathologies: abortion, colitis with severe diarrhea and death in mink (*Mustela vison*), severe diarrhea in raccoon (*Procyon lotor*), diarrhea in primates, enteritis and epatitis in ostrich (*Struthio camelus*). Prevalence of *C. jejuni* in wild birds was reported in USA in 6 avian families (7.2%), mainly in crows (*Corvidae*) (23%) and gulls (*Laridae*) (25%) [11]. In Italy was reported a positiveness of 38.8% [12]. Occurrence of *Campylobacter*-related gastroenteritis was reported in members of different animal orders: among mammals Artiodactyla 15%, and among birds Galliformes 15%, Anseriformes 30%, Ciconiformes 34% and Gruiformes 44% [13]. Due to the rising importance of the infection, based on the Commission Decision 516 2007/EC [14], the European Commission financed the first surveillance program in avian zootechnics, through sampling at slaughterhouses and verification of antibiotic resistance. This funding allowed to undertake detailed epidemiological studies. A survey conducted in 9 Italian regions on *Campylobacter* in avian meat in 2008 revealed 72.3% of positive slaughter lots, with percentages of positivity in carcasses up to 71.5% and 75.8 in Veneto and Marche regions, respectively [15]. The 52.1% of characterized isolates were *C. jejuni*; *C.*

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coli represented the 55.6% and *C. lari* 1.1%. The study revealed also very high levels of contamination: >10,000 colony forming units (CFU)/g.

Prevention relies on general hygienic measures. Food safety preventive measures are required at all the levels of food chain from primary production to retail, as well as good hygienic practices at household. The World Health Organization (WHO) is developing policies that will further promote the safety of food, promoting the strengthening of food safety systems, promoting good manufacturing practices and educating retailers and consumers about appropriate food handling and avoiding contamination. Education of consumers and training of food handlers in safe food handling is one of the most critical interventions in the prevention of food borne illnesses [16]. In countries without adequate sewage disposal systems, faeces and articles soiled with faeces may need to be disinfected before disposal. Measures to reduce the prevalence of *Campylobacter* in poultry include enhanced biosecurity to avoid transmission of *Campylobacter* from the environment to the flock of birds on the farm. This control option is feasible only where birds are kept in closed housing conditions. Good hygienic slaughtering practices reduce the contamination of carcasses by faeces, but will not guarantee the absence of *Campylobacter* from meat and meat products. Bactericidal treatment, such as heating (e.g. cooking or pasteurization) or irradiation is the only effective method of eliminating *Campylobacter* from contaminated foods [16].

The main problem is the high level of contamination of food. It is estimate that it is possible to reduce the 90% of the cases of human *Campylobacteriosis* limiting the level of contamination under 500 CFU per gram in raw poultry meat [2]. It is therefore of outmost importance the reduction of the bacterial burden of raw meat to ensure a radical decrease of clinical forms. Pathogen reduction treatments (PRTs), implying the use of physical treatments or chemical products as such as chlorate compounds, are efficiently applied on poultry carcasses at the end of the slaughtering process to obtain a diminution of pathogens on the surface of the meat [17,18]. However, exception made for the use of lactic acid as PRT in beef plants recently authorized by the European Union (EU) [19], these practices are forbidden by the EU food law [20]. This determined long term disputes between EU and USA [21]. Therefore, research efforts should be focused on the achievement of such objective through alternative means.

Taking into account that meat is contaminated by bacteria from caecal intestine contain despite application of hygienic measures during slaughtering and subsequent evisceration, direct interventions on primary production should be considered. An optimal theoretical approach should be the reduction of *Campylobacter* among intestinal bacterial flora. For example, the anti bacterial effects of plants which can be used to integrate animal feed diet should be investigated. This approach find basis on the phytotherapy concept. A number of vegetal species have been recognized to posses anti bacterial activity. Among them, olive tree (*Olea europaea*) showed to contain oleuropein, its most important phenolic compound characterized by a potent antioxidant action [22] with different pharmacological features such as antibacterial actions against a variety of pathogens, including several species of *Lactobacilli, Bacillus cereus, Salmonella typhi, Vibrio parahaemolyticus, Staphylococcus aureus* (including penicillin-resistant strains), *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli* [23-26]. These observations suggest the potential for an effective anti bacterial action also against *Campylobacter*, to be determined experimentally. Olive oil extraction generates several by-products (primarily olive oil cake) that can be used to feed animals. Similarly, by-products such as olive leaves from annual pruning or the young shoots coming out from the base of the tree can be also used. The 98% of the production of olive oil is done in Mediterranean countries. Spain, Italy and Greece are the main producers (75% of the total production). The olive oil production in 2009 was 2.91 million tons [27], thus, representing a large accessible resource of by-products for animal feeding in Europe and the Mediterranean region. Different trials have been conducted in poultry to determine the olive by-product supplement optimal ratio in diets. Broilers consuming 100 g olive pulp/kg (a 10% inclusion level in the diet replacing maize) gave the highest average live weights in starters and finishers (1-21 and 22-35 days of age) and had no effect on carcass quality (visceral organ mass, gastrointestinal tract weight, carcass cuts, carcass composition, and dressing percent) [28]. A 7.5% inclusion level was previously recommended [29]. Replacement of 15 and 30 g wheat bran/kg with olive leaves in the starter and finisher diets produced no significant effect on performance and carcass characteristics of chickens [30]. Laying hens could be fed on olive pulp at 9% dietary level with no deleterious effects on bird's performance [31]. Rising up to 16% olive pulp inclusion in laying hens diets, adverse effect were observed on egg protein quality, eggshell weight and yolk color, but not on egg production, feed intake and egg mass [32]. However, the above mentioned ratios have been determined on the entire productive cycles. For the purpose of the reduction of intestinal bacterial burden, still remains to be clarified the possibility to increase the quantity of olive by-products in the diet at the end of the production cycle, before slaughtering, avoiding side effects and obtaining the maximum beneficial effect of anti bacterial activity. In addition, attention should be paid to the feed processing to ensure palatability and preserve anti bacterial efficiency of active principles which might be hampered for example by high temperature applied in the pelleting process.

Similar researches should be conducted also on other plants candidates for their therapeutical and preventive potential due to anti bacterial activity such as bamboo (*Phyllostachys heterocycla* is the most dominant among a variety of bamboo species) or mango (*Mangifera indica*). Researches on *bamboo* extractives have mostly focused on shoots, roots, and leaves for the bioactive components with antioxidant activity and antimicrobial activity [33-36]. Antimicrobial activity of the extract of the fresh leaves of bamboo, evaluated against both Gram positive and Gram-negative bacterial strains, revealed effective inhibitory action against *S. aureus* [37]. The ethanolic extract of Petung bamboo (*Dendrocalamus asper*) leaves was the most effective to inhibit all tested *E. coli* strains [38]. Mangiferin, a mango's polyphenolic antioxidant, showed in vitro activity against 7 bacterial species, *Bacillus pumilus, B. cereus, Staphylococcus aureus, S. citreus, Escherichia coli, Salmonella agona* and *Klebsiella pneumoniae* [39]. The methanolic extracts of mango exhibited antimicrobial activities at a concentration of 20 mg/ml [40]. Their use is possible in tropical and subtropical regions were they are widely available. In particular bamboo is one of the fast-growing forest plants. Furthermore, the use of by-products is economically interesting thus reducing the production costs. Nevertheless, such by-products, currently considered cheap feed, should be used also as therapeutic and preventive nutritional components through a precise application strategy (e.g. higher doses at the end of production cycles), before slaughtering, to reduce bacterial burden. This strategy might be applied not only in zootechnic industry but also at rural level directly and easily by farmers. In conclusion, this approach might represents an innovative alternative for a sustainable prevention strategy in the full respect of the EU food law and coping with the increasing consumers' demand of naturally produced and healthy food without use of chemical compounds or antibiotics.

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