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Campylobacteriosis in poultry

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بسم الله الرحمن الرحيم

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُل رَّبِّ زِدْنِي عِلْمًا)

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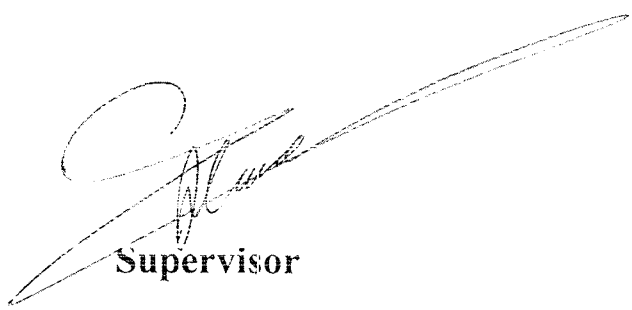

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Thank god lord of the worlds may Allah bless Muhammad and aspects of my thanks and appreciation to my professors valued all of what have made me of valuable guidance opinions sound as Iam pleased to draw my thanks and gratitude to Assist. Prof. Alaa Abdul Aziz

Dedication

To ..my parents

And to all members of my family

To ..my friends

I dedicate this work

Jaafer

List of Abbreviations

Campylobacter	C.
species	spp.
Gastrointestinal	GI.
Marek's disease	MD.
polymerase chain reaction	PCR.
United Kingdom	UK.

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Summary

Summary

Campylobacter jejuni, a widespread food-borne pathogen is responsible for enteritis in the populations of both industrialised and developing nations and is acquired by consumption of contaminated water, milk and food products. Contaminated poultry meat is regarded as an important source of campylobacteriosis, with both commercial broiler and turkey growing flocks infected at two to three weeks of age by direct and indirect horizontal exposure. Non-chlorinated water is regarded as a vehicle of infection, followed by rapid intraflock dissemination. Intensification in the poultry industry has contributed to the increased prevalence rates on carcasses associated with increased stocking density and mechanised processing which are inherent to the high efficiency dictated by a competitive market.

Currently, pre- and post-harvest control measures may ameliorate the problem of *Campylobacter* infection in consumers. Refrigerated storage and transport of red meat and poultry, appropriate handling and food preparation, and thorough cooking reduce the possibility of food-borne infection. In view of the world-wide distribution of *C. jejuni* infection and the multiplicity of sources, including non-pasteurised milk and contaminated water, it is inappropriate to impose trade restrictions on poultry meat based on the detection of campylobacters.

Chapter One

Introduction

Chapter 1

1. INTRODUCTION

Campylobacter jejuni and C. coli are spiral, curved, or s-shaped rods that are 0.2 to 0.8 μm in width and 0.5 to 5 μm in length and the most important human enteropathogens among the campylobacters, affecting an estimated 2.4 million cases each year in the U.S. alone (1, 2). In the U.S., there were approximately 5,712 laboratory-confirmed cases of campylobacteriosis in 2006, according to the Centers for Disease Control and Prevention; second only to Salmonella infections (1). Although C. jejuni, C. coli, C. lari, and C. upsaliensis are all capable of causing sickness in humans, most cases of campylobacteriosis are caused by C. jejuni (85%) with the majority of the remaining caused by C. coli (1, 3, 2). Risk factors for contracting campylobacteriosis include consumption and handling of raw and undercooked poultry, most commonly chicken and turkey products, cross contamination with other foods, and contaminated milk and water (4, 5, 6, 7, 8).

Both C. coli and C. jejuni colonize the intestines of food-producing animals and humans (9). The favored environment appears to be the intestines of avians, which may be due the body temperature of 42° C, which is the optimal growth temperature for Campylobacter. There have been many investigations into the possible sources of contamination of poultry flocks with Campylobacter, but no definitive factor has been determined to explain its incidence (5, 8). Since Campylobacter is ubiquitous, it is easy for the flock to be exposed to many potential sources of contamination; current sampling and culture methods may not be adequate to recover all bacterial cells present. In avians, Campylobacter is a commensal that colonizes the gut. After one member of a flock has become contaminated, Campylobacter is transmitted from that animal to other animals vertically and horizontally (10, 11).

C. jejuni and C. coli can be found on 90% of poultry in the U.S. (12), and from 18% to >90% of poultry in Europe, varying from country to country (13). Carry over contamination from a positive flock to a negative one is a source for contamination in processing (14, 13). Cross contamination in the slaughter plant due to contaminated equipment and processing water, is very difficult to control. Finished products that are heavily contaminated with high numbers of microbes are considered undesirable from a food safety and quality point of view. There are various steps in processing designed to eliminate or control these microbes, such as multiple washes and chilling. In some instances, processing aids are used to further reduce the microbial load on chicken carcasses by removing surface contamination.

Measures taken to reduce or eliminate the prevalence of Campylobacter are being researched and applied in the food industry. Prevalence in foodstuffs is likely to have a huge impact on the health care industry, as Campylobacter-related illness and hospitalization costs are $8 billion in the United States alone (15). The underlying principle for reduction of Campylobacter infection in humans is to prevent colonization of poultry, or to reduce the prevalence on carcasses during processing. Information relative to the diversity of Campylobacter and nature of infection helps in the investigations of adaptation. Molecular subtyping is an important tool for epidemiological studies; it helps in tracing sources and routes of transmission of human infection, identifying and monitoring specific strains over time and different regions with important characteristics. Molecular subtyping also contributes to the development of strategies to control transmission, elucidate sources, and determine possible routes of contamination in the food chain (16, 17, 18).

The objectives of this study were

1. The importance of Campylobacter in poultry and human
2. Determine the genetic diversity of Campylobacter on poultry

Chapter two

Literature Review

Chapter 2

2.Literature review

2.1. Contamination of poultry meat with *Campylobacter*

The high prevalence of *Campylobacter* on poultry meat and derived products is of significance to consumers (19). Records of the occurrence of C. *jejuni*and the less frequently isolated species of *Campylobacter* (C.*coli,* C.*lari* and C.*fetus)* during the period 1980 to 1990 are documented in a review of forty-two publications concerning seventeen countries and five poultry species(20).

Live broilers (21), turkeys (22) and ducks (23) are delivered to processing plants with high levels of faecal contamination (24). A study conducted in the USA confirmed that 20% of live broilers yielded C. *jejuni*from cloacal swabs obtained at the time of delivery to two plants (25). Unwashed transport coops may contribute to surface contamination of plumage and feet (26), resulting in recovery rates of 80% to 100% from the caecum for clinically healthy broiler flocks(24).

The practice in the USA of withholding feed from broiler flocks for periods of 6 h to 10 h to reduce contamination of carcasses with ingestaduring evisceration, may exacerbate the introduction of C. *jejuni*contamination into plants via the crop. The recovery of the organism increased from 25%of 360 crops before feed withdrawal, to 62% at the time of harvesting. During an eight-hour period, levels of C. *jejuni*in the caeca of the subject birds remained constant (27). Subsequent studies using a fluorescent dye gavaged into the crop confirmed the extent of dissemination of ingesta among carcasses and the eviscerating environment (28, 29). Surveys conducted at three processing plants demonstrated cross contamination of carcasses during defeathering and

evisceration, but a decrease in level of C. *jejuni*on the skin surface associated with scalding and immersion chilling (30). On-line washing of turkey carcasses with chlorinated water reduced levels of contamination(31).

A recent study of whole, processed packed and refrigeratsd carcasses and portions at point of sale yielded a*Campylobacter* recovery rate of 26%. The products were derived from five countries of the European Union (EU) with similar methods of flock management and processing (32). The values recorded in the study in Belgium are generally in agreement with surveys which yielded a 28% recovery rate in a survey conducted in Germany (33). Higher levels of C. *jejuni*were documented in a study in the USA using enrichment culture of samples derived from whole carcasses offered for sale. Recovery ranged from a low of 7% in December, to 97% in June and July, with an average annual rate of 64%among the thirty samples examined by whole carcass wash (34). Previous surveys over the period from 1980 to 1988 to quantify the levels of C. *jejuni*contamination on broiler carcasses reveal a generally high rate of recovery rangingfrom 14% to 88%, with an unweighted mean of 57% (TableII).The recovery rate from carcasses may be influenced by the proportion of flocks infected, the degree of intraflockcolonisation, seasonal and climatic factors, configuration and operation of immersion tanks and processing plant equipment, chlorination and chemical treatment of water and carcasses and microbiological techniques used for sampling, isolation andidentification.Quantification of the level of *Campylobacter* on carcasses, portions and derived products can be influenced by handling and storage (35). Freeze-thaw and heat stress injury following exposure to disinfectants or acids can lower recovery of C. *jejuni,* unless appropriate enrichment and isolation techniques areapplied.*Campylobacterjejuni*is relatively tolerant to freezing (36). A reduction of 0.5 to 2.0 log was recorded over a two-week period on broiler carcasses held at -20°C,with inoculation levels of 10^3 to 10^5 CFU/g.

Viability of C. *jejuni*persisted on drumsticks contaminated at a level of 4.8 x 10^3 CFU/cm^2 , for an extreme shelf life of ten days at both 9°C and -12°C.At-20°C,the level of *C.jejuni*declined from 9.9 x 10^2CFU/cm^2 to 4.5 x 10 CFU/cm^2 in seven days, but persisted through the twenty-sixth week of storage with a terminal level of 0.2 X 10 CFU/cm^2(37). *Campylobacter jejuni*survived for up to twenty-eight days in vacuum-packed processed turkey rolls and hams held at 4°C (38). A statistically significant decrease was reported in the level of C. *jejuni*over time, and differences in viability were recorded among three isolates. The organism survived in sliced turkey roll under carbon dioxide enriched packaging for eighteen days at 4°C, confirming that processed poultry products may serve as a vehicle forinfection.

2.2. C. Jejuni Colonization Pattern in Broiler Chicks

It is generally accepted that C. jejuni colonizes the aviangut as a commensal and colonized broilers carry a large number of bacteria in their ceca (generally around 106 to 108 cfu/g), the predominant site for colonization [39,40]. Ingestion of C. jejuni numbers as few as 35 cfu can be sufficient for successful colonization of chicks [41]. After ingestion, the bacterium reaches the cecum and multiplies, resulting in an established colonizing Campylobacter population within 24 hours after entrance [42]. Most flocks become colonized only at an age of two to four weeks [43,44], probably due to the presence of maternallyderived antibodies in young chicks conferring protection against colonization [45]. Once flock colonization is detected, the majority (> 95%) of the birds of that flock is colonized within several days [46] and stay so until slaughter [42,47]. C. jejuni isolates can have different colonization potential [41,48,49]. Isolates from humans have been reported to be less successful in colonizing chickens than poultry isolates [49,50]. C. jejuni isolates from poultry have been divided in three colonization phenotypes. Strains of the first

phenotype fail to colonize 14-day-old chickens. In the second phenotype, strains can colonize but are readily eliminated and are classified as transient. The third phenotype contains strains that show efficient and sustained colonization [48,50]. These three colonization phenotypes were found to be stable and independent of in vivo passages and the amount of viable bacteria in the inoculum. Although C. jejuni strains did show enhanced colonization capacity (i.e. the minimal infective dose required for maximal colonization decreased) after passage through the avian gastrointestinal (GI) tract, their colonization phenotype did not change [49]. Enhanced colonization capacity and increased virulence after in vivo passage through chicks has been shown in several other studies as well [41,51,52]. This variability in colonization capacity, but the fixedness of the colonization phenotype of a given strain indicates that C. jejuni genes involved in initial and sustained colonization are not identical. However, in contrast to this stable colonization phenotype [49], it has been previously reported that after several in vivo passages a poorly colonizing isolate was able to consistently colonize chicks [41].

2.3. Risk factors and sources of infection for poultry

Since Campylobacter is horizontally transmitted into broiler flocks, primary control measures should be implementedat the farm level. However, before targeted intervention strategies can be implemented, the sources and routes of infection for broiler flocks must be identified (53). In many different studies, several common risk factors were identified for the introduction of Campylobacter into a

broiler flock. Contamination of flocks increases with the

following:

–the age of the animals

–the number of broiler houses on a farm

–the presence of other animals on the farm or in the

direct vicinity (54, 55, 56.(

In a Dutch study on ten broiler farms that were screened for the presence of Campylobacter for ten subsequent cycles, the risk for a flock to become positive increased when a former cycle tested positive (57). Recently, a systematic review on risk factors, based on United Kingdom data, and comprising (58) research papers, was published (59). Depopulation schedules (thinning) and multiple broiler houses on farms were identified as factors associated with increased risk. Disease prevention andhygiene measures, the presence of more than onegeneration of chicks (i.e. broilers and their parents) andcertain seasons of hatching were all associated with Decreasing risk.

2.4.Clinical signs:

Symptoms of campylobacteriosis include diarrhoea (sometimes bloody), nausea, abdominal pain, fever, muscle pain, headache, and vomiting. The incubation period before onset of disease is usually 2–5 days, with illness generally lasting for 2–10 days. The unique feature of the disease is the severity of abdominal pain which may become continuous and sufficiently intense to mimic acute appendicitis (60;61). As a consequence of *C. jejuni* infection a small number of individuals develop a secondary condition such as reactive arthritis or Guillain-Barré syndrome, in which a harmful immune response of the body attacks part of the peripheral nervous system leading to symptoms of muscle weakness or paralysis (62).

2.5.Post mortem changes:

In chicks, distention of the jejunum, disseminated hemorrhagic enteritis and focal hepatitis may be seen .

The chickens examined were either spontaneously dead or euthanized by the owner. The most common postmortem findings were Marek's disease (MD) and colibacillosis. All the chickens examined tested negative for *Salmonella* spp. One or more ectoparasite species was found from 19% of the chickens and one or more endoparasite species was found from 40% of the chickens. Eight of the chickens studied in 2011 were investigated in the case of infectious bronchitis virus and all tested were negative(127).

2.6.RESERVOIRS AND TRANSMISSION OF CAMPYLOBACTER

Because the ideal growing environment for Campylobacter is at a temperature of 42U and has an obligate host requirement (63), it does not proliferate easily outside of the gut (64). Therefore, reservoirs provide critical links to human disease. Livestock, domestic animals, and birds are some of the commonly known reservoirs for Campylobacter spp.

65, 66) and are shed in the feces of these animals in various concentrations throughout the year. Campylobacter jejuni can be isolated year round from slurry tanks around sheep farms (66). Land application of fecal waste could lead to further contamination of the environment and possible runoff into nearby waterways.

Despite its obligate host requirement, Campylobacter are routinely found in environmental sources such as water and sewage (67, 68, , 69, 70). For short periods of time, Campylobacter spp. can survive in sterile water but their survival increases when associated with a biofilm and at lower temperatures (67). In sterile water at 37o C Campylobacter survived an average of 21.8

hours while at lower temperatures the survival times went up with highest survival in sterile water at 4o C (201.6 hours). When autochthonous microflora were added to the microcosms to better represent the natural environment, survival rates increased significantly to ~ 200 hours at 30o C and ~ 550 hours at 4 o C (67).

One proposed environmental model for the transmission of campylobacteriosis to humans (71) suggests that humans are exposed to the pathogen through feces, food, and aquatic environments. While Campylobacter has been found in all these environments, the modes of movement between them are not fully understood.

2.7.Incidence of Campylobacter infection in humans

The United States Department of Health, Centers for Disease Control and Prevention has recently completed an extensive survey of food-bome disease in the USA (72). Figures were collected from ten national and regional databases including the Food-bome Disease Active Surveillance Network ('Food Net') established in 1996. An estimate of the incidence of campylobacteriosis in 1998 was based on active surveillance among a population of 20 million. The incidence rate from1996 to 1997, of 24/100,000 was extrapolated to the entire population of the USA following application of a multiplication factor of thirty-eight to represent the proportion of non-reported to diagnosed cases. The total estimated number of cases in the USA exceeded 2.5 million, with 13,000 hospital admissions and 124 deaths in 1997. Campylobacteriosis represented 14.2% of all diagnosed food-borne infections including bacterial, viral, protozoal and metazoalaetiologies, and exceeded paratyphoid salmonellosis (9.7%) in incidence. The most recent compilation of data on food-borne Campylobacter infections in the USA updates previous reports on occurrence and causation of outbreaks covering the period from 1973 to 1992 (73, 31 74 , 75). The campylobacteriosis incidence rate in the USA of 100,000/1,020

population, estimated in 1992 (76), is strongly supported by the latest, more structured evaluation.The differential between diagnosed and non-reported cases of campylobacteriosis complicates estimates of economic losses associated with infection. Based on incidence rates and hospital records pertaining to the mid-1980s, the direct and indirect cost of the disease ranges from US$700 million to US$1,400 million (77). A comparative value of US$150 million was estimated for the United Kingdom (UK), based on an incidence rate of 1,100/100,000 and prevailing medical costs in that nation (78).

2.8.Diagnosis:

2.8.1.Isolation and identification of thermophilic Campylobacter spp.

The genus Campylobacter was established in the early 1970s (79), based on morphological and biochemical characteristics including serological typing (80). Subsequent developments in molecular biology have facilitated revision of the genus and differentiation from Helicobacter and Arcobacter (81).The three thermophilic Campylobacter species of humanhealth significance, C. jejuni , C. coli and C. lari, require selective media, incubation at 42°C and a microaerobic environment comprising a low level of oxygen (5% to 10%) and elevated carbon dioxide (1% to 10%). Methods of specimen collection to avoid desiccation, and subsequent culture and identification are reviewed in laboratory manuals (82). The three thermophilic species of Campylobacter can be differentiated by biochemical characteristics (83) and hydrogen sulphide production (84). The Penner serotyping scheme is based on heat-stable antigens derived from surface lipopolysaccharides (85). The alternative Lior serotyping scheme using heat-labile H antigens (86) is practical under laboratory conditions to differentiate among C. jejuni isolates derived from flocks and patients (87).The relative efficiency of ten alternative methods to distinguish among Campylobacter isolates in epidemiological investigations

was based on extensive studies undertaken at the United States Centers for Disease Control and Prevention, Atlanta (88). Techniques included Penner and Lior serotyping, multilocus enzyme electrophoresis, deoxyribonucleic acid (DNA) restriction endonuclease analysis, phage typing, plasmid analysis and ribotyping. Serotyping was determined to be the most discriminating phenotypic method, but all the procedures required specialised laboratory equipment and trained technicians consistent with reference centres. Pulse field gel electrophoresis is frequently applied to distinguish C. jejuni from C. coli and in molecular epidemiological studies (89). Flagella typing using restriction fragment length polymorphism (RFLP) analysis can discriminate among isolates and is regarded as a practical typing method for epidemiological investigations (90). Highly sensitive polymerase chain reaction (PCR) procedures are being developed to detect C. jejuni in food products (91). This has specific implications for regulations which impose a zero tolerance for C. jejuni on imported poultry, since the high sensitivity of this technique will detect the organism at extremely low prevalence. In a study conducted in Switzerland, Campylobacter was detected in 4% of a series of 231 litter samples using conventional microbiology, compared to 68% detection using PCR (92). In a trial conducted on faecal samples derived from hospitalised patients with enteric infections, the sensitivity and specificity of the PCR procedure as compared to conventional culture was determined to be 91% and 97% respectively (93). Alternative methods of detection and identification of Campylobacter include immunomagnetic separation and identification of pathogen-specific ions by mass spectrometry (94).

2.8.2-Serotyping Serotyping separates bacteria based on antibody-antigen reactions. Campylobacter serotyping was developed in Canada in the 1980's by Penner and Lior (95). Penner schemes are based on the heat stable antigens using passive hemagglutinations (96) and Lior is based on the heat labile antigens using bacterial agglutination (97). The Penner scheme is used as the

basis for the typing scheme in the Laboratory of Enteric Pathogens (LEP), Public Health Laboratory Service in the United Kingdom (98). Advantages of serotyping include the wide acceptance as a valid typing method (99). Disadvantages of serotyping include a high level of untypeable strains, in human and veterinary samples (up to 20% in some instances), culturing conditions may render an isolate untypeable, ambiguous results, transient antigen expression, and cross-reactivity between certain antigens (100). The method requires a panel of antisera that is costly to maintain, is laborious and requires at least 5 to 7 days to complete (100, 101, 102), considering the need to repeatedly subculture isolates before testing. Using the LEP scheme, up to 40% of poultry isolates are untypeable .

2.8.3.Phagetyping

Phagetyping uses viruses as markers to identify microorganisms. Since bacteriophages are host-specific, identification to subspecies is possible. Campylobacter phage typing has many different schemes. The primary typing scheme was developed in the U.S. and uses common poultry phage. The scheme was adapted by other countries, with the basic or main phages remaining the same, but other phages added that were isolated from Campylobacter positive birds in that particular country. Disadvantages of phagetyping include the appearance of a unique phage pattern of a strain that Reacts with phage but Does Not Conform to a designated type (RDNC) (103). An advantage of phagetyping is the host specificity of phages. Host specificity reduces the chance of phages interacting with the wrong bacteria. Another advantage of phagetyping is its application as a complement to serotyping. Serotyping classifies bacteria into 20 broad groupings and phagetyping can further classify 6 to 29 subgroups from each of those 20 serogroups, thereby enhancing the discriminatory power of this epidemiological tool (98). There are 66 known serotypes and 76 known phage types for a total of 5016 possible

combinations (104) In Frost's study, (98) 336 phage-serotype combinations were identified among a sample of 2407 isolates, which may be indicative of the relationship between phage and serotypes .

2.8.4Biotyping

Biotyping is used to distinguish isolates according to biochemical reactions and metabolic activities. Differentiation is based upon the results of the biochemical tests. The discriminatory index depends on the number of tests administered (105). There are many types of tests or combinations of tests that can be used in order to discriminate, which is an advantage of biotyping. The discriminatory power may be increased as the number of tests administered. Biotyping is very common and widely accepted as a separatory method. For example, Skirrow (106) developed a typing scheme based on hippurate hydrolysis, a rapid H2S test in iron-containing media, and resistance to nalidixic acid for the differentiation of campylobacters into C. jejuni, C. coli, and a third group, the nalidixic acid-resistant thermophilic campylobacters. Disadvantages of biotyping include cost in supplies and labor. Cost and labor increases as discriminatory power increases, due to the increased number of tests performed. However, recent developments have made it possible to automate and perform many tests at one time. Biotypes may be dependent upon growth conditions and therefore reproducibility may be difficult, inter and intralaboratory .

2.8.5.Genotypic methods

2.8.5.1.Polymerase Chain Reaction (PCR)

PCR is used as the basis of many genotypic typing schemes. PCR is an automated, rapid, sensitive way to amplify DNA. PCR is comprised of three basic

steps—denaturation, annealing, and extension. The original double stranded DNA is denatured to form a single strand to which a primer can anneal. After annealing, a complimentary strand is formed. In theory, a single piece of DNA is enough to begin a reaction that multiplies exponentially to contain over one million pieces of DNA, depending on the number of cycles performed by the thermal cycler. PCR may amplify the whole genome or a specific gene; it is dependent upon the primers used in the reaction. Primer selection is depends on the target gene and reaction conditions. Specificity of amplification reaction depends on selectivity of primers, enzymes used, condition of primers and enzymes, and reaction conditions. PCR has become increasingly automated and more complex. Examples of this include real time PCR, reverse transcriptase PCR, and hot start-cold end PCR. PCR is also used in combination with other methods to increase discriminatory power. Limitations in PCR include inhibitors present in the sample matrix, condition of the primers and enzymes, and product assumption. Some samples are taken from a food matrix, especially in the case of Campylobacter, without enrichment. Some matrix constituents may have an inhibitory effect on the PCR efficiency. Advantages of PCR include automation of procedure, quick turnaround time, usefulness in detection. Disadvantages include high capital input for equipment, and cell viability is not known, as DNA can be amplified from dead or living cells .

2.8.5.2.Random Amplified Polymorphic DNA (RAPD)

RAPD is a whole genome typing method in which a single oligonucleotide is used to amplify a gene. The primer has to have the ability to bind in both forward and reverse directions. When performing RAPD, annealing temperatures must be taken into account .

Primer selection is the greatest determinant of the number of bands, since the primer acts on DNA in random places, random bands are produced. RAPD can

have high discriminatory potential due to its ability to determine polymorphism in the entire genome (99). However, RAPD can have low reproducibility due to random digestion. Low reproducibility is also due to the ratio of DNA to primer concentration, model of thermal cycler, magnesium concentration or brand of taq DNA polymerase, which all effect banding patterns .

A combination of fla and RAPD typing have successfully been applied to Campylobacter (107, 108, 109, 110). However, disadvantages such as minor differences in band patterns and weak band patterns make RAPD's discriminatory capacity somewhat poor, which may lead to subjective interpretation of results (111, 112, 99)

2.8.5.3.PCR-Restriction fragment length polymorphism (RFLP)

PCR-RFLP is a method in which a particular sequence is amplified and enzymes are used to digest genomic DNA at certain sites. The results are precise band lengths, which produce identifiable electrophoretic patterns that can theoretically be reproduced because of the specificity of restriction enzymes used. Advantages of RFLP are simple setup, low maintenance costs, and rapid processing of samples. The level of discrimination can be improved with multiple restriction enzymes. Disadvantages of RFLP are difficulty in choosing an appropriate target for amplification; prior knowledge of genome is needed, and a small section of genome is examined. RAPD analysis has been compared to fla typing in a study, and it was found that fla typing is more discriminatory when analyzing broiler liver and intestine samples for C. jejuni and C. coli (113).

2.9.Treatment :

Many cases of campylobacteriosis are self-limiting and require only supportive therapy, poultry infections were often treated by mass administration of enrofloxacin and sarafloxacin for single instances of

infection. The FDA banned this practice, as it promoted the development of fluoroquinolone-resistant populations(114)

2.10.Strategies to prevent the introduction of Campylobacter into a flock

The control of Campylobacter along the food chain is most effective when the colonisation of living animals can be prevented. Reducing the prevalence of Campylobacter infection in the primary production phase decreases high numbers of Campylobacter in the following steps. This may result in a low concentration or absence of Campylobacter on the final product. Identifying risk factors for the introduction of Campylobacter means that specific intervention strategies can be implemented. In the following sections, the authors discuss the possibilities and effects of biosecurity‹ multispecies farming and thinning, as well as competitive exclusion, vaccination and genetic resistance. Biosecurity Theoretically, a high level of biosecurity on the farm should protect against Campylobacter. Some correlation has been found (115), but even an extremely high level of biosecurity does not guarantee a Campylobacter-free flock at the time of slaughter (W.F. Jacobs-Reitsma, personal communication). Educating farmers on improved disease prevention measures and hygiene may lead to a lower prevalence of Campylobacter. However, conflicting reports come from two Scandinavian countries: Norway reports a positive effect from its education programme but Iceland has not observed any effect (116, 117.(The effects of improved hygiene are hard to quantify. As part of the Campylobacter Risk Management and Assessment project (CARMA, available at: htt://www. rivm.nl/carma/index_eng.html) in the Netherlands, a mathematical model was developed to describe Campylobacter dynamics in the primary production phase. Improving biosecurity was evaluated as an intervention strategy and identified as a potentially effective approach. However, this could only be established in theory as there is a lack of knowledge on methods that are effective in practice. No researcher can recommend to a farmer how to improve their biosecurity and

indicate a specific percentage of anticipated reduction of Campylobacter. As increased biosecurity cannot be broken down into specific control measures, it is not clear what investments are needed. Campylobacter strains are continuously present around broiler houses and even if biosecurity measures)such as anterooms, disinfection facilities for boots and separate clothing and utensils for each house/worker) are in place, they must be consistently applied to prevent colonisation. Thus, apart from the technical aspects of disease prevention, there is also a behavioural aspect involved, which has not been studied so far. Research has been conducted on the role of flies in transmitting Campylobacter. Flies can act as vectors for Campylobacter and the fly 'traffic' in and out of broiler houses is huge, so flies are a clear risk factor (118, 119.(Controlling flies leads to both delayed and reduced Campylobacter infection in poultry flocks (120).

2.11. Competitive exclusion

Competitive exclusion has been shown to be successful in Salmonella control programmes in poultry. Several studies on the use of competitive exclusion to control Campylobacter have been published but the results are variable. As yet, there is no commercial product that claims good results against Campylobacter. One recent development is the use of a bacteriocin added to feed to control C. jejuni in chickens (121). This approach claims to be effective in preventing colonisation but it is not yet commercially available.

2.12. Vaccination

There are no commercially available vaccines against Campylobacter in poultry. The development of these vaccines is hampered by three main problems :

–the antigenic variety of strains

–the lack of knowledge of antigens which induce

a protective immune respons the requirement to provide protection in the very early life stages of the bird. Several scientific studies show a (partial) protective effect of the humoral response under experimental conditions. A vaccine against Campylobacter in broilers must be effective in the very early life stages of the bird, requiring either protective maternal immunity or an innovative approach to induce protective immunity in the young chick.

2.13. Genetic resistance

Differences between genetic lines for susceptibility to Campylobacter infection are suggested but the data are limited and the molecular background is not yet clear (122). Strategies to eliminate Campylobacter infections from flocks Once an infection is established in a flock, close to 100% of the birds become colonised and shed high numbers of Campylobacter (> 106 CFU per gram of faeces.(Over an extended time period, there is a slight reduction in shedding, suggesting that immunity plays a role. However، in the normal lifespan of a commercially housed chicken, this may be limited to approximately one log reduction of Campylobacter concentration in the faeces unpublished data.(A few approaches are reported to reduce shedding in a well-colonised flock. The first is phage therapy. This method uses lytic phages that specifically attach themselves to and lyse Campylobacter cells. Under experimental settings, this approach has been shown to reduce Campylobacter shedding by two to three logs(123,124)

Risk assessment models predict a significant reduction of risk for the consumer, with a two-to-three log reduction in thecaecal level of Campylobacter in poultry (125). However، since the large-scale practical implementation of this method involves several problems (e.g. its application، resistance), phage therapy is not expected to be commercially available within

the next few years. Althoughbacteriophages already exist wherever Campylobacter ispresent, including in poultry, and these phages are safe for human health, using a virus to control Campylobacter may not meet with public acceptance unless it is capably and comprehensively presented (126).

A second approach may be the already mentioned use of bacteriocins. Bacteriocins have been proposed as a curative treatment but the results have not yet been published (121).

Chapter three

Conclusions & Recommendations

Chapter 3

3.Conclusions &Recommendation

3.1. conclusion

1. No significant loses among birds especially adults ,although the colonization of intestine is high.

2. The infection rate or incidence were high among poultry and poultry products.

3. There is increase of public health concern because world elevation of Campylobacter infection among human .

3.2.Recommendation

1. Increase the poultry sanitary and biosecurity .
2. Using of probiotic and/or prebiotic during rearing birds.
3. Pelleted feed is preferable to used.
4. Acidifers solutions can be used during processing to minimize the contamination.