وما أوتيتم من العلم إلا قليلاً

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SHEDDING AND COLONIZATION OF CAMPYLOBACTER COLI IN BROILERS FROM DAY OF HATCH TO SLAUGHTER

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I-INTRODUCTION

Campylobacter coli and Campylobacter jejuni have been recognized since that date 1970s as important agents of gastrointestinal infections through out the world, in the United states these infections affected approximately 1% of the population each year *(***Tauxe et al., 1992).**

In many industrialized countries the incidence of Campylobacteriosis exceeds Salmonellosis. Campylobacter bacteria are transmitted to human mainly throught food especially poultry meat products *(***Rivoal et al., 2005).**

Campylobacters are carried in the intestinal tract of a wide variety of wild and domestic animals especially birds. They can establish a temporary symptomatic carrier state as well as illness in human. Consumption of food and water contaminated with untreated animal or human wastes accounts for 70 % of Campylobacter related illness as each year.

The food includes unpasteurized milk, meats, poultry, shellfish, fruits and vegetables **(Abeyta and Kaysner, 1987 and Abeyta, 1998).**

Once **Campylobacter** colonization occurs in birds within a flock, ceacal can colonized within 72 hours **(Newell , et al . 1983)** , reach high numbers in ceacal contents as high as 10^9 cfu in experimentally challenged birds **(Wassenaar, et al . 1993).** This level may also increased during evisceration of carcasses , washing and processing due to contamination by personnel **(Oosterom , et al . 1983)** . Several serotypes were identified in one flock , some of these were not found again on further examination of the same flocks during the growing period **(Pokamunski . et al 1986)** . However the horizontal transmission is generally the most significant cause of broiler flock **(Pattison , 2001)** varied consider with season and region **(Hofshagen , 2005) .**

Campylobacter is one of the most common causes of bacterial gastroenteritis in humans world and yet is still a poorly understood bacterial pathogen, little is known about the prevalence of different virulence factors and the ability to produce toxin among Campylobacter isolates obtained from different sources **(Bang et al., 2003).**

The presence of **Campylobacter** Spp. on broiler carcasses and in closed tank water in a commercial poultry processing facility was monitored at monthly intervals from July to December **(Hinton et al., 2004).**

Species within the genus **Campylobacter** have emerged over the last three decades as significant clinical pathogens particularly of human public health concern where the majority of acute bacterial; enteritis in the western world is due to those organisms of particularly concern ,the species C. jejuni and C. coli which are responsible for most of these gastrointestinal related infections.Although these organisms have already emerged as causative agents of zoonoses. **(Moore et al., 2005).**Yet , there is no medium available to allow growth of C. jejuni and inhibit C. coli or vice versa **(Martin et al ., 2002)**

Enteritis due to **Campylobacter** is the most common cause of acute bacterial diarrhoea worldwide. In most cases infection occurs as a result of consuming contaminated water or food especially raw meat of fowls. **(Seyed et al., 2005).**

There are no validated serological tests developed for the identification of infected mammals or birds **(Cawthraw et al ., 1994)** . Due to some technical difficulties such as long incubation time uncertainely in results and some a typical strains which are mattily to culture , the detection of campylobacters is more complicated and genome based detection methods like PCR have gained a premenant importance to compare the resultus with other data **(seyed et al ., 2005)**

The present work was designed to cover the following points.

1-Incidence of Campylobacter coli infection in broiler chickens from day of hatch to slaughter.

2-Identification of isolates of Campylobacter coli by bacteriological methods.

3 _ Comparison of PCR and confential laboratory methods for diagnosis of Enteropathogenic Campylobacter spp. in Fowl feaces .

II-REVIEW OF LITERATURE

II-1.Incidence of Campylobacter:-

Oosterom et al. (1983) believed that, the source of bacterial contamination of poultry meat is essentially with intestine or gut content which may come in contact with carcasses already in the broiler house and during transport and slaughter either directly or indirectly through a vehicle such as transport and processing equipment. High levels of bacterial cross contamination may occur especially during defeathering and water chilling with intestinal contamination apparently being the only source. However, these level may also increased during evisceration of carcasses washing and processing due to contamination by personnel.

Milakovic-Novak et al. (1984) isolated C. Jejuni from the small intestines of 12 (6.6 %) out of 180 and the large intestines 12 (2.2 %) out of the 558 broilers that had died and from the cloaca of 28 (24 %) of 122 healthy laying hens and 47 (40 % of 118 hens with clocitis (vent gleet). Of the 29 isolates that were types 8 were C. jejuni biotype 1 and 21 were C. coli.

Neill et al. (1984) showed that C. Jejuni was not usually present in intestines of broiler chicks less than two weeks old but they were present in most older birds.

Pokamanski et al. (1985) examined six flocks for Campylobacter from hatching to slaughter. A total of 1530 cloacal swabs and ceacum samples were collected from 768 chicks at one-day and 1, 4 and 8 weeks old Campylobacter was not isolated from 360 chicks (healthy condemned unhatched) at hatching only one flock was negative all samples of 172 isolates 147 were C. Jejuni and 25 were C. coli.

Pokamanski et al. (1986) examined 1440 samples. From 720 broilers (From 5 flocks) for C. jejuni. Campylobacter species were not isolated from day old chicks and were only isolated from one week old chicks in one flock. In three flocks Campylobacter species were isolated from all chicks sampled at 4 weeks of age. In the 4^{th} flock all chicks sampled were negative until 8 weeks of age when all were positive. The 5^{th} flock remained negative through the 8 weeks of its life eleven broiler flocks were examined only at slaughter, 24 caecal samples were examined from each flock. In three flocks Campylobacter was not isolated in one flock one broiler chick was positive and in 7 flocks (From 58 to 100 %) of sampled broiler chickens were positive of the 146 isolates typed, 123 were C. Jejuni and 23 were C. coli. The authors concluded that in some flocks , several serotypes were identified, some of these were not found again on further were identified, some of these were not found again on further examinations of the same flocks during the growing period.

Varga et al. (1986) isolated 62 Campylobacter strains from hens with hepatitis from the cecum of clinically healthy hen and from the surface of slaughtered chickens. About 5 % had diarrhea but the mean mortality rate did not exceed 2.3 %. Sixty strains were identified as C. Jejuni one as C. coli. Most C. jejuni strains had typical growth and biochemical characteristics.

Acevedo et al. (1987) cultured samples of intestine, gall bladder and liver from 161 chickens of different ages, 85 with diarrhea and 75 apparently healthy yielded Campylobacter from 43 (26 %).Most isolates were from the small intestine and ceacum very few from liver and gall bladder C. jejuni was isolated more often than Campylobacter coli and was of the same serotypes as those known to occur in man. Isolation were obtained from chickens older than 3 weeks but not from younger ones. There was no relationship between the presence of Campylobacter and diarrhoea.

Hoope and Ehrsam (1987) examined a large poultry farming organization over 18 months for C. jejuni and C. coli. Incidence of infection ranged 4 to 78 %. In 4 of 34 broiler units (12 %). Campylobacter shedders were found in 6 of 153 flocks (4 %) first isolations occurring between the 34^{th} and 42^{nd} of fattening 16 % of 625. Skin samples from birds prepared fort sale were positive. It was concluded that Campylobacter was introduced into broiler flocks as a result of inadequate protection and that the slaughter process was responsible for contamination of poultry.

Torre Manas et al. (1987) studied the incidence of Campylobacter in the cloacal contents of broiler chickens. The study revealed three species, C. jejuni (37. 24 %) C. coli (32.28 %) and thermophilic nalidexic acid resistance, Campylobacter (10.93%).

Kaino et al. (1988) showed that the intestines of poultry are easily colonized with C. coli and C. jejuni. Day old chickens can be colonized with a few 35 organisms. Most chickens in commercial operation are colonized by 4 weeks **(Humphrey et al., 1993)**

Kapperud et al. (1993) indicated that, vertical transmission from breeder flocks to progeny has been suggested in one study but is not widely accepted **(Pearson et al., 1996).**

Jacobs-Reitsma et al. (1995) found that, the vertical transmission of Compylobacter to flocks via contaminated egg breeder hens are usually colonized by multiple strains of C. jejuni. The recovered from various segments of the reproduction tract.

Stern et al. (1995) investigated the colonization of the ceca and the contamination of chicken carcasses by Campylobacter species. Samples were taken on the farm and after transport and handling. In the first set of experiments, 20 chickens obtained from each of 10 broiler farms were collected from houses containing 6 to 7 week old birds. Half of the birds were slaughtered at the farm, the other birds were slaughtered at the farm, the other half was transported (10 birds per chicken coop) to a holding facility and killed within 16 to 18 h. Caeca from birds in 9 of the 10 farms. Sampled were positive for Campylobacter species . Colonization level ranged from 10 (4.11) to 10 (2.28) CFU Campylobacter species / g caecal matter except on one farm, where the organism was not isolated. The mean count on the farm was 10 (9.44) CFU Campylobacter species /g caecal material and after transport the mean was 10 (6.15) CFU/g.

Berndtson et al. (1996) showed that, the prevalence of flock positively is also depend on flock size and the system. Flock positively is generally higher up to 100. In organic and free compared to intensively reared flocks. This presumably reflects the level of such birds as well as the increased age of the birds at slaughter.

Cawthraw et al. (1996) explained that, C. jejuni close relative C. coli. In chickens C. Jejuni colonizes the mucus overlying the epithelial cells primarily in the ceca and the small intestine but may also recovered from elsewhere in the gut and from the spleen and liver. Experimentally the dose of viable C. jejuni required to colonize chicks and chickens can be as low as 40 CFU. However this dose and the kinetics of colonization may be dependent on both the bacterial strain and the chickens strain. Once colonization is established Campylobacters can rapidly reach extremely higher numbers in the caecal contents, as high as 10^9 CFU experimentally challenged birds **(Wassenarr et al., 1993),** although this level may be lower in naturally colonized birds.

Chuma et al . (1997) found the incidence of C. jejuni and C. coli in broiler farms was 33.9% (19/56) C. jejuni positive flock accounted for 20.0 % (17/85) and C. coli positive ones was 4.7 % (4/85).

Gregory et al. (1997) found that, Campylobacter most establish at transient low level of infection but more typically colonization of the broilers occurs between days 14 and 49 of the grow out period with almost complete. Flock involvement by day 49. Whether the broiler house was new or had previously housed broilers influenced neither the time nor rate of colonization of the flock. Although various species of wild life around the grow-out houses were found to be positive for Campylobacter. There was no correlation between this and the onset of flock colonization.

In Denmark, **Nielsen et al. (1997)** examined 929 cloacal swabs between (1995 – 1996) and found that 36 % were positive for thermophilic Campylobacter.

Willis and Murray (1997) examined seasonal variation of the level of Campylobacter contamination of broiler chickens . Campylobacter have been reported to exhibit a cyclical pattern of contamination of poultry. We release the level of contamination consistently increased and decreased depending on the season of the year.

Andrews (1998) found that Campylobacter species are intestinal commensales of many different wild animals, domestic livestock and pets to include cow, cattle, poultry (Turkeys and chickens), sheep , dogs and cats. The organism regularly shed in the feces of these animals and consequently has associated with inadequately pasteurized milk and improperly handled, cooked meat.

Byrd et al. (1998) evaluated the effect of pre-slaughter feed withdrawal on the incidence of Campylobacter isolation from the crop was determined immediately before and after feed withdrawal in 7 weeks old broiler chickens obtained from each of nine separate broiler house. Ceca were collected from broilers in six of the same flocks for comparison with the crop samples. Feed withdrawal caused a significant ($P < 0.025$) increase in Campylobacter positive crop samples in seven of the nine houses sampled. A total number of Campylobacter positive crops increased significantly ($P < 0.001$) from 90/360 (25 %) before feed removal to 224 / 359 (62.4%0 after the feed withdrawal period.

Achen et al. (1998) mentioned that at 43 days old only 37.5 % of birds were shedding C. jejuni in their faeces, and enumeration of C. jejuni in the crop, jejunum and ceacum on day

43 revealed the ceacum was the major colonization site and out of 24 birds carried C. jejuni in their intestinal tract.

Colin et al. (1999) examined the colonization of the ceaca and organ invasion by different isolates of C. jejuni were investigated in day-of-hatch leghorn chicks. This model of Campylobacter colonization of the ceaca demonstrates that **(Hirn and Aho, 1988)** day-of hatch birds don't naturally contain cecal Campylobacter ceaca can be colonized with C. jejuni by oral gavage and not by cloacal inoculation. C. jejuni can be recovered from the ceca up until at least 7 days post-inoculation **(Berndtson et al., 1996)** ceacal colonization when as little as 10^2 colony forming units is orally inoculated into chicks **(Demming et al., 1987)**. Different C. jejuni isolates via both in their ability to colonize the ceaca and in their ability to invade the liver.

Pattison (2001) considered that the horizontal transmission is generally the most significant cause of broiler flocks. Campylobacter are ubiquitous in the environment and could the house by a number of vehicles including human activity associated with management. However, this has yet to be proven by reproductive observation genotyping of strains in the environment with subsequently result in flock.

Richard et al. (2001) examined ten genotypically strains of Campylobacter strains coli were isolated from a swine production facility. These porcine isolates were then orally inoculated into day-of hatching horn chicks and were excellent colonizers of the chick ceacum. C. coli recovered from inoculated chickens were genotypically identical to the challenge strain. The absence of the host specificity suggests a possible movement of strains among swine, field animals, birds and poultry houses.

Stern et al. (2001) found the proportion of broiler flocks colonize with Campylobacter varies, this variation may reflect at least in different sampling and indicated that nearly 90% of flock. In Europe this prevalence varies from 18 to 90 % with the Northern most countries lower figure than Southern most countries. In Sweden for National survey indicates the flock positively is less than 100 %. The reason for this is unknown. Zootechnical parameters including the climatic conditions and distance between farms may all influence flock pre possible that. The poultry industries in these northern European countries are there fore exploit hewer facilities and more closely regulated thus elsewhere is seen whether similar trends occur in other regions of the world.

Ringoir and Korolik (2002) detected the colonization and shedding in poultry has not been fully accepted that colonization in chickens persists at least for the life span of a cycle reared birds. This is usually less than 47 days , however in experimentally persistence of colonization may vary among Campylobacter strain.

Newell and Fearnley (2003) indicated the epidemiological investigations of commercial flocks the flock Campylobacter colonization is age dependent. Newly hatched chicks appear to be free. Europe this negatively persists until at least 10 days of age become infected only 2 to 3 weeks after the placement of chicks into a broiler colonized chicks into a broiler colonized. Chickens usually show no absorbable clinical symptoms of infected chicks are exposed to high doses under experimental condition.

In Europe **(Takkinen and Ammon, 2003)** studied the prevalence of Campylobacter species in poultry and cattle has been shown to be C. jejuni in swine, C. coli and in dog C. upsalinensis. Most human infections (90 – 95 %) are due to C. jejuni. Broiler flocks are after contaminated with C. jejuni and C. coli there is strong evidence for frequent cross contamination during slaughtering and product processing.

Hofshagen (2005) examined 10803 flocks from 562 broiler farms were tested altogether 521 (4.8 %) of the flocks were identified as positive for Campylobacter Spp., primarily C. Jejuni. The positive flocks originated from 257 (25.7%) of the farm . During the period 2002 to 2004 there was a large and steady reduction in flock prevalence. From 6.3 % in 2002 to 3.3 in 2004. the proportion of flocks positive for Campylobacter Spp. varied consider with season and region. The proportion of farms producing flocks positive for Campylobacter Spp. each year reduced substantially from 28.4 % in 2002 to 17.8 % in 2004.

Keller et al. (2005) examined samples for C. coli and C. jejuni were derived prior to slaughter from 100 randomly selected flocks (Five birds per flock) raised on three different farm types. The observed flock prevalence was 54 %., in total with 50 % for conventional and 69 % for free – range farms. Birds held on farms with a confined roaming area had the lowest prevalence of 37 % Campylobacter isolates were characterized by amplified fragment length Polymorphism (AFLP).

Ring et al. (2005) examined ten conventional and four extensive out door broiler flocks distributed over nine farms were investigated twice per week during a 35 – 58 days rearing period to observe the dynamics of Campylobacter Spp. Spread within these flocks. Spread within these flocks. A total of 4112 samples were collected 157 (3.8 %) of these samples were Campylobacter positive with all C. jeujni. The positive samples were distributed over three conventional and two extensive out door flocks on five farms. These five positive flock farms were colonized the fifth to the seventh week of age and remained colonized until slaughter. The data revealed to a single source from environment may have been responsible for the colonization of each flock.

Tesfaye et al. (2005) determined the prevalence of thermophilic Campylobacter Spp. in various food animals in Jimma Zone South West Ethiopia by studied urban and rural farm animal setting in Jimma collected. Fecal specimens from 485 various food animal. Cattle n = 205, Poultry n = 1991, Pigs n = 18, Sheep n = 71 and culture using standard method. The results Campylobacter Spp. were isolated from 192 (39.8 %) out of 485 fecal specimens. The highest isolation rate was recorded among chickens (68.1 %), followed by pigs (50.0 %), sheep (38. %) and cattle (12.7 %). Among the 192 thermophilic Campylobacter isolated 135 (70.3 %) were identified to be C. jejuni, 51 (26.6 %) were C. coli and 6 (3.1 %), C. lari C. jejuni was the most prevalent species in chickens (80.8 %), followed by sheep (59.3 %) and cattle (53.8 %). All isolates found in pigs were identified to be C. coli (100 %).

Wesley et al. (2005) said that, the Campylobacter is a major bacterial foodborne pathogen with consume contaminated poultry regarded as a major risk factor for human infections. The stress is feed withdrawal catching-crating transport and holding at the abattoir prior to slaughter been shown to alter the levels of Campylobacter. They reported

that 6 – 95 % of market Campylobacter as determined by cloacal swabs of birds both prior to transport at the abattoir the population of C. coli increased after transport the five trials. They also noted that the population of C. coli were more diverse than those of based on DNA profiles.

Rasschaert (2006) examined the relation between internal carriage and surface contamination with thermophilic Campylobacter species in broiler chickens. Samples were collected from 39 flocks in three Belgian poultry slaughter houses from each flock crop swabs before slaughtering and intestines and neck skin during slaughter were collected isolates were identified as C. jejuni (90 %), C. coli (8.37%) and C. lari (2.2 %). Seventy two percent of the flocks arriving at the abattoir were colonized with Campylobacters after slaughter 79 % of the flocks had contaminated neck skin. Four of these flocks were initially free of Campylobacter. These four flocks may have no contaminated carcasses after logistic slaughtering.

Hansson (2007) analyzed and identified sources and risk factors for colonization of Campylobacter Spp. in broilers at both farm level and 5 laughter groups with a low within group prevalence were identified split slaughter was confirmed as a risk factor and contamination of carcasses was shown to occur both during transport and during slaughter process. Environmental Capmylobacter load was comparable on high and low incidence. Forms indicating that hygiene regime are of greater importance than environmental load.

Sarah and Laura (2008) detected the prevalence of infection in broiler breeder flocks has been found to be as high as 80 % but Campylobacters are isolated from hatcheries or newly hatched chicks at the prevalence reported in broiler flock varies according to age and isolation technique or reason. The prevalence reported in broiler flocks also varies between countries Sweden, Finland and Norway report relatively low rates infection (5 – 20 %) whereas the UK, other European countries and the U.S.A appear to have higher levels of infection with up to 90 % of broiler flocks infected. C. jejuni is the most frequently isolated species from poultry but occasionally C. coli and C. lari are found.

2-Pathology and pathogenicity of Campylobacter in broiler chickens:

Newell et al. (1983) found the poultry is to be colonized primarily with C. jejuni (65 – 95 %) less often with C. coli and rarely with other species. Colonization rates in broiler chickens are age related. Most flocks are negative until 2 – 3 weekly of age. Once Campylobacter colonization occurs in a broiler flock, transmission via coprophagy, is extremely rapid and up to 100 % of birds within a flock can become colonized within 72 hours. Samples from liver birds, destined for the food chain, should therefore be taken as close to slaughter as possible the majority of birds shed large numbers of organisms ($> 10^6$ colony-forming units/g faeces). Campylobacters can be isolated from fresh faeces / caecal droppings or cloacal swabs.

Establishment of C. jejuni in the intestinal tract results in extensive fecal excretion **(Welkos, 1984).** Found that about 83 % of the birds excreted organisms within 83 % of the bird's excreted organisms within 24 h of challenge. However, all birds did not excrete organisms , formally on all sampling days a cyclical or intermittent fecal shedding pattern was observed similar observation were made in the study of **(Achen et al., 1998).** The