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CAMPYLOBACTER JEJUNI INFECTION IN JAPANESE QUAIL (*COTURNIX COTURNIX*) "ISOLATION, PATHOGENICITY AND PUBLIC HEALTH IMPLICATIONS"

(With 2 Tables and 2 Figures)

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إصابة السمان اليابانى بالكامبيلوباكتر جيوجينى " العزل والضراوة والأهمية الصحية "

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أجريت هذه الدراسة لمعرفة مدى تواجد ميكروب الكامبيلوباكتر المعوى فى طائر السمان اليابانى الذى يربى ويذبح للاستهلاك الآدمى. الأماكن التى تم العزل منها بالترتيب هى الأمعاء والمسحات المجمعية والكبد بنسبة ١٦-١٨% ، ١٠-١٤% ، ٤%. تم العزل من الأعمار التى تم دراستها والتى تراوحت من ٦-٢٤ أسبوع. اعتمد التصنيف البيوكيميائى على الإنتاج السريع لثانى أكسيد الكبريت وتحلل الهيبيورات والحساسية لحمض الناليديكس والنمو عند ٤٢ درجه مئوية بالاضافه إلى بعض الاختبارات الأخرى. عند حقن الميكروب فى أجنة بيض الدجاج عن طريقى كيس المح والغشاء الكوريوألآنتويس، تراوح زمن النفوق من ٤٨-٦٠ و٤٨-٧٢ ساعة بعد الحقن. ظهرت الآفات التشريحية على شكل التواء الأجنة وتضخم كيس المح واحتقانه وتغيرات موضعيه فى الكبد فى حالة الحقن عن طريق كيس المح بينما فى حالة الحقن عن طريق الغشاء الكوريوألآنتويس حدث احتقان شديد وتضخم فى الكبد مع وجود أنزفه على الأجنة. وعندما تمت عدوى الطيور عن طريق الفم كانت نسبة النفوق حوالى ١٥% فقط أما الأعراض فكانت عبارة عن إسهال وتشبع الريش فى منطقة المجمع بالزرق فى حوالى ٦٥% من الحالات. أظهرت الآفات التشريحية شكل التهابات معوية فى معظم الحالات المصابة وتغيرات موضعيه فى الكبد فى بعض الحالات. بدأ إفراز الميكروب فى زرق الطيور عند اليوم الثانى بعد العدوى وحتى نهاية التجربة من خلال المسحات المجمعية كما تم إعادة عزل الميكروب من المجمع والأمعاء فى كل الحالات كما تم العزل من قناة البيض والكبد فى بعض الحالات. كانت نسبة العزل من الأشخاص الذين يعانون من الاضطرابات المعوية ١٧.٩% بينما تم عزل الميكروب من ٨.٣% من الأشخاص الأصحاء ظاهريا. نوقشت

الأهمية الصحية والوبائية لعزل الميكروب من السمان والإنسان وكذلك الإجراءات الوقائية لحماية صحة الإنسان من الإصابة بالكامبيلوباكتر جيوجينى.

SUMMARY

Existence of *Campylobacter* species that colonize quail gastrointestinal tract raised and slaughtered as a source of food for human consumption were studied. The higher the frequency of isolation was obtained from intestinal tract & cecum, coloacal swabs, and liver in order. Percentage of isolation of *Campylobacter jejuni* was 16-18% from intestinal tract, 10-14% from coloacal swabs and 4% from liver. Isolation was done from different ages (6-24 weeks old) investigated in this study. *C. jejuni* was the only identified *Campylobacter* species during this study. Time to death of chicken embryo in case of yolk sac inoculation ranged from 48-60 hours post-inoculation, and 48-72 hours post-inoculation in case of chorio-allantoic membrane (CAM) route. Died embryos showed severe enlargment and congestion of yolk sac, curling and focal hepatic involvment in case of yolk sac route. Severe congestion and enlargment of liver as well as congestion of embryos in case of CAM infected embryos. Oral infection in 12 weeks old quail revealed, only 15% of birds were died during observation period. Diarrhoea and saturation of vent plumage were only noticed clinical signs in 65% of birds. Most of examined birds showed enteritis and increased intestinal contents specially at dudenum, and jejunum, while some birds showed focal hepatic changes. Excreation of *C. jejuni* started from second day post-infection till the end of observation period. *C. jejuni* was reisolated from intestinal tract and coloaca in all infected birds, while isolation from oviduct and liver was done from some cases. Stool swab cultures from human resulted in isolation of *C. jejuni* from 17.9% of examined persons with bowl incontinences and 8.3% from apparently healthy persons dealing with quail either in rearing or in slaughter shops.

***Key words**: Campylobacteriosis, C. jejuni, Quail, pathogenicity, Zoonoses.*

INTRODUCTION

Campylobacteriosis is one of bacterial infections affecting wide range of exotic and free-living birds and farm animals. This condition was retrospectively attributed to *Campylobacter jejuni* infection (Tauxe, 1992). Poultry serve as primary reservoir hosts of thermophilic campylobacters. Various species of *Campylobacter* have been isolated from free-ranging pigeons, game birds, marine birds, waders and

migratory anseriformes (Salem *et al.*, 1986; Maruyama and Katsube, 1988; Shane, 1997). The epidemiologic evidence fails to support any association between *C. jejuni* and classic hepatopathy syndrome (Soerjadi-Liem *et al*., 1984). Adayel (1993) reported the age susceptibility at 6-12 weeks of age in summer and autumn. The association of campylobacters with poultry meat represents a significant potential source for human food-borne infection under conditions of defective handling, inadequate refrigeration and improper preparation (Istre *et al.*, 1984). The correlation between specific *C. jejuni* and *C. coli* serotypes in poultry and in diarrhic humans has been documented (Annan-Prah and Janc, 1988). While *Campylobacter* infection in human can occur by direct contact with animal and human carriers of the organism, the principle source of infection are non-chlorinated water, raw milk, and raw of undercooked meat or poultry and other avian species (Blaser, 1997). Since various species of domestic poultry serve as reservoir hosts of *C. jejuni*, infection is significant primarily in relation to food-borne enterocoloacitis in consumers of broilers, turkeys and potentially eggs (Maruyama *et al.*, 1995).

The major clinical manifestation of *Campylobacter* enteritis in human is an acute diarrhoeal illness often with acute abdominal cramping and fever. Other symptoms often present are nausea, headache and muscle pain. The illness usually occurs 2-5 days after ingestion of contaminated food or water (Blaser, 1997). *Campylobacter* spp. are among the most frequently reported causes of bacterial entritis in the developing countries. The WHO recommended the relative importance of the different potential sources of human *Campylobacter* infection needs to be elucidated and noted that this may vary from one country to another (WHO, 1994). The number of human cases of campylobacteriosis has increases dramatically in recent years in many countries (Nielsen *et al*., 2000; Anonymous, 2000). Due to the previous investigations on *Campylobacter* species which could be isolated from human (Istre *et al*., 1984) and animal sources (Shane, 1997), this study was planned to isolate *C. jejuni* as a causative agent increminated in daily quail mortality in quail farms and local slaughter shops, experimental infection in quails and its public health importance.

MATERIALS and METHODS

Collection of samples

Quails: Fifty samples were collected from daily mortalities of Japanese quail obtained from animal production farm, Faculty of Agriculture, Assiut University. Two hundred samples were obtained from local

slaughter shops in Assiut Governorate. Birds were of different ages. Freshly dead birds were pathologicaly examined; intestinal tract and liver were subjected for isolation of *Campylobacter* species. Coloacal swabs from living birds were also applied for *C. jejuni* isolation

Human: Stool specimens from 52 persons working at quail farm and local slaughter shops were examined. Twenty-eight persons with bowl incontinience, while 24 persons were apparantly healthy. Samples were collected into sterile plastic containers. Swabs from these samples were cultured on suplemented brucella blood agar and incubated in microairophilic atmosphere at 42ºC for *Campylobacter* isolation.

Isolation of *Campylobacter* species

Coloacal, cecal, intestinal swabs and liver tissues from quails from different sources as well as human stool were plated on brucella blood agar (10% sheep blood). *Campylobacter* selective supplement containing vancomycin 5 mg; trimethoprim 2.5 mg and polymixin B 1250 IU (Skirrow, SR0069E, Oxoid) was added as 1 vial for 500 ml. *Campylobacter* agar with charcoal and deoxycholate plus supplement (CCDA, Oxoid) were used. Plates were incubated for 48 hours in a microaerophilic condition at 42ºC. The microaerophilic condition was created in an anaerobic jar using gas generating kits (Oxoid), producing 5% oxygen, 10% CO_2 and 85% nitrogen. The grown bacteria were subcultured and incubated at 25ºC for 48 hours (Mossel, 1985).

Morphological and Biotyping criterea

Staining: *Campylobacter* spp. staining was done using a modified Gram stain with 0.8% solution of basic carbol fuchsin instead of safranine.

Motility test: The test was carried out using brucella semisolid agar (0.2% agar) and incubated for 36-48 hours at 42ºC.

Haemolysis: Through culturing on on brucella blood agar plates.

Oxidase test: A portion of a colony from recently inoculated CCDA was smeared with an inoculating needle onto an oxidase strip. The oxidase positive indicated by developing of dark purple color in 5-10 seconds.

Catalase test: A portion of a colony from recently inoculated CCDA was mixed with a drop of 30% H_2O_2 on a clean glass slide.

Rapid production of hydrogen sulfide: Rapid production of hydrogen sulfide (H_2S) was carried out according to Lior, (1984) using semisolid H_2S test medium containing (brucella broth, 0.2% agar, disodium hydrogen phosphate, dihydrogen potasium phosphate, ferrous sulfate, sodium metabisulfite and sodium pyruvate). A large ball-like inoculum from a 24 hours culture was gently suspended into the upper third of the

test medium without mixing. The tube was incubated in a 37ºC waterbath for 2 hours. A rapid blackening around the bacterial mass represents a positive reaction.

Hippurate hydrolysis: Colonies from Muller Hinton blood agar culture was inoculated into 0.5 ml of sterile sodium hippurate solution in a small screw capped tube. Tubes were incubated including an uninoculated control, aerobically at 37ºC for 3 hours. Overlayed with 0.2 ml of ninhydrin solution without mixing. Development of a deep purple color within 5 min, indicates a positive result while development of a slight bluish color regarded as negative results.

Nalidixic acid sensitivity: According to Lior (1984), 2-3 typical colonies were streaked on brucella blood agar plate. Nalidixic acid sensitivity disc (30 µg) was placed on streaked surface. The plate was incubated at 42ºC for 36 hours in a microaerobic environment. Inhibition zone surrounding the disc indicates sensitivity to nalidixic acid. This test can differentiates between *C. jejuni*, *C. coli* and *C. lari*.

Propagation using embryonated chicken eggs

Typical morphologically and biochemically suspected colonies were picked up into brucella broth containing selective agents, incubated at 42ºC for 48 hours. 0.2 ml of broth culture of each isolate was inoculated in 7 and 11-day-old embryonating chicken eggs in yolk sac and on CAM, incubated at 38ºC in humidity, frequent turning, and twice daily candling. Died embryos were exposed and examined (Field *et al*., 1986; Lam *et al*., 1992).

Experimental infection

Two groups of 12 weeks old Japanese quail proved by coloacal swab culture to be free from campylobacters. The first group consisted of 20 birds, orally infected with brucella broth culture containing 10^7 CFU/ml of isolated strain were kept and observed for two weeks. Coloacal sawbs were cultured on CCDA plates every day for tracing the *Campylobacter* excreation. Deathes were recorded and at the end of second week, living quails were sacrificed and subjected for *Campylobacter* isolation from different organs. Another group of 5 birds was kept as non-infected control and recieved sterile broth (Maruyama and Katsube,1990).

RESULTS

The necropsy findings were not pathognomonic and noticed as enteritis specially in small intestine and cecum, contents were dark and watery. Sometimes intestinal tract and liver were congested.

In case of daily mortalities obtained from quail farm, isolation was done from cecum & intestinal tract, coloacal swabs and liver with percentage of 16%, 14% and 4% respectively. Campylobacters were isolated from quails in slaughter shops with percentage of 18% and 10% from intestinal tract and coloacal sawbs respectively. Isolation was done from different ages (6-24 weeks old) investigated in this study. (Table1).

Stool swab cultures from human with bowl incontinence resulted in isolation of *C. jejuni* from five persons (5/28) with percentage of 17.9%. Two out of 24 apparently healthy persons were positive for *C. jejuni* isolation from their stool (8.3%) (Table 1).

Isolated *Campylobacter* was small Gram negative curved or spiral rods, motile in semisolid brucella agar and non-haemolytic when grown on brucella blood agar. All isolates grown at 42ºC and did not grow at 25ºC. Oxidase positive, catalase positive, rapidly produced H_2S within 45-60 minutes, hydrolyse hippurate, and produced inhibition zone of 12-14 mm in nalidixic acid sensitivity testing (Table 2).

Time to death in case of yolk sac inoculation ranged from 48-60 hours post-inoculation, and 48-72 hours post-inoculation in case of CAM. Died embryos showed severe congestion and enlargment of liver (Fig 1A), focal hepatic involvment (Fig. 1B), congestion and curling of embryos (Fig. 1C), and severe enlargment and congestion of yolk sac (Fig. 1D).

In experimental infection trial via oral route, only 3/20 (15%) of birds were died during observation period. Diarhoea and saturation of vent plumage were only noticed clinical signs in 13/20 birds (65%). Most of examined birds showed enteritis and increased intestinal contents (Fig. 2). Excreation of *Campylobacter* started from second day post-infection till the end of observation period. *Campylobacter* was reisolated from intestinal tract and coloaca in all diseased birds, while isolation was done from few cases from oviduct and liver.

DISCUSSION

In this work existence of *Campylobacter* species that colonize Japanese quail gastrointestinal tract raised and slaughtered as a source of food for human consumption were studied. Infection has been recorded among game birds such as quail, patridges and pheasants (Minakashi *et al.,* 1988).

Regarding to present results, the higher the frequency of isolation was obtained from intestinal tract, cecum, coloacal swabs, and liver in

order. Percentage of isolation of *C. jejuni* was 16-18% from intestinal tract, 10-14% from coloacal swabs and 4% from liver. Isolation was done from different ages (6-24 weeks old) investigated in this study. Several authors come in agreement with the present results, Wallace *et al*., (1997) analysed *Campylobacter* colonization at different sites along the gastrointestinal tract of mature turkeys at slaughter shops and stated that numbers increased with distance from beak and were highest in the ceca. Thermophilic *Campylobacter* can be isolated from feces, cecal and jujenal contents. With systemic infection, the organism can also be recovered from liver tissue, bile and blood (Misawa *et al.,* 1996; Shane, 1997). Carvalho *et al*., (1997) concluded that the liver may be the organ of choice for isolation in presence of diarrhoea in chickens. Stephens *et al*., (1998) isolated *C. jejuni* and *C. coli* from disease causing high mortality and morbidity from young ostriches aged (2-8 weeks old) in Australia. Those were suffering from bright green diarhoea and severe necrotic hepatitis.

C. jejuni was the only identified *Campylobacter* species during this study. All tested isolates gave rapid production of H_2S, hippurate hydrolysis and sensitivity to nalidixic acid. Isolation and biotyping and growth time and incubation at 42-43ºC agreed with Skirrow and Benjamin, (1980). The incubation period for detecting growth generally exceed 24 hours, with low concentration of organism in the inoculum specialy in presence of inhibitory medium, incubation for up to 72 hours may be required to observe colony formation (Morris *et al*., 1982). Colonies are nonhaemolytic on blood agar (Smibert, 1984).

Time to death in case of yolk sac inoculation ranged from 48-60 hours post-inoculation, and from 48-72 hours post-inoculation in case of CAM. Died embryos showed severe enlargment and congestion of yolk sac, curling and focal hepatic involvment in case of yolk sac route. Severe congestion and enlargment of liver as well as congestion of embryos in case of CAM infected embryos. Victoria (1991) stated that, the pathogenicity of the various *C. jejuni* and *C. coli* isolates was investigated using chicken embryo model, and found a marked variation in pathogenicity for chicken embryo. Similar findings were described by several authors; Field *et al*., (1986) and Lam *et al*., (1992) concluded that fertile chicken eggs serve as a convenient system for isolation and propagation of *Campylobacter* via yolk sac, CAM, and intravenous routes, death produced with hepatic necrosis and generalized haemorrhages.

Orally infected quails aged 12 weeks old, only 3/20 (15%) of birds were died during observation period. Diarrhoea and saturation of vent plumage were only noticed clinical signs in 13/20 birds (65%). Most of examined birds showed enteritis and increased intestinal contents specially at dudenum, and jejunum, while some birds showed focal hepatic changes. Excreation of *Campylobacter* started from second day post-infection till the end of observation period. *Campylobacter* was reisolated from intestinal tract, coloaca, oviduct and liver. Shane, (1997) found clinical picture similar to that observed in present study. Distention of the intestinal tract extending from the distal dudenal loop to the cecal biforcation with accumulation of mucous and watery fluid occurs, haemorrhages may be present, liver involvment may occurs due to infection with toxigenic and invasive strains of *C. jejuni*.

Poultry and related avian species are a major source of *Campylobacter* infections in humans, but how the organism gains access to broiler facilities is unclear. Theories include spread from animal reservoirs (Ziprin *et al.,* 2003b), presence of *Campylobacter* in water (Stern *et al.,* 2002), presence of viable but noncultrable forms in water (Ziprin *et al.,* 2003a), spread by rodent and insect vectors (Shane, 1997), contamination of hatcheries (Wallace *et al.,* 1997), and vertical transmission through breeder stock (Cox *et al.,* 2002). It is possible that each of these plays some role.

Campylobacters are often found in the intestine of animals raised for food production. Poultry and other avian species are primary sources of campylobacteriosis. The high body temperatures of birds are thought to be more suitable for the growth of *Campylobacter*. It is estimated that 1% of the general population is infected each year (Kaminstein, 1999). During the last decade, *Campylobacter* has showen to be an important cause of diarrhoea worldwide, affecting persons of all ages, in both industrialized and developing nation. Data obtained from developing countries demonstrated that *Campylobacter* species are more frequently isolated, but rates of recovery from healthy carriers are often similarly high (Blaser, 1997).

Stool swab cultures from human with bowl incontinence resulted in isolation of *C. jejuni* from 17.9% of examined persons, while 8.3% of apparently healthy persons were positive for *C. jejuni* isolation (Istre *et al.,* 1984). Izat and Gardner, 1988 stated that staff of poultry processing plants are exposed to campylobacteriosis by handling contaminated material and the condition may be regarded as occupational disease. Also Takahiko *et al*., (1997) isolated *C. jejuni* from 39% of stool swabs

tacken from enteric patients. El-Prince *et al.*, 1998 stated that campylobacters were recovered from 26.7% of babies diarrhial stools in Assiut Governorate, Egypt. Moreover, in Netherlands, Wit *et al.*, 2000, found that *Campylobacter* were cultured in 4.5% in patients with gastrienteritis as well as Mclver *et al.*, 2001 isolated *C. jejuni* (12%) from children under 6 years with diarrhoea.

Sporadic nature of human campylobacteriosis and the ubiquitous distribution of the bacteria have traditionally hindered the unequivocal identification of sources of infection (Nielsen *et al*., 2000). Tauxe, 1992 stated that contaminated, undercooked poultry meat is believed to be a sigificant vector of sporadically detected human disease. A study on infectious intestinal disease in the population in England between 1993 and 1996 revealed that *Camlylobacter* spp. were isolated from 4.2% of cases in the community. *C. jejuni* and *C. coli* accounted for 88% and 9% of *Camlylobacter* isolates (Madden, 1998). Also in the United States, *Camlylobacter* is the most common bacterial cause of diarrhoea. The Centers for Disease Control and Prevention estimates there are more than 2 million cases of camlylobacteriosis each year. In addition, Iceland, with its insulated poultry industry, was the ideal place to research the source of *Camlylobacter* (Durham, 2001). Campylobacters are carried in the intestinal tract of a wide variety of wild and domestic animals, especially birds and other related avian species. They can establish a temporary asymptomatic carrier state, as well as illness, in human. This is especially prevalent in developing countries (Nachamkin *et al*., 1992). During processing, poultry and other related avian species carcasses may contaminated by the release of intestinal contents. Levels greater than 10^6 CFU per carcass can be enumerated from commercial product. The infectious dose of *C. jejuni* for humans may be as few as 500 cells. The organism cannot grow under 32 degrees, consequently, the levels found on the carcass represents an important consumer exposure and the risk for infection (Norman, 1998).

In brief, *C. jejuni* infection in poultry in general causing enteritis and diarrhoea, hence during preliminary diagnosis or feild diagnosis may be passed as one of common enteric diseases like clostridial infection, salmonellosis, coccidiosis and consequently misdiagnosed and treatment failiure occurs. For that reasons, campylobacteriosis should be listed as on of the most important avian pathogens and serious zoonotic infection. Moreover, infection of quail represent a mode of spreade of infection or reservoir of infection to other domestic birds and human beings.

Good hand washing technique as well as proper preparation and cooking of food is the best way to prevent campylobacteriosis in human. In addition, refrigerate foods promptly, minimize holding at room temperature, wrap fresh meats in plastic bags at the market to prevent blood from dripping on other foods, cutting broads and counters used for preparation should be washed immediately after use to prevent cross contamination with other foods; houses, floors, and incubators in quail farms should be cleaned and disinfected at intervals; avoid contact with dropping of quails; hygienic disposal of quail excreta; people with bowel incontinence, unable to control bowel function, should be isolated until they are free from symptoms of diarrhoea as well as exclude symptomatic patients from food hanling generally until asymptomatic and specific antimicrobial therapy for patients should be adopted (Ebeid *et al*., 1999).

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Table 1: Epidemiological data of *C. jejuni* isolation from quails and human

Number	Source	Isolation	Rate	Percent
50	Quail farm	Intestine	8/50	16%
		Coloacal swabs	7/50	14%
		Liver	2/50	4%
200	Slaughter shops	Intestine	36/200	18%
		Coloacal swabs	20/200	10%
28	Persons with bowel incontinence	Fecal swabs	5/28	17.9%
24	Apparently healthy persons	Fecal swabs	2/24	8.3%

Table 2: Biotyping of *C. jejuni* isolated from quails

Test	Result
Carbol fuchsin-based staining	Gram negative, curved rods
Motility in semisolid media	Positive
Growth at 42ºC	Positive
Growth at 25ºC	Negative
Hemolysis	Negative
Oxidase	Positive
Catalase	Positive
Rapid H_2S production	Positive within 1 hour
Hippurate hydrolysis	Positive
Nalidixic acid sensitivity	Positive 12-14 mm

Fig. 1: Chicken embryo inoculated on CAM, died embryos showing severe congestion and enlargment of liver (Fig 1A), focal hepatic involvment (Fig 1B), congestion and curling of embryos (Fig 1C), chicken embryo inoculated via yolk sac; died embryos showing severe enlargment and congestion of yolk sac (Fig 1D).

Fig. 2: Japanese quail orally infected with 10^7 CFU/ml of broth culture of isolated *C. jejuni*. Arrow indicates distention of duodenal and jejunal portion and hepatic changes