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# OCCURRENCE AND MOLECULAR DETECTION OF *CAMPYLOBACTER JEJUNI* AND *COLI* IN TABLE EGGS AND STOOL OF DIARRHEIC PATIENTS

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## ABSTRACT

Inappropriate handling of eggs can still result in infection from bacteria such as *Campylobacter*, which mostly contaminates the surface of the eggshell, so this study focused on the molecular detection of *Campylobacter jejuni* and *Campylobacter coli* in table eggs and stool of diarrheic patients. The conventional PCR was carried out on 40 egg samples, 20 from each farm and baladi types equally, and 20 stool samples from diarrheic patients. It was found that *Campylobacter* spp. infection was prevalent in farm and baladi eggshell samples, and positive *C. jejuni* DNA was detected in 45% and 15%, respectively. In comparison, 50% and 40% of farm and baladi egg shell samples were positive *C. coli*. However, neither *C. jejuni* nor *C. coli* DNA was detected in the egg contents of either farm or baladi eggs. The prevalence of Campylobacter spp. infection was 70% and 50% in farm and baladi eggshell samples, respectively. *C. coli* was detected in 50% of diarrheic patients' stool, while *C. jejuni* wasn't detected. The prevalence of *C. coli* infection was higher in females than males. The 3Y-5Y age group had the highest prevalence (75%) and the lowest (40%) was in the more than 5Y group. The prevalence of *C. coli* infection in diarrheic patients was highest in patients with vomiting, followed by dehydration, abdominal pain, and fever. The study recommended good hygienic practices inside poultry farms and farmer's houses and increasing awareness of poultry farm workers and farmers from infection.

***Key Words:*** Eggs, stool, *C. jejuni*, *C. coli*, PCR

## INTRODUCTION

Gram-negative *Campylobacter* species come in a variety of morphologies, including spiral, curved, and rod-shaped bacteria (Mobaien *et al.*, 2016). Depending on the species, they can have a single polar flagellum, bipolar flagellum, or none at all. According to reports, there are at least 12 different species of *Campylobacter* that may infect humans, with *Campylobacter jejuni (C. jejuni)* and *Campylobacter coli* (*C. coli*) being the most prevalent ones (Ansari-Lari *et al.*, 2011). Eggs are important to the diet of Egyptians and are one of the supply-managed commodities in Egypt. Despite

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their high nutritional content, eggs have been linked to multiple outbreaks and are a means of disease transmission to consumers (Hangombe *et al.,* 1999 and Gast *et al.,* 2004). Chicken products are considered the main vehicles for human infections and foodborne illness with campylobacteriosis (Kim and Lee, 2017). *Campylobacter*, a foodborne pathogen closely associated with poultry, is one of the bacterial etiologic agents of human gastroenteritis (Hiett *et al*., 2013). *C. jejuni* and *C. coli* are responsible for food poisoning in humans (Casalino *et al*., 2022).

Many factors can lead to contaminate eggs, including ovary infections caused by bacterial pathogens, which can infect eggs before they are laid in a bird's genital system. Following the bird's laying, the shell quickly becomes contaminated with a variety of organisms by contact with food, feces from the bird, washing water, handling, or maybe the accumulation of eggs (DeReu *et al.,* 2008). Avian vibrionic hepatitis is a contagious disease that affects both young and mature chickens and is due to *C. jejuni*. It is characterized by low mortality, substantial morbidity linked to a chronic course, and poor growth and productivity (Peckham, 1984).

One of the most common sources of *Campylobacter* as a zoonotic disease is poultry (Rahimi and Tajbakhsh, 2008). *Campylobacter* infection has spread along with the development of the poultry industry in the world, as poultry is the main source of spread for *Campylobacter* spp. (Amjad, 2023).

*Campylobacter* spp. is one of the most commonly reported bacterial causes of acute diarrheal disease in humans throughout the world (Wang *et al*., 2001). Approximately 90% of the world's human campylo-bacteriosis cases are caused by *C. jejuni*, while the remaining 10% are caused by *C. coli*. The disease's symptoms include fever, abdominal pain, and occasionally bloody diarrhea (Rastyani *et al.,* 2015). Serious long-term side effects include reactive arthritis, Miller Fisher syndrome, and peripheral neuropathies are rare. The most frequent cause of neurological sequelae is infection with *C. jejuni* (Rastyani *et al.,* 2015).

Cultural methods of identifying *Campylobacters* and differentiating between species within the genus are difficult, time-consuming and challenging due to their fastidious and restricted growth requirements and very inert biochemical properties (Penner, 1988, On and Holmes, 1992, and Sanders, 1998). Bacterial inoculum size (On and Holmes, 1991), which can be hard to regulate, has an impact on the precision of certain biochemical assays. Furthermore, *Campylobacter* cells are typically found in extremely small quantities and are susceptible to damage from food and natural water, rendering them unsuitable for cultivation (Humphrey, 1986, Rollins and Colwell, 1986, Beumer *et al.,* 1992; and Medema *et al.,* 1992). These factors led to the development of nucleic acid-based detection techniques as choices for *Campylobacter* identification. PCR is a rapid and sensitive method for the detection of *C. jejuni* and *C. coli* from eggs (Kim and Lee, 2017).

Serious health hazard outbreaks linked to egg consumption continued even after important control measures for food safety regarding consumer health had been implemented. Therefore, the purpose of the study is to direct molecular detection of *C. jejuni* and *C. coli* in table eggs and stool of diarrheic patients in Assiut city, Egypt.

## MATERIALS AND METHODS

### 1. Ethical approval

The Scientific Research Committee and Ethics Board of the Faculty of Medicine, Assiut University, Assiut, Egypt, conducted an ethical evaluation and gave their approval to the research. IRB No 04-2024-300513 is the number for ethical approval. To take part

in this study, the patients gave their written informed consent.

## 2. Sample collection and preparation

### 2.1. Collection of egg samples

Forty fresh hen`s egg samples (pooled samples), including poultry farm´s eggs and farmer´s house eggs (baladi eggs), were collected for molecular detection of *C. jejuni* and *C. coli* on egg shells, and contents (20 pooled samples for each type, each pooled sample was represented by 3 eggs) (Pande *et al.,* 2016). These samples were collected from June to September 2024. Farm egg samples were purchased from different shops and supermarkets, while Baladi egg samples were purchased from farmer´s houses. All the samples were obtained randomly from different locations in Assiut city, Egypt. These samples were transferred in plastic bags in a tank to the laboratory. Whole eggs were stored in a refrigerator at 4˚C until DNA extraction.

### 2.2. Preparation of egg samples

According to (Pande *et al.,* 2016), the 3 eggs of each pooled egg sample were washed in 15 ml of sterile saline in a sterile beaker of 1000 c.c. capacity, rubbed by a sterile swab, and taken as an eggshell sample in a sterile falcon tube of 15 ml, from which 200 µl was taken in a sterile Eppendorf tube for DNA extraction. The three eggs were sprayed with 70% alcohol and exposed to flame on their broad ends and aseptically broken. Their contents were taken into a sterile beaker and thoroughly homogenized and mixed by a sterile fork. 200 µl was taken in a sterile Eppendorf tube as an egg content sample. The egg shell and egg content samples were stored at -20°C until DNA extraction. This part of the work was done in the Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

### 2.3. Collection of stool samples

Twenty stool samples were collected from diarrheic patients of various ages in different hospitals and medical laboratories in Assiut city, Egypt, from June to September 2024. The stool samples were collected in sterile cups, transferred in a tank to the laboratory, and preserved at -20˚C until molecular examination for the presence of *C. jejuni* and *C. coli* infection.

## 3. Molecular detection.

### 3.1. DNA extraction.

From eggshells, content, and stool samples, DNA extraction was performed according to the manufacturer's instructions for the ABT Genomic DNA Mini Extraction Kit (Cat. No. EX01). The whole DNA of samples was extracted and stored at -20°C.

### 3.2. PCR amplification and primer design.

PCR was used to amplify the *C. jejuni* genome's $mapA_{C.jejuni}$ gene with a length of 589 bp PCR products and *C. coli* genome's $ceuE_{C.coli}$ gene with a length of 462 bp PCR products were produced. The total volume of the PCR reaction was 20 µl. The reaction contained the following 10 µl 2X ABT red master mix (Applied Biotechnology, Egypt) (Cat. No. AMP 01), 1 µl for each forward and reverse primer (10 pmol) and 8µl template DNA. The conditions for primers during PCR started with initial denaturation at 94˚C for 5 min, followed by 45 cycles including the denaturation phase at 94˚C for 30 sec, annealing phase for 60 sec at 56.5°C and 58°C for *C. jejuni* and *C. coli* primers, and the extension phase at 72˚C for 40 sec. At the end, the final extension was carried at 72˚C for 10 min. The PCR amplification was performed in a thermal cycler (Kyratec. Thermal Cycler, Adelab Scientifi, Australia). The primers for detecting DNAs of the genus *Campylobacter* (*C. coli* and *C. jejuni)* directly in samples were developed as shown in the following Table.

**The Primers used in PCR reaction.**

| PCR target and gene | Primer name (internal EURL no.) | $T_m$ (°c) | Sequence (5′ to 3′) | Size (bp) | Reference |
|---|---|---|---|---|---|
| ***mapA*** $_{C.jejuni}$ | MDmapA1 (3034) | 58 °c | 5'-CTA TTT TAT TTT TGA GTG CTT GTG-3' | 589 bp | **(Eunju and Lee, 2009)** |
| | MDmapA2 (3035) | | 5'-GCT TTA TTT GCC ATT TGT TTT ATT A-3' | | |
| ***ceuE*** $_{C.coli}$ | COL3 (3036) | 58 °c | 5'-AAT TGA AAA TTG CTC CAA CTA TG -3' | 462 bp | |
| | MDCOL2 (3037) | | 5'-TGA TTT TAT TAT TTG TAG CAG CG-3' | | |

### 3.3. Gel electrophoresis

For visualizing, 8 µl of amplified PCR products were subjected to 40 minutes of gel electrophoresis at 150 V and 116 mA in a 1.5% agarose molecular grade, from GENETIX biotech ASIA PVT.LTD. (Cat.:PG-40005). It is prepared according to Sambrook *et al*. (1989), and then mixed with ethidium bromide solution 0.5g/ml (2 µl / 100 ml gel) (applied Biotechnology, Cat.No.EL01) for electrophoresis using Electrophoresis Equipment (Cleaver Scientific & multiSUB Midi, Midi Horizontal Electrophoresis System, UK). The gell was documented and visualized on a UV transilluminator (Gel Imager - Azure 200, USA), to view the amplicons after their size was determined using size marker DNA of 100 bp (BERUS 100 bp, WF-10407002). The part of molecular detection was made in Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

### 4. Statistical analysis

Chi-square tests were applied using the statistical package for the social sciences (SPSS) Statistics software (Version 16, 2007) to determine the prevalence of *C. jejuni* and *C. coli* in eggshell and stool samples, and also to obtain the influence of each risk factor separately on the molecular detection of *C. coli* in the diarrheic patients under study (i.e., sex, age, and accompanied symptoms). A probability value (P-value) of $P<0.05$ was deemed statistically significant.

## RESULTS

### 1. Molecular detection of *Campylobacter* spp in egg samples.

### 1.1. Prevalence of *Campylobacter* spp. in egg types

According to Table (1), positive *C. jejuni* DNA was detected in 45% and 15% of farm and baladi eggshell samples, respectively **(**Figure 1), with no significance difference, while positive *C. coli* DNA was observed in 50% and 40% of farm and baladi eggshell samples, respectively **(**Figure 2), with no significant variation. Fortunately, both *C. jejuni* and *C. coli* DNA weren't detected in the egg contents of either farm or baladi eggs.

**Table 1:** The prevalence of *Campylobacter* spp. infection in egg samples.

| Egg samples | | No. of examined samples | *Campylobacter* spp positive samples | | *P value* |
|---|---|---|---|---|---|
| | | | *C. jejuni* | *C. coli* | |
| Farm eggs | Shell | 20 | 9 (45%) | 10 (50%) | 0.279 |
| | Content | | 0 (0%) | 0 (0%) | |
| Baladi eggs | Shell | 20 | 3 (15%) | 8(40%) | |
| | Content | | 0 (0%) | 0 (0%) | |

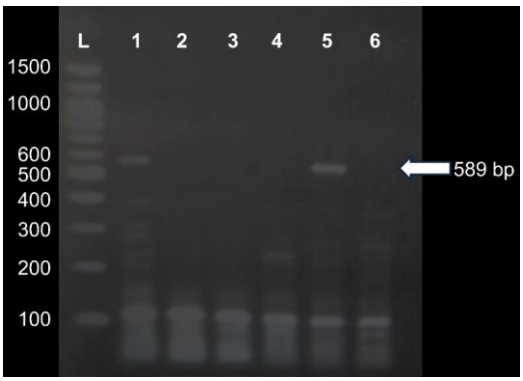


**Figure 1:** Amplification of *C. jejuni* genome's mapA*C.jejuni* gene with 589 bp length PCR products. Lane L. DNA ladder (100 bp), Lane 1 *C. jejuni positive* farm eggshell sample, Lane 5 *C. jejuni positive* baladi egg shell sample and Lane 2,3,4,6 *C. jejuni* negatives samples.

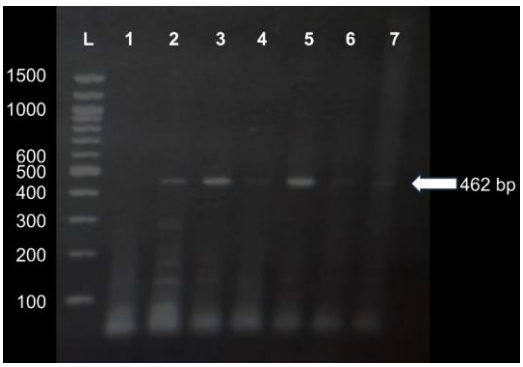


**Figure 2:** Amplification of *C. coli* genome's ceuE*C.coli* gene with 462 bp length PCR products. Lane L. DNA ladder (100 bp), Lane 1 *C. coli* negative sample, Lane 2,3 *C. col positive* farm egg shell samples, Lane,4,5 *C. coli positive* baladi egg shell samples and Lane 6,7 *C. coli* stool positive samples.

**1.2. Correlation between the detected eggshell samples according to *Campylobacter* spp. infection.**

From the results obtained in Table (2), 4 (20%), 5 (25%), and 5 (25%) of farm eggshell samples contained *C. jejuni* only, *C. coli* only, and mixed *Campylobacter* spp. DNA, respectively, with total *Campylobacter* spp. detection of 70% (14/20) in farm eggshell samples. Also, from the data demonstrated in Table (2), *C. jejuni* was only detected in 2 (10%) of baladi eggshell samples, *while C. coli* only was observed in 7 (35%). Only one (5%) baladi eggshell sample contained mixed *Campylobacter* spp. infection, with total *Campylobacter* spp. detection of 50% (10/20) in baladi eggshell samples. There was no significant variation between both egg types according to *Campylobacter* spp. detection.

**Table 2:** The correlation between the detected eggshell samples according to *Campylobacter* spp. infection.

| Samples types | No. of examined samples | *Campylobacter* spp. positive samples | | | | *P value* |
|---|---|---|---|---|---|---|
| | | *C. jejuni only* | *C. coli only* | Mixed infection | Total | |
| Farm egg shell | 20 | 4 (20%) | 5(25%) | 5(25%) | 14(70%) | 0.214 |
| Baladi egg shell | 20 | 2 (10%) | 7(35%) | 1(5%) | 10(50%) | |
| | 40 | 6 | 12 | 6 | 24(60%) | |

**2. Molecular detection of *Campylobacter* spp. in diarrheic patient samples.**

**2.1. The prevalence of *Campylobacter* spp. infection among diarrheic patients**

Out of the 20 diarrheic patient samples, 0% (0/20) was positive for *C. jejuni* and 50% for *C. coli* (10/20). According to *Campylobacter* infection detected in our study in diarrheic patients, samples belonged to *C. coli* only (Table 3 and Figure 2).

**Table 3:** The prevalence of *C.coli* infection among diarrheic patients

| No. of examined samples | *Campylobacter* spp | *Campylobacter* positive samples | |
|---|---|---|---|
| | | No. | % |
| 20 | *C. jejuni* | 0 | 0 % |
| | *C. coli* | 10 | 50% |
| Total | | 10 | 50% |

### 2.2. The prevalence of *C. coli* infection in diarrheic patients according to sex

According to Table (4), the prevalence of *C. coli* was higher in females than males by a percentage of 55.55% and 45.45%, respectively. There was no significant variation between males and females.

**Table 4:** The prevalence of *C. coli* infection in diarrheic patients according to sex.

| Sex | No. of examined samples | *Campylobacter* positive samples | |
|---|---|---|---|
| | | No. | % |
| Males | 11 | 5 | 45.45% |
| Females | 9 | 5 | 55.55% |
| Total | 20 | 10 | 50% |
| *P value* | 0.653 | | |

### 2.3.The prevalence of *C. coli* infection in diarrheic patients according to age

Belonging to the ages, it was noted that the highest prevalence of *C. coli was* detected in age group 3Y-5Y (75%) followed by 8M-2Y (50%) and the lowest prevalence of *C. coli* found in more than 5Y group (40%), Table (5). There was no significant variation between age groups.

**Table 5:** The prevalence of *C. coli* infection in diarrheic patients according to age

| Age | No. of examined samples | *Campylobacter* positive samples | |
|---|---|---|---|
| | | No. | % |
| 8M -2 Y | 6 | 3 | 50% |
| 3 Y – 5 Y | 4 | 3 | 75% |
| 5 Y-15Y | 10 | 4 | 40% |
| Total | 20 | 10 | 50% |
| *P value* | 0.497 | | |

### 2.4.The prevalence of *C. coli* infection in diarrheic patients according to accompanied symptoms

In Table (6), it was observed that 36.36% of diarrheic samples were accompanied by other symptoms, and 66.67% of samples were diarrheic only without significant variation between the two groups. According to Table (7), a correlation between *C. coli* infection in diarrheic patients and vomiting was found in a prevalence of 100% in *C. coli* infected samples, followed by dehydration in a prevalence of 33.33% in *C. coli* infected samples, abdominal pain in a prevalence of 27.27% in *C. coli* infected samples, and fever in a prevalence of 25% in *C. coli* infected samples.

**Table 6:** Prevalence of *C. coli* infection in diarrheic patients according to symptoms.

| Accompanied symptoms | No. of examined samples | Positive samples | *P value* |
|---|---|---|---|
| | | No. (%) | |
| With accompanied symptoms | 11 | 4 (36.36%) | 0.178 |
| Without accompanied symptoms | 9 | 6 (66.67%) | |
| Total | 20 | 10 (50%) | |

### 2.5.The correlation between positive *C. coli* infection in diarrheic patients

Depending on the illustrated data in Table (8), 25% of positive *C. coli* diarrheic patients suffered from fever and abdominal pain, others from vomiting and dehydration, others from vomiting and abdominal pain, and others from abdominal pain only. It was noted that the

abdominal pain symptom (75%) was the most common accompanied symptom in positive *C. coli* diarrheic patients, then vomiting (50%). Finally, fever and dehydration (25%) were the less commonly accompanied symptoms in positive *C. coli* diarrheic patients with accompanied symptoms.

**Table 7:** The prevalence of *C. coli* infection in diarrheic patients according to accompanied symptoms.

| Accompanied symptoms | No. of examined patients | *Campylobacter* positive samples | |
|---|---|---|---|
| | | No. | % |
| Fever | 4 | 1 | 25% |
| Vomiting | 2 | 2 | 100% |
| Abdominal pain | 11 | 3 | 27.27% |
| Dehydration | 3 | 1 | 33.33% |

**Table 8:** The correlation between positive *C. coli* infection in diarrheic patients according to accompanied symptoms.

| Positive patients with accompanied symptoms (4) | Accompanied symptoms | | | |
|---|---|---|---|---|
| | Fever | vomiting | Abdominal pain | Dehydration |
| 1 | √ | | √ | |
| 1 | | √ | | √ |
| 1 | | √ | √ | |
| 1 | | | √ | |
| Total | 1 (25%) | 2 (50%) | 3 (75%) | 1 (25%) |

## DISCUSSION

Little information was available at the time about *Campylobacter* contamination of table eggs, so a better understanding of the egg's role in the spread of *C. jejuni* and *coli* is necessary (Fonseca *et al*., 2014). When the shell passes through the vent, it may already contaminate. According to a theory, bacteria that are deposited on the egg's surface after it has been laid may penetrate the shell and contaminate the egg's contents (Messens *et al.*, 2007). Depending on the species, they can have a single polar flagellum, bipolar flagellum, or none at all (Ansari-Lari *et al.*, 2011). In the eggs stored at 18°C, *Campylobacter* can survive for up to 14 days (Sahin *et al*., 2003). The common etiological agent of human diarrhea is now known to be *C. jejuni* and *coli*. In broiler farms, these microbes are widely distributed (Wieliczko, 1995). The egg passage can mean more than vertical, transovarian transmission of *Campylobacter* spp.. The eggs that have contaminated shell with *Campylobacter* spp., represent serious problems for poultry farms, because fecal bacteria, including *Campylobacter*, can contaminate the shell, shell membranes, and albumen of freshly laid fertile eggs. This contamination is drawn through the shell by temperature differential, aided by the presence of moisture (the "sweating" of the egg). When the chick emerges from the egg, it can ingest *Campylobacter*, become colonized, and spread this contamination to flock mates in the grow house (Cox *et al*., 2012).

Also, the eggs that have contaminated shells with Campylobacter spp. represent public health significance, because they may transmit infection to humans through manual separation of egg yolk from egg white using the eggshell, which is a common practice in private households. The egg is cracked, and both components are separated by passing the egg yolk back and forth between the two halves of the eggshell, allowing the egg white to drip down while the egg yolk remains in the shell. During this process, the

egg content naturally gets in contact with the outside of the eggshell, which might lead to a cross-contamination with its micro-organisms (Dorn-In *et al.*, 2024).

However, positive *C. jejuni* DNA was detected in 45% and 15% of farm and baladi eggshell samples, respectively, and positive *C. coli* DNA was observed in 50% and 40% of farm and baladi eggshell samples, correspondingly. There was no significant variation between both egg types. Fortunately, *C. jejuni* and *coli* were not detected in egg contents (Table 1) (Lin 1988; Ahmed and Ahmed 1994; Adesiyun *et al.*, 1994; Vashin *et al.*, 2008; Messelhausser *et al.*, (2011). In contrast with the current study, Ansarifar et al. (2023) demonstrated that *C. jejuni* (6.3%) was more than *C. coli* (1.3%) in avian eggs, and Gharbi et al. (2022) noted that the prevalence of *Campylobacter* infection in eggshells was 25.6%, with *C. jejuni* accounting for 81.9%, followed by *C. coli* at 18.2%.

The results in Table (2) showed *Campylobacter* spp. infection; 20%, 25%, and 25% of farm eggshell samples contained *C. jejuni* only, *C. coli* only, and mixed *Campylobacter* spp. DNA, respectively, with total *Campylobacter* spp. detection of 70% in farm eggshell samples. Also, it was found that *C. jejuni* was detected in 10% of Baladi eggshell samples, while *C. coli* was only observed in 35%. Only 5% of baladi eggshell samples contained mixed *Campylobacter* spp. infection, with total Campylobacter spp. detection of 50% in baladi eggshell samples.

Compared to other studies, the obtained result (total *Campylobacter* spp. 70% farm egg and 50% baladi egg) was lower than those in Finland (86%) (Sulonen *et al.*, 2007), but higher than those in Greece 13.3% (Jones *et al.*, 2016) and Australia 11% (Fonseca *et al.*, 2014). During the production process, this high rate of contamination may be a source of contamination for egg products, particularly cracked eggs (Sato and Sashihara, 2010; Messelhausser *et al.*, 2011). A batch of unpasteurized liquid eggs could potentially become contaminated by a single infected egg, posing a serious risk to the health of consumers (Jones et al., 2016).

In this study, the prevalence of *Campylobacter* spp. infection in diarrheic patient samples was 50% positive for *C. coli* (10/20), while *C. jejuni* was not detected (Table 3). So, the overall infection in humans in this study was caused by *C. coli* only. Similarly, Abushahba et al. (2018) noted 0% detection of *C. jejuni* in stool samples by using multiplex PCR and Mohamed. (2019), found that the prevalence of *C. coli* infection in 50% of human diarrhea using multiplex PCR.

The obtained results were higher than those reported in Sokoto State, Nigeria, which recorded 24% prevalence rates of *C. coli* infection in human samples (Nwankwo *et al.*, 2016). A study reported by Awadallah et al. (2014), revealed that *C. coli* was identified from 3.2%, and a study from Germany, recorded that the *C. coli* prevalence rate was 18.6% (Gürtler *et al.*, 2005). While there was also a study in Zuru Kebbi State, Nigeria, which recorded a 60.63% humans *C. coli* prevalence rate (Gwimi *et al.*, 2015), which appears higher than the present prevalence.

According to sex in this study, females recorded a higher prevalence rate of *C. coli* infection (55.55%) than males (45.45%) (Table 4). This may be due to females having more contact with poultry than males, especially in the home-rearing system. In agreement with our result, Abushahba et al. (2018) recorded that the C. coli prevalence rate in females (11.76%) was higher than in males (3.03%). Contrary to ours, another study from Sokoto State, Nigeria, recorded that prevalence rates of *C. coli* infection in males were 40% higher than in females (38%) (Nwankwo *et al.*, 2016) and Gwimi et al. (2015) reported that the Campylobacter prevalence rate in males (52.76%) was higher than females (47.33%).

From Table (5), which indicated the prevalence rate according to age, it was noted that the highest prevalence rate of *C. coli* infection was detected in age group 3Y-5Y (75%), followed by 8M-2Y 50%, and the lowest prevalence of *C. coli* found in more than 5Y group (40%). Although it is hard to pinpoint the cause of this discrepancy, it may be connected to dietary habits, immunity development, and preferences, as well as closeness to birds. Our results agreed with the former study in Assiut, Egypt, which illustrated the highest prevalence rate of *C. coli* infection detected in the age group 1.1 Y-2Y 9.09%, followed by ≤ 1Y 6.06%, and *C. coli* infection was not detected in the age group >2Y group 0% (Abushahba *et al.*, 2018). While it contradicts a study from Zuru Kebbi State, Nigeria, that reported higher isolates between the ages of 10 and 29 years (Gwimi *et al.*, 2015).

The prevalence rate of the accompanied symptoms with diarrhea was vomiting in a prevalence of 100% in *C. coli* infected samples, followed by dehydration (33.33%), abdominal pain (27.27%), and fever (25%), as illustrated in Table (7). In contrast to our results, Abushahba et al. (2018) demonstrated that abdominal pain was recorded in 100% of infected patients, followed by fever and vomiting in 87.5% and 25% bloody diarrhea. In Table (8), it was noted that the abdominal pain symptom 75% (3/4) was the most common accompanied symptom in positive *C. coli* diarrheic patients, then vomiting 50% (2/4). Fever and dehydration, 25% (1/4), were the less commonly accompanied symptoms in positive *C. coli* diarrheic patients.

## CONCLUSION

The results obtained from the study indicated that hygienic and technical compliance is needed in poultry farms and farmer's houses to produce eggs free from Campylobacter microbes to prevent public health hazard, because contamination by *Campylobacter* spp. represents a serious threat to the poultry industry. Although *Campylobacter* spp. was not found in the egg content in the present study, the high percentage of eggs with infected shell is regrettably indicative of unhygienic farm systems and worker approaches. Keeping cooked and raw food separate may prevent the development of egg-borne illnesses caused by bacterial contamination of table eggs. The manual separation technique of egg white and yolk by the eggshell should be avoided, especially if raw egg white or yolk is used for the preparation of insufficiently heated foods, where contaminating pathogens are not inactivated during processing. Proper biosecurity of farms and awareness programs for workers and consumers are necessary for the control of infection. Proper washing of eggs to get rid of fecal matter and from any debris. Sufficient boiling and frying must be done to reduce Campylobacter infection through eggs. An integrated HACCP plan must be applied in the poultry farm and farmer's houses for the prevention of *C. jejuni* and *coli* infections.

## REFERENCES

*Abushahba, M.F.; Ahmed, S.O.; Ibrahim, A.A. and Mosa, H.A. (2018):* Prevalence of zoonotic species of *Campylobacter* in broiler chicken and humans in Assiut governorate, Egypt. *Approaches in Poultry, Dairy and Veterinary Sciences, 3*(4), 260-268.

*Adesiyun, A.A.; Ojo, M.O.; Mohammed, K.; and Garcia, G. (1994):* Frequency of isolation of *Campylobacter* and *Samonella* from live broilers reared by contact farmers in Trindad. *Bulletin of Animal Health and production in Africa, 42*(3): 167-172.

*Ahmed, M.M. and Ahmed, F.A. (1994):* Occurrence of *Campaylobacter* species in broilers and laying hens suffering from diarrhea. *Assiut Veterinary Medical Journal, 32*(63): 119-125.

*Amjad, M. (2023):* Poultry as a source and reservoir for Campylobacteriosis.

*European Journal of Veterinary Medicine, 3*(1), 11-17.

*Ansari-Lari, M.; Hosseinzadeh, S.; Shekarforoush, S.S.; Abdollahi, M. and Berizi, E. (2011):* Prevalence and risk factors associated with *Campylobacter* infections in broiler flocks in Shiraz, southern Iran. *International Journal of Food Microbiology, 144*(3):475–479.

*Ansarifar, E.; Riahi, S.M.; Tasara, T.; Sadighara, P. and Zeinali, T. (2023): Campylobacter* prevalence from food, animals, human and environmental samples in Iran: a systematic review and meta-analysis. *BMC Microbiology, 23*(1), 126.

*Awadallah, M.A.I.; Ahmed, H.A.; El-Gedawy, A.A. and Saad, A.M. (2014):* Molecular identification of *C. jejuni* and *C. coli* in chicken and humans, at Zagazig, Egypt, with reference to the survival of *C. jejuni* in chicken meat at refrigeration and freezing temperatures. *International Food Research Journal, 21*(5), 1801-1812.

*Beumer, R.R.; De Vries, J. and Rombouts, F.M. (1992): Campylobacter jejuni* non-culturable coccoid cells. *International Journal of Food Microbiology, 15*(1-2), 153-163.

*Casalino, G.; Bozzo, G.; Dinardo, F.R.; D'Amico, F.; Dimuccio, M.M.; Camarda, A. and Circella, E. (2022):* Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from laying hens housed in different rearing systems. *Animals, 12*(21), 2978. https://doi.org/10.3390/ani12212978

*Cox, N.A.; Richardson, L.J.; Maurer, J.J.; Berrang, M.E.; Fedorka-Cray, P.J.; Buhr, R.J. and Doyle, M.P. (2012):* Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. *Journal of Food Protection, 75*(10), 1896-1902. https://doi.org/10.4315/0362-028.jfp-11-322

*DeReu, W.; Messens, M.; Heyndrickx, T.B.; Rodenberg, M.; Uyttendaele M. and Herman, L. (2008):* Bacterial contamination of table eggs and the influence of housing systems. *World's Poultry Science Journal, 64*(1), 5-19.

*Dorn-In, S.; Daldrup, E.; Mang, S.; Esteban-Cuesta, I.; Gareis, M. and Schwaiger, K. (2024):* Viable *Campylobacter jejuni* on Eggshells and Its Potential to Cross-contaminate Egg White and Yolk When Using a Manual Separation Technique, Determined by Culture and Propidium Monoazide (PMA) qPCR. *Journal of Food Protection, 87*(4), 100246. https://doi.org/10.1016/j.jfp.2024.100246

*Eunju, S. and Lee, Y. (2009):* Comparison of three different methods for *Campylobacter* isolation from porcine intestines. *Journal of Microbiology and Biotechnology, 19*(7), 647-650. PMID: 19652510.

*Fonseca, B.B.; Beletti, M.E.; Melo, R.T.D.; Mendonça, E.P.; Coelho, L.R.; Nalevaiko, P.C. and Rossi, D.A. (2014): Campylobacter jejuni* in commercial eggs. *Brazilian Journal of Microbiology, 45*(1), 76-79. https://doi.org/10.1590/s1517-83822014000100011

*Gast, R.K.; Guardo Bouldin, J. and Holt, P. S. (2004):* Colonization of reproductive organs and internal contamination eggs after expriemental infection of laying hens with *Salmonella heidelberg* and *Salmonella enteritidis*. *Avian Diseases, 48*(4): 863-869.

*Gharbi, M.; Béjaoui, A.; Ben Hamda, C.; Alaya, N.; Hamrouni, S.; Bessoussa, G.; Ghram, A. and Maaroufi, A. (2022): Campylobacter* spp. in eggs and laying hens in the North-East of Tunisia: High prevalence and multidrug-resistance phenotypes. *Veterinary Sciences, 9*(3), 108. https://doi.org/10.3390/vetsci9030108

*Gürtler, M., Alter, T., Kasimir, S and Fehlhaber, K. (2005):* The importance of *Campylobacter coli* in human campylobacteriosis: prevalence and

genetic characterization. *Epidemiology and Infection, 133*(6), 1081-1087.

*Gwimi, P.B.; Faleke, O.O.; Salihu, M.D.; Magaji, A.A.; Abubakar, M.B.; Nwankwo, I.O. and Ibitoye, E.B. (2015):* Prevalence of *Campylobacter* species in faecal samples of pigs and humans from Zuru Kebbi State, Nigeria. *International Journal of One Health, 1*(1), 1-5. https://doi.org/10.14202/IJOH.2015.1-5

*Hangombe, B.M.; Sharma, R.N.; SKjerver, E. and Tuchili, L.M. (1999):* Occurrence of *Salmonella enteritidis* in pooled table eggs and market– ready chicken carcasses in Zambia. *Avian Diseases, 43*(3), 597-599.

*Hiett, K.L.; Rothrock Jr, M.J. and Seal, B.S. (2013):* Characterization of the *Campylobacter jejuni* cryptic plasmid pTIW94 recovered from wild birds in the southeastern United States. *Plasmid, 70*(2), 268-271.

*Humphrey, T.J. (1986):* Techniques for the optimum recovery of cold injured *Campylobacter jejuni* from milk or water. *The Journal of Applied Bacteriology, 61*(2), 125–132.

*Jones, D.R.; Guard, J.; Gast, R.K.; Buhr, R.J.; Fedorka-Cray, P.J.; Abdo, Z. and Karcher, D. (2016):* Influence of commercial laying hen housing systems on the incidence and identification of *Salmonella* and *Campylobacter*. *Poultry Science, 95*(5), 1116-1124. https://doi.org/10.3382/ps/pew036

*Kim, J.Y. and Lee, J.L. (2017):* Development of a multiplex real-time recombinase polymerase amplification (RPA) assay for rapid quantitative detection of *Campylobacter coli* and *jejuni* from eggs and chicken products. *Food control, 73*, 1247-1255. https://doi.org/10.1016/j.foodcont.2016.10.041

*Lin, Y.J. (1988):* Survey for *Campylobacter fetus subspecies. jejuni* infection in domestic fowls in Fujian province. *Chinese Journal of Veterinary Science and Technology, 6*, 18-20.

*Messelhausser, U.; Tharigen, D.; Elmer–Englhard, D.; Bauer, H.; Schreiner, H. and Hollar, C. (2011):* Occurrence of thermotolerant *Campylobacter* spp. On egg shell: a Missing link for food – Borne infection? *Applied and Environmental Microbiology, 77*(11), 3896 -3897. https://doi.org/10.1128/aem.00145-11

*Medema, G.J.; Schets, F.M.; Van De Giessen, A.W. and Havelaar, A.H. (1992):* Lack of colonization of 1 day old chicks by viable, non-culturable *Campylobacter jejuni*. *The Journal of Applied Bacteriology, 72*(6), 512–516.

*Messens, W.; Grijspeerdt, K.; De Reu, K.; Deketelaere, B.; Mertens, K.; Bamelis, F.; De Baerdemaker, J.; Decuypere, E. and Herman, L. (2007):* Egg shell penetration of various types of hens' egg by various microorganisms. *Journal of Food Protection 70*(3), 623-628.

*Mobaien, A.; Moghaddam, F.; Talebi, S.; Karami, A.; Amirmoghaddami, H. and Ramazani, A. (2016):* Studying the prevalence of *Campylobacter jejuni* in adults with gastroenteritis from northwest of Iran. *Asian Pacific Journal of Tropical Disease, 6*(12), 957-960. https://doi.org/10.1016/S2222-1808(16)61164-7

*Mohamed, K. (2019):* Prevalence of *Campylobacter* spp. and its pathogenic genes in poultry meat, human and environment in Aswan, Upper Egypt. *Assiut Veterinary Medical Journal, 65*(161), 151-158. https://doi.org/10.21608/avmj.2019.168777

*Nwankwo, I.O.; Faleke, O.O.; Salihu, M.D.; Magaji, A.A.; Musa, U. and Garba, J. (2016):* Epidemiology of *Campylobacter* species in poultry and humans in the four agricultural zones of Sokoto State, Nigeria. *Journal of Public Health and Epidemiology, 8*(9), 184-190.

https://doi.org/10.5897/JPHE2016.0809

*On, S.L. and Holmes, B. (1991):* Effect of inoculum size on the phenotypic characterization of *Campylobacter* species. *Journal of Clinical Microbiology, 29*(5), 923-926.

*On, S.L. and Holmes, B. (1992):* Assessment of enzyme detection tests useful in identification of *Campylobacteria*. *Journal of Clinical Microbiology, 30*(3), 746-749.

*Pande, V.V.; Devon, R.L.; Sharma, P.; McWhorter, A.R. and Chousalkar, K. K. (2016):* Study of *Salmonella Typhimurium* infection in laying hens. *Frontiers in Microbiology, 7*, 203. https://doi.org/10.3389/fmicb.2016.00203

*Peckham, M.C. (1984):* Avian vibrio infections. In: Hofstad, M. S., Barnes, H. J., Calvek, B. W., Reid, W. M., & Yoder, Jr., H. W. (eds): Disease of poultry, 8th ED., pp 221-231. Iowa State Univ. Press Ames.

*Penner, J.L. (1988):* The genus *Campylobacter*: a decade of progress. *Clinical Microbiology Reviews, 1*(2), 157–172.

*Rastyani, S.; Alikhani, M.Y.; Sedighi, I.; Kazemi, S.; Kohan, H.F and Arabestani, M.R. (2015):* *Campylobacter jejuni* and *Campylobacter coli* in children with acute diarrhea in health centers of Hamadan, Iran. *Avicenna Journal of Clinical Microbiology and Infection, 2*(4), 29791-29791. https://doi.org/10.17795/ajcmi-29791

*Rollins, D.M. and Colwell, R.R. (1986):* Viable but nonculturable stage of *Campylobcter jejuni* and its role in survival in the natural aquatic environment. *Applied and Environmental Microbiology, 52*(3), 531-538.

*Sambrook, J.; Fritscgh, E.F. and Mentiates, T. (1989):* Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, New York.

*Sanders G. (1998):* Isolation of *Campylobacter* from food. In: Warburton, D. (eds): Compendium of Analytical Methods. Vol 3. HPB laboratory procedure MFLP-46. Polyscience Publications, Laval Québec, Canada.

*Sato, M. and Sashihara, N. (2010):* Occurrence of *Campylobacter* in commercially broken liquid egg in Japan. *Journal of Food Protection, 73*(3), 412-417.

*Sahin, O.; Luo, N.; Huang, S. and Zhang, Q. (2003):* Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. *Applied and Environmental Microbiology, 69*(9), 5372-5379.

*Sulonen, J.; Kärenlampi, R.; Holma, U. and Hänninen, M.L. (2007):* *Campylobacter* in Finnish organic laying hens in autumn 2003 and spring 2004. *Poultry science, 86*(6), 1223-1228.

*Vashin, I.; Stoyanchev, T. and Roussev, V. (2008):* Prevalence of microorganism of the *Campylobacter* genus in Quail (*Coturnix Coturnix*) eggs. *Bulgarian Journal of Veterinary Medicine. 11*(3): 213-216.

*Wang, H.; Ng, L.K. and Farber, J.M. (2001):* Detection of *Campylobacter jejuni* and thermophilic *Campylobacter* spp. from foods by polymerase chain reaction. In: Spencer, J. F.T., & Ragout de Spencer, A.L. (eds) Food Microbiology Protocols. Vol 14, pp 95-106.

*Wieliczko, A. (1995):* The role of *Campylobacter* in poultry pathology. Part 1. Epidemiological studies on *Campylobacter* infections in poultry. *Medycyna Weterynaryina., 51*(3), 150-152.

# نسبة حدوث والكشف الجزيئي عن كامبيلوباكتر جيجوني وكولي في بيض المائدة وبراز مرضى الإسهال

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قد يؤدي التعامل غير السليم مع البيض إلى الإصابة بعدوى من البكتيريا مثل كامبيلوباكتر، والتي تلوث سطح قشرة البيض في الغالب. لذلك تركز هذه الدراسة على الكشف الجزيئي عن كامبيلوباكتر جيجوني وكولي في بيض المائدة وبراز مرضى الإسهال .تم إجراء تفاعل البلمرة المتسلسل التقليدي على ٤٠ عينة بيض، ٢٠ من كل من بيض المزارع والبيض البلدى بالتساوي، و٢٠ عينة براز من مرضى الإسهال .وقد وجد أن عدوى كامبيلوباكترفى المجمل كانت منتشرة في عينات قشر البيض في بيض المزارع والبيض البلدى، وتم الكشف عن الحمض النووي الإيجابي لكامبيلوباكتر جيجوني في ٤٥٪ و١٥٪ على التوالي .وبالمقارنة، كانت ٥٠% و٤٠ % من عينات قشر البيض في بيض المزارع والبيض البلدي إيجابية لبكتيريا كامبيلوباكتركولي .ومع ذلك، لم يتم الكشف عن الحمض النووى لبكتيريا الكامبيلوباكتر جيجوني أو كامبيلوباكتر كولي في محتويات البيض لكل من بيض المزارع والبيض البلدي .بينت الدراسة ان معدل انتشار عدوى كامبيلوباكترفى المجمل٧٠% و٥٠% في عينات قشر البيض في بيض المزارع والبيض البلدي على التوالي .كما تم الكشف عن الحمض النووى لكامبيلوباكتر كولي في ٥٠% من براز مرضى الإسهال، بينما لم يتواجد الحمض النووى لكامبيلوباكتر جيجوني . وتبين ان انتشار عدوى كامبيلوباكتر كولي فى الإناث أعلى منه فى الذكور. بينما كان أعلى معدل الانتشار في الفئة العمرية ٣-٥ سنوات بنسبة (٧٥%) وأقل معدل حوالى (٤٠%) في الفئة العمرية أكثر من ٥ سنوات .كذلك اظهرت الدراسة ان عدوى كامبيلوباكتر كولي في مرضى الإسهال المصحوبة بالاعراض المرضية مثل القيء أعلى من المصحوبة بالجفاف وآلام البطن والحمى . لذلك توصي الدراسة بتطبيق واتباع قواعد صحية جيدة داخل مزارع الدواجن ومنازل الفلاحين وزيادة الوعي لدى عمال مزارع الدواجن والفلاحين لتطبيق التدابير الصحية لتقليل العدوى.