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# Overview on Campylobacter Situation in Pets

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**ABSTRACT**

Bacteriological examination of 200 collected fecal swap from both human (80) and pet animal (120) show that the rate of campylobacter positive sample was 49 sample (24.5%) with (20%) in pet animal and (31.25%) in human, the result of isolation was (21.5%). Animal was grouped to healthy and symptomatic with nearly equal rate of isolation, convential PCR used for campylobacter spp. Detection using *C. coli* ceuE and C. jejuni mapA where all the samples was positive for c.jejuni, campylobacter pathogensity was examined using the following virulence genes (*flaA, wlaN, VirB11),* gene sequencing was performed to detect identify between both human and pet samples.

## INTRODUCTION:

Campylobacter is Gram-negative, non-spore forming, curved S-shaped rods, micro-aerobic, catalase and oxidase positive bacteria. *Campylobacter* is one of the most common causes of zoonotic gastroenteritis in developed and developing countries **(ECDC. 2015)**.

Pet animals can be colonized with numerous *Campylobacter* spp including mainly *C. jejuni*, *C. upsaliensis,* and *C. helveticus*. Pets are known as a carrier since about 50% of the healthy dogs can shed the campylobacter spp. in feces without any clinical signs with similar rate of isolation between symptomatic and asymptomatic dogs **(Smith and Taylor 2019).**

*FlaA* sequence typing is a genotyping method that is increasingly used in epidemiological investigations of *Campylobacter* species *FlaA* sequencing is another commonly used, cost-effective, and well-accepted sequence based typing method for *Campylobacter* with low levels of non-typeability **( Kittl et al. 2013).**

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Campylobacter infection in pets and humans from a common source is possible. However, the importance of pets as a source of *Campylobacter* infections to human remains unclear as genetic studies have shown that human and canine strains are distinct, suggesting that host-specific genotypes may exist among strains **( Damborg et al. 2008).**

Dogs feeding diet containing raw meat especially poultry are considered to be one of the most important risk factor **(LeJeune and Hancock 2001).** Feeding of poultry slaughterhouse offal is the main source of pet animals feeding in Egypt.

In addition, there are no studies undertaken to highlight the campylobacter infection in pet animals in Egypt.

**So the aim of this work was :**

Isolation of campylobacter species from pet animals and human. Prevalence of Campylobacter in fecal samples in healthy and diarrheic pet animals and human. Biochemical and molecular identification of isolated strains. Molecular detection of some virulence genes of isolated stains. Gene sequencing for determination of similarity between isolated strains in pet animals and human to identify the possibility of transmission between them.

## MATERIAL and METHODS

**Sampling: (OIE 2008)**

The total number and classification of samples was illustrated in table (1).

Table 1. The total number and classification of samples:

| Species | Healthy | Diarrhoea | Total |
|---|---|---|---|
| Dog | 40 | 32 | 72 |
| Cat | 27 | 21 | 48 |
| Human | ---- | 80 | 80 |

**Isolation and Identification: according to (ISO 2006):**

**Molecular Identification:**

**DNA extraction : using QIAamp DNA Mini Kit** ( Catalogue no.**51304)**

**Oligonucleotide primers sequences used in cPCR**

Table 2. Oligonucleotide primers sequences used for PCR assay :

| Target gene | Primer sequence (5'-3') | Length | Reference |
|---|---|---|---|
| 23S rRNA | TATACCGGTAAGGAGTGCTGGAG<br>ATCAATTAACCTTCGAGCACCG | 650 bp | Wang *et al*., 2002 |
| flaA | AATAAAAATGCTGATAAAACAGGTG<br>TACCGAACCAATGTCTGCTCTGATT | 855 bp | Datta *et al*., 2003 |
| VirB11 | TCTTGTGAGTTGCCTTACCCCTTTT<br>CCTGCGTGTCCTGTGTTATTTACCC | 494 bp | |
| wlaN | TTAAGAGCAAGATATGAAGGTG<br>CCATTTGAATTGATATTTTTG | 672 bp | Kordinas *et al*., 2005 |
| C. coli ceuE | AAT TGA AAA TTG CTC CAA CTA TG<br>TGA TTT TAT TAT TTG TAG CAG CG | 462 bp | Eunju and Lee, 2009 |
| C. jejuni mapA | CTA TTT TAT TTT TGA GTG CTT GTG<br>GCT TTA TTT GCC ATT TGT TTT ATT A | 589 bp | |

Table 3. Preparation of PCR Master Mix for cPCR :

| Component | Volume/reaction |
|---|---|
| Emerald Amp GT PCR mastermix (2x premix) | 12.5 $\mu l$ |
| PCR grade water | 4.5 $\mu l$ |
| Forward primer (20 pmol) | 1 µl |
| Reverse primer (20 pmol) | 1 µl |
| Template DNA | 6 $\mu l$ |
| Total | 25 $\mu l$ |

- **Cycling conditions of the primers during cPCR**

Temperature and time conditions of the two primers during PCR are shown in Table (4).

Table 4. Cycling conditions of the primers during cPCR :

| Gene | denaturation | | Annealing | Extension | No. of cycles | Final extension |
|---|---|---|---|---|---|---|
| | Primary | Secondary | | | | |
| *23SrRNA* | 94˚C 5 min. | 94˚C 30 sec. | 55˚C 40 sec. | 72˚C 45 sec. | 35 | 72˚C 10 min. |
| *flaA* | 94˚C 5 min. | 94˚C 30 sec. | 53˚C 40 sec. | 72˚C 50 sec. | 35 | 72˚C 10 min. |
| *VirB11* | 94˚C 5 min. | 94˚C 30 sec. | 53˚C 40 sec. | 72˚C 45 sec. | 35 | 72˚C 10 min. |
| *wlaN* | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 45 sec. | 35 | 72˚C 10 min. |
| *C coli.* | 94˚C 5 min. | 94˚C 30 sec. | 58˚C 45 sec. | 72˚C 45 sec. | 35 | 72˚C 10 min. |
| *C. jejuni* | 94˚C 5 min. | 94˚C 30 sec. | 55˚C 45 sec. | 72˚C 45 sec. | 35 | 72˚C 10 min. |

**purification of the PCR Products:**

Using QIAquik PCR product purification protocol, Using QIAquick PCR Product extraction kit. (Qiagen Inc. Valencia CA).

**Sequencing reaction**:

A purified RT-PCR product was sequenced in the forward and/ or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). Using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit. (Perkin-Elmer/Applied Biosystems, Foster City, CA), with Cat. No. 4336817.

A BLAST® analysis (Basic Local Alignment Search Tool) **(Altschul et al. 1990)** was initially performed to establish sequence identity to GenBank accessions. The sequence reaction was done according to the instruction of the manufacture as following:

Table 5. Preparation of master mix using Big dye Terminator V3.1 cycle sequencing :

| Amount | Reagent |
|---|---|
| 2µl | Big dye terminator v.3.1 |
| 1µl | Primer |
| From 1 to 10 µl | Template according to quality of band and concentration of DNA |
| Complete till to total volume become 20µl | Deionized water or PCR grade Water |
| 20µl (Mix well, spin briefly) | Total volume |

**Phylogenetic analysis:**

A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 1.83 of MegAlign module of Lasergene DNAStar software Pairwise, which was designed by ( **Bacon et al. 2002)** and Phylogenetic analyses were done using maximum likelihood, neighbour joining and maximum parsimony in MEGA6 (**Tamura et al. 2013).**

## RESELTS:

Table 6. Prevalence of campylobacter species in fecal samples of pet animals and human :

| Species | % of Positive samples | % of Negative samples |
|---|---|---|
| Dog | 20.8 (15/72) | 79.2 (57/72) |
| Cat | 18.17 (9/48) | 81.25 (39/48) |
| Human | 31.25 (25/80) | 68.75 (55/80) |

Table 7. Prevalence of campylobacter species in fecal samples of pet animals and human according to Health status:

| | Total samples | Healthy | | Diarrhoea | | Total Positive |
|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative | |
| Dog | 72 | 37.5 (15/40) | 62.5 (25/40) | 21.8 (7/32) | 78.2 (25/32) | 30.5 (22/72) |
| Cat | 48 | 18.5 (5/27) | 81.5 (22/27) | 19 (4/21) | 81 (17/21) | 18.7 (9/48) |
| Human | 80 | 0.0 | 0.0 | 31.2 (25/80) | 68.8 (55/80) | 31.2 (25/80) |

Table 8. Molecular characterization of Campylobacter spp. in pet animals and human ;

| Sample | Origin | *C. jejuni* | *C. coli* |
|---|---|---|---|
| 1 | Human | Nd | Nd |
| 2 | Human | Nd | Nd |
| 3 | Pet animal | + | - |
| 4 | Pet animal | + | - |
| 5 | Pet animal | + | - |
| 6 | Pet animal | + | - |
| 7 | Human | + | - |
| 8 | Pet animal | + | - |
| 9 | Pet animal | + | - |
| 10 | Pet animal | + | - |

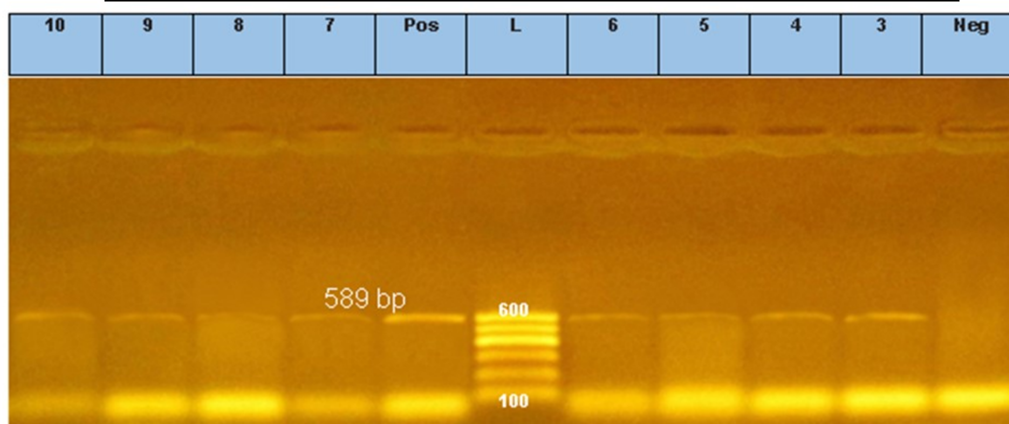


Fig 1. Agarose gel electrophoresis of conventional PCR for detection of *C. jejuni mapA* gene in 10 examined samples from pet animals and human showing amplification of 589 bp. fragment.
L (lader): 100 bp plus DNA ladder (100-3000 bp).
(Pos): positive control. (*Campylobacter jejuni* strain identified by RLQP).
(Neg): negative control. (Master Mix without DNA).
Lanes (3, 4, 5,6, 7, 8, 9 and 10): positive samples. Lane (6): negative sample.

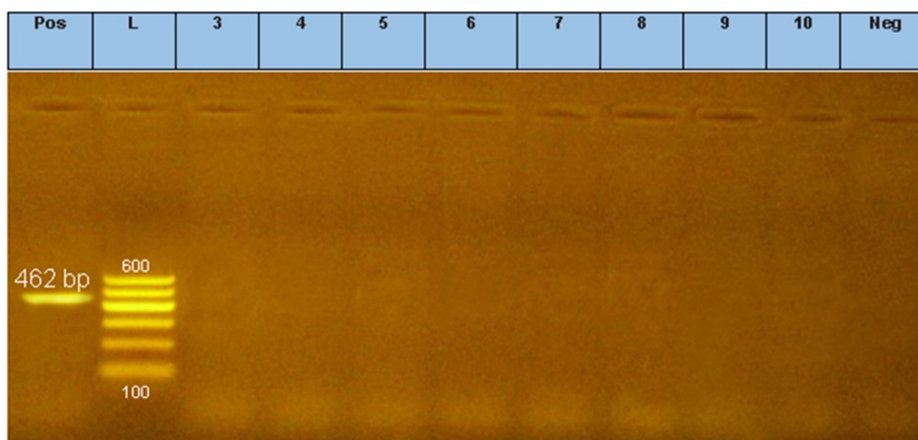


Fig 2. Agarose gel electrophoresis of conventional PCR for detection of *C. coli* ceuE spp. in 10 examined samples from pet animals and human showing negative resluts.
L (lader): 100 bp plus DNA ladder (100-3000 bp). (Pos): positive control. (*C. coli* strain identified by RLQP). (Neg): negative control. (Master Mix without DNA).

**Molecular detection of *C. jejuni* virulence genes:**

Molecular charaterization of 7 different virulence genes (23S rRNA, wlaN, VirB11, flaA) were illustrated in table (9).

Table 9. Moleculer detection of *C. jejuni* virulence genes :

| Sample | Origin | 23S rRNA | wlaN | VirB11 | flaA |
|---|---|---|---|---|---|
| 1 | Human | + | Nd | Nd | Nd |
| 2 | Human | + | Nd | Nd | Nd |
| 3 | Pet animal | + | - | - | + |
| 4 | Pet animal | + | - | - | - |
| 5 | Pet animal | + | - | - | + |
| 6 | Pet animal | + | - | - | - |
| 7 | Human | + | - | - | + |
| 8 | Pet animal | + | - | - | - |
| 9 | Pet animal | + | - | - | - |
| 10 | Pet animal | + | - | - | - |

Nd: not detected

***23s RNA* gene**

All the ten representive samples were examined for the presence of 7 virulance genes. Result showed that 8 samples (no. 3,4,5,6,7,8,9,10) was positive for *23s rRNA* (80%) at 6500 bp.

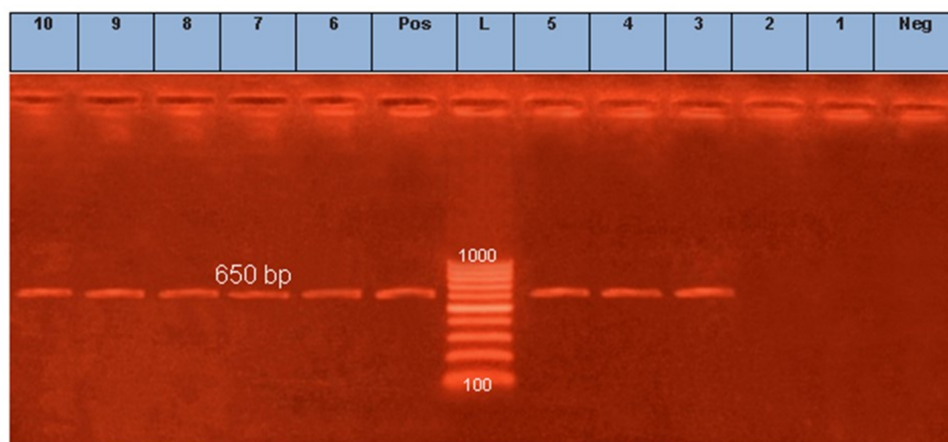


Fig 3. Agarose gel electrophoresis of conventional PCR for detection of C. jejuni 23s rRNA gene in 10 examined samples from pet animals and human showing amplification of 6500 bp. fragment. L (lader): 100 bp plus DNA ladder (100-3000 bp).
(Pos): positive control. (*Campylobacter jejuni* strain identified by RLQP).
(Neg): negative control. (Master Mix without DNA). Lanes (3, 4, 5, 6, 7, 8, 9 and 10): positive samples. Lane (1,2): negative sample.

***wlaN* gene**

All the eight samples show negative result to all of *wlaN*.

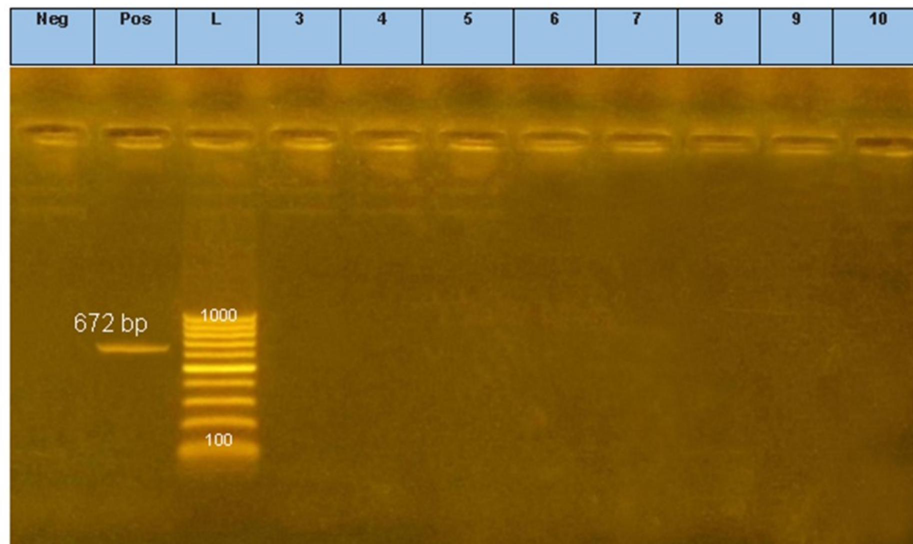


Fig 4. Agarose gel electrophoresis of conventional PCR for detection of *C. jejuni WlaN* gene in 10 examined samples from pet animals and human showing amplification of 672 bp. fragment. L (lader): 100 bp plus DNA ladder (100-3000 bp).
(Pos): positive control. (*Campylobacter jejuni* strain identified by RLQP).
(Neg): negative control. (Master Mix without DNA). Lanes (1 to 8): negative samples.

***Virb11* gene.**

All the eight samples show negative result to *Virb11* gene.

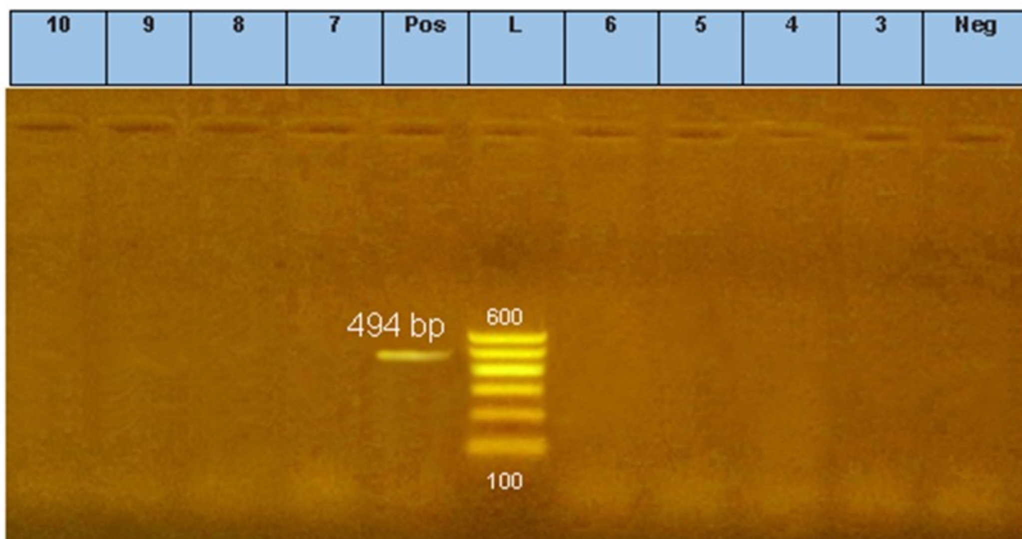


Fig 5. Agarose gel electrophoresis of conventional PCR for detection of *C. jejuni Vibr 11* gene in 10 examined samples from pet animals and human showing amplification of 449 bp. fragment. L (lader): 100 bp plus DNA ladder (100-3000 bp).
(Pos): positive control. (*Campylobacter jejuni* strain identified by RLQP).
(Neg): negative control. (Master Mix without DNA). Lanes (1 to 8): negative samples.

## ***FlaA* gene**

The result confirmed that only three samples out of the eight samples were positive for *flaA* gene (37.5%). Human samples (3, 7) and animal sample (5).

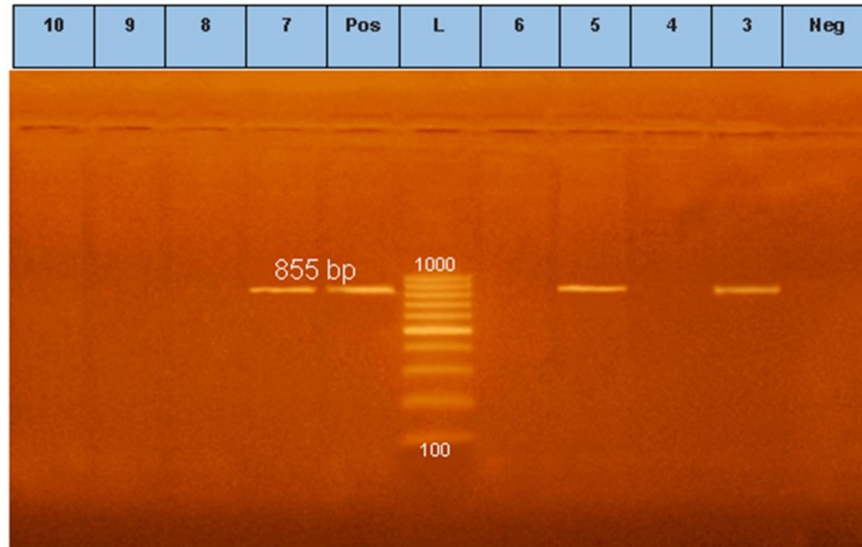


Fig 6. Agarose gel electrophoresis of conventional PCR for detection of *C. jejuni flaA* gene in 8 examined samples from pet animals and human showing amplification of 855 bp. fragment. L (lader): 100 bp plus DNA ladder (100-3000 bp).
(Pos): positive control. (Campylobacter jejuni strain identified by RLQP).
(Neg): negative control. (Master Mix without DNA). Lanes (3, 5, 7): positive samples.
Lane (4, 6, 8, 9, 10): negative samp

## ***FlaA* gene sequencing:**

A representative positive isolates from pet animal (L5) and human (L7) were were selected for *C. jejuni* characterization by *FlaA* gene sequencing and were submitted to NLQP for sequencing of the PCR product. The data of the chosen isolates was illustrated in table (14). It was conducted in forward directions and the nucleotide sequence of the *FlaA* gene from nucleotide positions 0 to 830 were determined for each isolate and used for nucleotide analysis and deduced amino acid analysis.

Table 10. Data of the chosen isolates for sequencing :

| Isolate no. | species | Health condition |
|---|---|---|
| L5 | Animal (dog) | Diarrhea |
| L7 | human | Diarrhea |

**_FlaA_ nucleotide sequencing**

The sequence analysis of the *FlaA* gene of the two isolates strains were used for comparison in this study. The result indicated that the identify percentage between the two representative isolates was 95%.

The flaA sequences of the two representive isolates were determined where BLAST analysis revealed significant homology with reported Campylobacter species at the NCBI GenBank database. The data of these referenced campylobacter species showed in table (15). the the result showed that the two chosen isolates were more closly related to isolates from different sources including human, chicken and turkey (Tab. 15 &Fig.18).

Phylogenetic tree was constructed from the nucleotide sequences of the flaA gene to assess the genetic relationship among the *C.jejuni* isolates (Fig. 7)

Phylogenic tree was separated into two main distinct branches, one branch include only *C.jejuni* strain CS0052 (JQ991582.1) and the other branch include the rest of isolates

The tree shows that our representive isolate (L7 human) was closely related to *C. jejuni* strain B30 KF846024.1 (isolated from human in Tanzania) and *Campylobacter jejuni* strain B24 KF846024.1 (isolated from human in Tanzania), Campylobacter jejuni strain Cjst25 KM396359.1 (isolated from human in Iran)

The tree also shows that our representive isolate (L5 dog) was closely related to *C. jejuni* strain L17 AF050193.1 (isolated from poultry in USA) and *C. jejuni* D2290 AF050189.1 (isolated from poultry in USA).

Table 11. Nucleotides identity of the chosen *C. jujeni* isolates from pet animal (L5) and human (L7) in comparison to commonly used reference isolates obtained from GenBank.

| | | Percent Identity | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| L5-c.jejuni-dog | 1 | | 94.9 | 98.6 | 98.6 | 98.4 | 98.1 | 97.9 | 97.8 | 96.2 | 96.2 | 95.4 | 91.8 |
| L7-c.jejuni-human | 2 | 94.9 | | 94.1 | 94.1 | 94.1 | 94.3 | 94.1 | 93.9 | 93.8 | 93.8 | 92.9 | 93.2 |
| C.jejuni_SSU9896_AF050196 | 3 | 98.6 | 94.1 | | 99.2 | 99.0 | 98.7 | 97.5 | 97.4 | 96.8 | 96.8 | 96.0 | 92.0 |
| c.jejuni_L17_AF050192 | 4 | 98.6 | 94.1 | 99.2 | | 99.7 | 99.5 | 97.1 | 96.9 | 97.5 | 97.5 | 96.7 | 92.7 |
| C.jejuni_CS0048_JQ991581 | 5 | 98.4 | 94.1 | 99.0 | 99.7 | | 99.2 | 96.8 | 96.7 | 97.3 | 97.3 | 96.5 | 92.5 |
| C.jejuni_D2290_AF050188 | 6 | 98.1 | 94.3 | 98.7 | 99.5 | 99.2 | | 97.3 | 97.2 | 97.1 | 97.1 | 96.2 | 93.0 |
| C.jejuni_L17_AF050193 | 7 | 97.9 | 94.1 | 97.5 | 97.1 | 96.8 | 97.3 | | 99.8 | 96.1 | 96.1 | 95.0 | 92.6 |
| C.jejuni_D2290_AF050189 | 8 | 97.8 | 93.9 | 97.4 | 96.9 | 96.7 | 97.2 | 99.8 | | 96.0 | 96.0 | 94.9 | 92.5 |
| C.jejuni_B30_KF846024 | 9 | 96.2 | 93.8 | 96.8 | 97.5 | 97.3 | 97.1 | 96.1 | 96.0 | | 100.0 | 98.6 | 93.1 |
| C.jejuni_B24_KF846020 | 10 | 96.2 | 93.8 | 96.8 | 97.5 | 97.3 | 97.1 | 96.1 | 96.0 | 100.0 | | 98.6 | 93.1 |
| C.jejuni_Cjst25_KM396359 | 11 | 95.4 | 92.9 | 96.0 | 96.7 | 96.5 | 96.2 | 95.0 | 94.9 | 98.6 | 98.6 | | 92.4 |
| C.jejuni_CS0052_JQ991582 | 12 | 91.8 | 93.2 | 92.0 | 92.7 | 92.5 | 93.0 | 92.6 | 92.5 | 93.1 | 93.1 | 92.4 | |

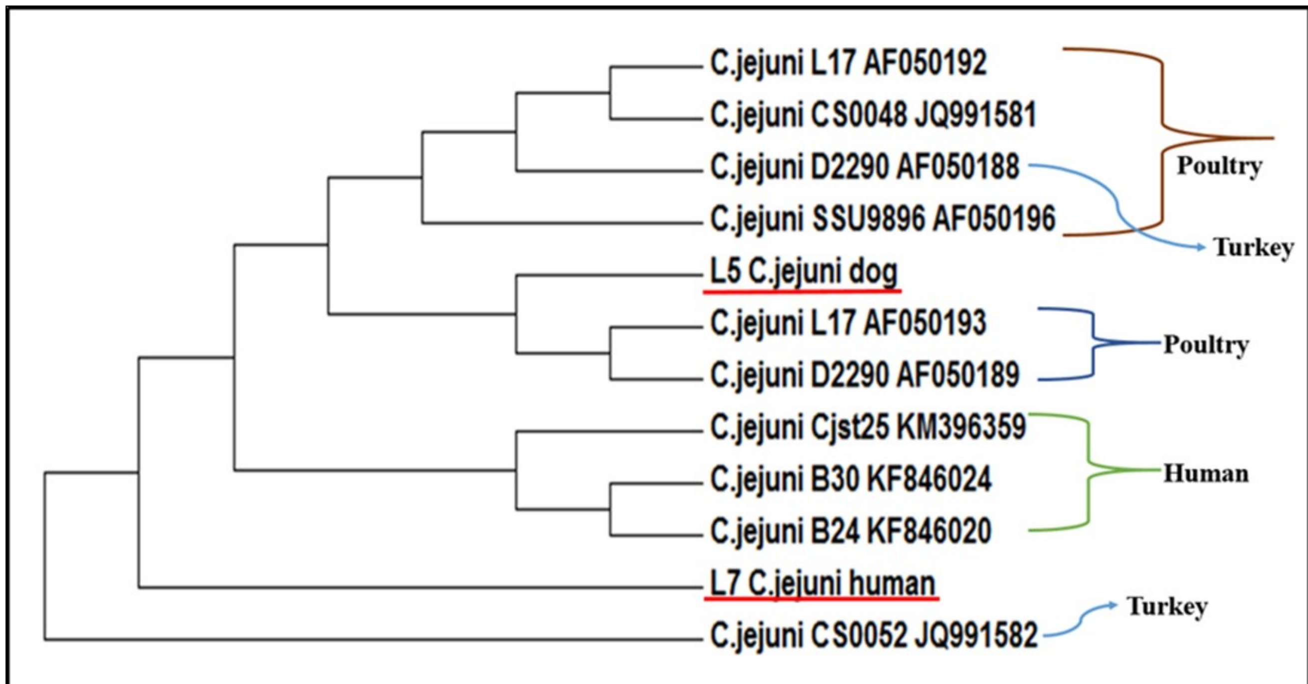


Fig (7): Phylogenitic tree of FlaA gene sequence from *C. jujeni* isolated from pet animals (L5_C.Jejuni_dog) and human (L7_ C.Jejuni_human) and closely related isolates obtained fom Gene Bank

## DISCUSSION

*Campylobacter* is one of the most common causes of zoonotic gastroenteritis in developed and developing countries **(ECDC 2015)**. Consumption of contaminated meat especially poultry, drinking contaminated water, contact with pets, also during traveling are the most likely sources of Campylobacter contamination **(Kittl et al. 2012)**.

Result indicated that the overall prevalence of *Campylobater* spp was 24.5% (49/200). The prevalence in human samples was 31.25% (25/80).

There is variation among campylobacter isolation in human population. Lower percentage of Campylobacter isolation was reported by **Rozynek and Dzierzanowska (1994)** who found that the mean frequency of C. jejuni infections did not exceed (9.1%) and **(Pazzaglia et al. 1995)** who reported 17.2% in hospitalized diarrheic children in Alexandria, Egypt.

On the other hand, higher prevalence of *C. jejuni* was approximately 46% among annual bacterial foodborne illnesses in the USA **(Frost et al. 2002).**

Concerning pet animals, our investigation revealed that the overall prevalence of *Campylobater* spp was 20% (24/120). The prevalence was 20.8% (15/72) in dog and 18.57% (9/48) in cat.

The rate of isolation of campylobacter in dog (20.8%) is little higher than that of the cat (18.75%) and this was in agreement with the previously reported results of **(Frost et al. 2002).** who reported higher prevalence in dogs than cat.

The study detected the rate of isolation in both healthy and diseased animal to confirm the ability of pets to be a carrier without any clinical signs and possibility of transmission to human. Some Previous studies showed that approximately 50% of the healthy dogs can shed campylobacter spp. in their feces without any clinical signs which indicates similar rate of isolation between symptomatic and asymptomatic dogs. The reason for equal rate of isolation between symptomatic and asymptomatic pets may be due to the ability of the pet animal to shed the bacteria in their feces for long period of time in contrast to human **(Romich, 2008).**

Molecular characterization of C. jejuni was carried out. In our study, molecular identification of 8 out of 10 reprehensive samples was confirmed to be *C. jejuni* through investigation of its characteristic *mapA* gene which indicates that the recovery of C.jejuni in the chosen isolates was 80%. In contrast, investigation of *C. coli* ceuE spp. gene in the examined samples from pet animals and human showing negative results .This was in agreement with **Couturier et al. (2012)** who state that *C. jejuni* is considered to be the most commonly isolated species out of the 17 species within the genus Campylobacter as it cause 90% of the infections .

The conventional PCR used for detection of campylobacter strain virulence genes revealed that all the 8 representive samples was positive for 23s rRNA (100%).wlaN gene was not detected in any sample where **(Bacon et al. 2002)** found that the detection rate of the wlaN gene from human clinical samples (25.0 %) was similar to that from poultry meat (23.8 %).All the 8 representive samples show negative result for *VirB11*. This was in accordance with **Bacon et al. (2002)**.

Three samples out of the eight representative samples showed positive results for the flaA gene (37.5%) and the positive samples was obtained from human (no. 3,7) and animal (no. 5).

FlaA sequence typing was performed. FlaA sequence is a genotyping method that is increasingly used in epidemiological investigations of thermo-tolerant Campylobacter species **(Kittl et al. 2012).**

Nucleotide sequence of the flaA gene showed 2 highly variable regions; one at positions 539 to 689 designated the *flaA* and a large variable region (flaA-LVR), at approximately positions 700 to 1,600 where The sequence data of the LVR showed similar results to those of the SVR; owing to the length of the sequence and the complexity of LVR data analysis, this region is not suitable for molecular epidemiological studies of Campylobacter infections **(Singh and Kwon 2013).**

The *flaA* sequences of the two representive isolates were determined and undergo BLAST analysis which revealed significant homology with reported Campylobacter species at the NCBI GenBank database, the result showed that the two chosen isolates were more closely related to isolates from different sources including human, chicken and turkey.

There is a shortage in the literature reviewing the molecular typing and gene sequencing of *C. jejuni* in pet animals. On the other hand, many studies were undertaken in especially in poultry.

Phylogenetic tree was constructed from the nucleotide sequences of the flaA gene to assess the genetic relationship among the c.jejuni isolates whereas the tree shows that our represENTive isolate (L7 human) was closely related to *C. jejuni strain B30 KF846024* (isolated from human in Tanzania) and *C. jejuni strain B24 KF846024* (isolated from human in Tanzania), *C. jejuni strain Cjst25 KM396359* (isolated from human in Iran) the high identity rate between our isolate (L7 human, Egypt) and human isolates from other countries indicates that *campylobacter* can be worldwide.

The tree also shows that our representative isolate (L5, dog) was closely related to *C. jejuni strain L17 AF050193* (isolated from poultry in USA) and *C. jejuni D2290 AF050189* (isolated from poultry in USA).

These high degree of identity between our isolate (L5 dog) and poultry show that dogs can acquire the infection from poultry as mentioned before by **LeJeune and Hancock (2001) who** state that dogs feeding diet containing raw meat especially poultry are considered to be one of the most important risk factor of campylobacter infection.

**Conclusion**

Consumption of contaminated meat, especially poultry considered to be one of the major source of Campylobacter infections in both human and pets so Veterinarians should advise owners of the potential dangers of using raw or undercooked protein sources for their pets.

human can acquire infection from their pets through the fecal oral route there for owners should practice appropriate hygienic measures. The high identity between the C. jejuni isolates from pet animal and human potentiate the hypothesis of zoonotic possibility of campylobacter transmission between pet animal and human.

**REFERENCES**

Altekruse SF, Stern NJ, Fields PI, Swerdlow DL. 1999. Campylobacter jejuni—an emerging foodborne pathogen. Emerging infectious diseases, 5(1), 28.

Bacon DJ, Alm RA, Hu L, Hickey TE, Ewing CP, Batchelor RA, Guerry P. 2002. DNA sequence and mutational analyses of the pVir plasmid of Campylobacter jejuni 81-176. Infection and immunity, 70(11), 6242-6250.

Couturier BA, Hale DC, Couturier MR. 2012. Association of Campylobacter upsaliensis with persistent bloody diarrhea. Journal of clinical microbiology, JCM-01807.

Damborg P, Guardabassi L, Pedersen K, Kokotovic B. 2008. Comparative analysis of human and canine Campylobacter upsaliensis isolates by amplified fragment length polymorphism. Journal of clinical microbiology, 46(4), 1504-1506.

Datta S, Niwa H, Itoh K. 2003. Prevalence of 11 pathogenic genes of Campylobacter jejuni by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. Journal of medical microbiology, 52(4), 345-348.

ECDC E. 2015. EMA, 2015. First joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producting animals, EFSA Journal, 13(1), 4006.

Frost JA, Gillespie IA, O'BRIEN SJ. 2002. Public health implications of campylobacter outbreaks in England and Wales, 1995–9: epidemiological and microbiological investigations. Epidemiology & Infection, 128(2), 111-118.

ISO 2006. Microbiology of food and animal feeding stuffs- Horizontal method for detection and enumeration of Campylobacter spp. ISO 10272. First edition. International Standards Organization, Geneva.

Kittl S, Heckel G, Korczak BM, Kuhnert P. 2013. Source attribution of human Campylobacter isolates by MLST and fla-typing and association of genotypes with quinolone resistance. PloS one, 8(11), e81796.

Kordinas V, Nicolaou C, loannidis A, Legakis 2005. Prevalence of four virulence genes in Jejune determined by PCR and sequence analysis. 9(4), 211-215

LeJeune JT, Hancock DD. 2001. Public health concerns associated with feeding raw meat diets to dogs. Journal of the American Veterinary Medical Association, 219(9), 1222-1225.

Pazzaglia G, Bourgeois AL, Mourad AS, Gaafar T, Diab AS, Hebert A, Murphy JR. 1995. Campylobacter diarrhea in Alexandria, Egypt. The Journal of the Egyptian Public Health Association, 70(3-4), 229-241.

Romich JA. 2008. Understanding zoonotic diseases (pp. 205-218). Clifton Park NY: Thomson/Delmar Learning..

Rozynek E, Dzierzanowska D. 1994. Distribution of biotypes and Lior serogroups of enteric Campylobacter jejuni/coli isolated from children in Poland(1986-1991). Alpe Adria Microbiology Journal, 3(1), 21-29.

Smith T, Taylor MS. 1919. Some morphological and biological characters of the spirilla (Vibrio fetus, n. sp.) associated with disease of the fetal membranes in cattle. Journal of Experimental Medicine, 30(4), 299-311.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution, 30(12), 2725-2729.

Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Rodgers FG. 2002. Colony multiplex PCR assay for identification and differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. fetus. Journal of Clinical Microbiology, 40 (12), 4744-4747.