**Studies on different recent techniques for diagnosis of *Campylobacter* in rabbit.**

**By**

**Ashraf A. Abd- El Tawab\*; Mohsen A. Agag \*, Mona, M. Sobhy\*\* and Marwa E. M. Radwan\*\*\***

**\*** Bacteriology, Immunology and Mycology Dept., Fac. Vet. Med, Benha Univ.

\*\* Reproductive diseases Dept., ARRI, ARC, Giza, Egypt.

Bacteriology, Immunology and Mycology Dept., Fac. Vet. Med, Benha Univ. \*\*\*

**Abstract:**

Rabbits can breed for the production of meat and fur. Their meat is considered as a source of human campylobacteriosis; caused by *Campylobacter* organism's has been recognized as the main etiological agent of human bacterial gastrointestinal disease.

Two hundred and thirty rabbit samples were collected including cloacal swabs from (130), liver (40), intestinal samples (40), water (10) and ration (10) from apparently healthy and diseased rabbits suffering from diarrhea in different farms. All rabbit samples were processed for isolation of *Campylobacters*. Each sample was homogenized in sterile Thioglycolate broth, incubated at 42 °C for 48 hrs under microaerophilic condition. All The isolates were subjected biochemical tests, such as catalase, oxidase, hippurate hydrolysis test, glycine, sodium chloride (NaCl) 3.5% tolerance test and susceptibility to cephalothin and nalidixic acid. Identified colonies were stored at -70 C in nutrient broths with 15% glycerol until subjected to molecular identification. The results of this study showed that overall *Campylobacter* isolates was 58 (25.22%) from the different sources sampled. The prevalence of *C. jejuni* was the most prevalent species 26 (11.30%) in samples taken from rabbits followed by *C. coli* was 15 (6.52%) then *C. lari* was 12 (5.22%) and *C. hyointestinalis* was 5(2.17%). The overall prevalence of *C.jejuni* and *C.coli* (74.3%) (25.70%); the difference was notably due to a positive hippurate test result for isolates identified as *C.jejuni* due to the absence of hippurate hydrolysis for *C.coli.* Multiplex PCR methods thegenus specific *(16S rRNA)* revealed that 51 (22.17%) *Campylobacter* species isolates; 27 (52.94%) as *C. jejuni* specific at323 bp while, 17(33.33%) produced th*e C. coli* specific at 126 bp and 7 (13.73%) other *Campylobacter* species.

We concluded that *C. jejuni* *and C. coli* are highly prevalent in rabbit farms in Egypt. Control measures for contamination of the rabbit supply should be identified to protect human exposure to *Campylobacter* spp. Further analysis of rabbit samples by using PCR assay are needed to evaluate the applicability of the method for detection of *Campylobacter* organisms exposed to an environment.

**Introduction:**

*Campylobacter* enteritis is a leading cause of acute bacterial gastrointestinal infection worldwide. The genus *Campylobacter* includes many species of which *Campylobacter jejuni and C. coli* are common pathogens and the majority of diagnosed human *Campylobacter* infections (**Skirrow, 1994**).

Campylobacteriosis is considered as the major important zoonotic gastrointestinal disease around the world and most of the cases are caused mainly by *C. jejuni*. Poultry play an important role in transmission of campylobacteriosis to human (**Fitzmaurice, *et al*. 2004** and **Gormley *et al*, 2008)**.

*Campylobacter* species were isolated from the caecal contents of rabbits (Oryctolagus cuniculus). All strains were initially identified as belonging to the genus *Campylobacter* by means of genus-specific PCR, but were identified PCR for known thermophilic species (**O'Sullivan, *et*** ***al.* 2000** and **Morris, *et al*. 2003).** Cells were spiral shaped with bipolar unsheathed flagella, with no periplasmic fibres, and appeared coccoid after 10-12 days of incubation. Phylogenetic analyses based on 16S rRNA gene revealed that all strains recognized *Campylobacter* species (**Zanoni, *et al*. 2009**).

Routine detection of *Campylobacter* species in clinical laboratories is based on culture on selective media and subsequent phenotypic identification. Culture methods are based toward the detection of *C. jejuni* and *C. coli*. The antimicrobial agents incorporated into used selective media may inhibit growth of some *Campylobacter* species (**Collins, *et al.* 2001** and **Reddington, *et al*. 2011**).

The true incidence of *Campylobacter* species may be under estimated because of the limitations of routine culture methods because conventional methods are relatively slow (**Engberg, *et al*. 2000**). Presumptive results may be available after 2 days. However, definitive species-level identification based on phenotypic methods may require a further 3 to 4 days (**O'Connor, *et al*. 2000**). Phenotypic identification can be challenging because of the fastidious growth requirements and the distinguishing of biochemical characteristics by *Campylobacter* species (**Goossens, & Butzler, 1992).**

Molecular methods based on PCR amplification may provide an alternative to culture methods for the detection of *Campylobacter* in different specimens. The application of PCR-based assays applied to the detection of *Campylobacter* species in rabbit specimens (**Maher, et al.** **2003**). Amplification of DNA targets including the *Campylobacter* flagellin gene, 16S rRNA and the 16S/23S rRNA intergenic spacer region (**Touzet, *et al*. 2009)**.

The aim of this study was to identification of *Campylobacter* species isolated from rabbit specimens by conventional methods. Molecular characterization for Campylobacter isolates by using PCR and amplification of DNA including the *Campylobacter* flagellin gene, 16S rRNA, virulence gene and cytolethal genes in rabbit specimens.

**Materials and Methods:**

**1- Sampling:**

Two hundred and thirty rabbit samples were collected including cloacal swabs from (130), liver (40), intestinal samples (40), water (10) and ration (10) from apparently healthy and diseased rabbits suffering from diarrhea in different farms. All rabbit samples were collected in sterile sample collection vials, transferred to the lab. As quick as possible, all samples kept at 4 °C and processed for isolation of campylobacters (**Table, 1**).

**2- Isolation and identification of Campylobacter species:**

About 10 g of each sample were homogenized in sterile Thioglycolate broth, incubated at 42 °C for 48 hrs under microaerophilic condition (5% O2, 10% CO2 and 85% N2). Microscopic examination for the incubated samples for detection of *Campylobacte*r microorganisms identified under phase contrast microscope using (4 00 x) magnification for detection of characteristic motility **(Smibert, 1984).** All The isolates were subjected to Gram’s staining and biochemical tests, such as catalase, oxidase, urease, nitrate reduction and indole acetate hydrolysis, hippurate hydrolysis test, glycine tolerance test, sodium chloride (NaCl) 3.5% tolerance test and susceptibility to cephalothin and nalidixic acid by the disc diffusion method according to **Holt *et al*. (1994**). Identified colonies

were stored at -70 °C in nutrient broths with 15% glycerol until subjected to molecular identification **Sheppard and Dallas (2009**).

**3- Molecular characterization of Campylobacter species:**

***3-1. Isolation of DNA:***

DNA extracts were prepared for each isolate by 8 minutes boiling of colonies in 10% Chelex 100 (Bio-Rad) in 10 mM Tris/HCl, 1 mM EDTA, pH 8. The crude DNA preparation was stored at 4°C until used (**Iroala *et al*., 2012**).

***3-2. Oligonucleotide primers used in cPCR***

Six pairs of primers were supplied from (Metabion). They have specific sequence and amplify specific products as shown in **Table (2).**

***3-3. Extraction of DNA:* according to QIAamp DNA mini kit**

20 μl QIAGEN protease were pipetted into the bottom of a 1.5 ml micro centrifuge tube. 200 μl of the sample was added. 200 μl buffer AL were added to the sample, mixed by pulse vortexing for 15 seconds. The mixture was incubated at 56˚C for 10 min. The 1.5 ml micro centrifuge tube was centrifuged. Ethanol (96%) 200 μl were added to the sample, and mixed again by pulse vortexing for 15 seconds. After mixing, the 1.5 ml micro centrifuge tube was briefly centrifuged. The mixture from step 6 was carefully applied to the QIAamp mini spin column (in a 2ml collecting tube) without wetting the rim. The cap was closed, and centrifuged at 8000 rpm for 1 min. The QIAamp mini spin column was placed in a clean 2 ml collection tube. The QIAamp mini spin column was carefully opened and 500 ml buffer AW2 was added without wetting the rim. The cap was closed, and centrifuged at full speed for 3 min. Centrifugation at full speed for 1 min was done. The QIAamp mini spin column was placed in a clean 1.5 ml micro centrifuge tube. The QIAamp mini spin column was carefully opened and 100 μl buffer AE were added. The QIAamp mini spin column was Incubated at room temperature (15-25˚C) for 1 min, and then centrifuged at 8000 rpm for 1 min.

***3-4. Preparation of duplex PCR Master:***

Mix for each of *(C. coli* *glyA* and *C. jejuni* *hipO*), (cdtB and cdtC) and (*virB11* and *flaA*) genes according to **GoTaq® Hot Start Green Master Mix.**

***3-5. Agarose gel electrophoreses***  **(Iroala *et al*., 2012).**

Electrophoresis grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, 0.5μg/ml Ethidium bromide was added and mixed thoroughly. Twenty μl of each PCR product samples, negative control and positive control were loaded to the gel. The gel was photographed by a gel documentation system and the data was analyzed.

**Results:**

Out of **230** rabbit samples screened for the presence of *Campylobacters*, **58** samples yielded characteristic *Campylobacter* colonies on CCDA plates after 48 hours of incubation (**Table, 4**). They had characteristic corkscrew motility observed by the phase contrast microscope. The biochemical tests for isolates were found positive for catalase, oxidase, and nitrate. None of the isolates revealed positive reaction for urease activity (**Table, 5**).

Out of the 51 (22.17%) *Campylobacter* isolates, 27 (52.94%) were identified as *C. jejuni (323bp)* , 17 (33.33%) isolates as *C.coli* (126 bp), and 7 (13.73%) isolates as other *Campylobacter* species based on the hippurate hydrolysis test and all were found to be sensitive to nalidixic acid and resistant to cephalothin (**Tables , 6 & Fig. 1,2 &3**).

**Discussion:**

Rabbits can breed for the production of meat and fur. Their meat is considered as a source of human campylobacteriosis; caused by *Campylobacter* organism's has been recognized as the main etiological agent of human bacterial gastrointestinal disease (**Adak *et al*., 2002; Lin, 2009; Hermans *et al.*, 2012).** *Campylobacter* will have a positive impact on consumers’ perceptions related to food safety, the food industry and public health agencies (**Moore *et al*., 2006; Wassenar *et al*., 2007**).

The results of this study showed that overall *Campylobacter* isolates was 58 (25.22%) from the different sources sampled. The prevalence of *C. jejuni* was the most prevalent species 26 (11.30%) in samples taken from rabbits followed by *C. coli* was 15 (6.52%) then *C. lari* was 12 (5.22%) and*C. hyointestinalis* was 5(2.17%) (T**able, 4 & fig. 1).** The overall prevalence of *C.jejuni* and *C.coli* (74.3%) (25.70%); the difference was notably due to a positive hippurate test result for isolates identified as *C.jejuni* due to the absence of hippurate hydrolysis for *C.coli (***Nicholson &Patton1993 and Sincinschi, 1995)**.

Data recorded in **table (4)** revealed the high incidence of *Campylobacter* in intestinal content (40%) followed by water (30%) then in cloacal swabs was (26.92%) and in liver (10%). The high incidence of *Campylobacter* in intestinal content and cloacal swabs may be due to the normal inhabitant of *Campylobacter* organisms in intestine of rabbits without any diseased signs **(Atanssova and Ring 1998 and Bi, *et al*. 2008).** The high incidence of *Campylobacter* in diseased rabbit samples was **(**32.04%) followed by apparently health rabbit samples (19.69%). The high incidence of *Campylobacter* isolates in apparently health rabbit samples was in the intestinal content (46.67%) may be due to the normal inhabitant of *Campylobacter* organisms in intestine without any diseased signs. Followed by cloacal swabs was (21.33%) then in drinking water samples (14.29%) and liver (7.69%).Also, *Campylobacter* contamination increases during untreated drinking water **(Sahin, 2003** **and Barakat, *et al*. 2015).**

In this present study, according to the Multiplex PCR methods and amplification parameters, 58 *Campylobacter* isolates yielded the genus specific (16S *rRNA*) revealed that 51 (22.17%) *Campylobacter* species isolates*;* 27 (52.94%) *as C. jejuni* specific at323 bp while, 17 (33.33%) produced the *C. coli* specific at 126 bp and 7 (13.73%) other *Campylobacter* species **(Table, 5 and Fig. 1).** *C. jejuni* isolates from liver and water (100%), followed by cloacal swabs (50%) and intestinal samples (42.86%) were confirmed by mPCR, while C*. coli* isolates were confirmed by the amplification of *glyA* gene as cloacal swabs (37.5%) and intestinal samples (35.71%). The hippurate hydrolysis assay is dependent upon the inoculums size of the bacterium, which means that the assay is unable to detect low level of hippuricase product (**Linton *et******al*., 1997**). Therefore, the detection of the gene by PCR instead of the phenotypic detection of the hippuricase product is considered a reliable alternative method for the discrimination of *C. jejuni* isolates (**Nayak *et al*., 2005** **and Asmaa, *et al*. 2017).**

*Vir B11* is a pathogenic gene responsible for the expression of invasion. In the present study, the confirmed *C. jejuni* isolates *Vir B11* gene was high incidence in liver and intestinal samples 50% while in cloacal swabs was 35.71%. A putative virulence gene associated with adhesion of the pathogen to intestinal epithelial cells (**Rosenquist *et al*., 2003).** This gene is 100% conserved among *C. jejuni* and *C. coli* isolates of diverse sources; therefore, it was used to detect virulent isolates of both species (**Datta *et al*., 2003**). Cytolethal distending toxin (CDT) is widely distributed among Gram-negative bacteria (**Guo *et al*., 2010**) and is the best characterized of the toxins produced by *Campylobacter* spp. It has been described as an important virulence factor of this pathogen (**Asakura *et al*., 2008**). *CDT* holotoxin, composed of three subunits encoded by the *cdtA, cdtB and cdtC* genes, causes eukaryotic cells to arrest in the G2/M phase of the cell cycle, preventing them from entering mitosis and consequently leading to cell death (**Zilbauer *et al*., 2008).** *C.jejuni* cytolethal distending toxins showed at 555 bp for *CdtC* and at 495 bp for *CdtB* of the genus *Campylobacter* 555 bp and 495 bp amplicons were confirmed**.**

*C. lari* strains were about the same size as the *C. jejuni and C. coli* products during the PCR step did not amplify this product. Thus, the assay could be used to discriminate between *C. lari* and the *C. jejuni*-*C. coli* isolates. The remaining seven *Campylobacter* strains were belonging to other *Campylobacter* species in PCR assays. However, reducing the primer concentration from 0.25 to 0.2 μm during the first PCR step was sometimes necessary to reduce the number of nonspecific amplicons **(Woz´niak and Wieliczko, 2011)**.

We concluded that*C. jejuni* *and C. coli* are highly prevalent in rabbit farms in Egypt. Control measures for exposure and contamination of the rabbit supply should be identified so that methods can be developed to protect human exposure to *Campylobacter* spp. Further analysis of rabbit samples by using PCR assay are needed to evaluate the applicability of the method for detection of *Campylobacter* organisms exposed to an environment.

**References:**

**1- Adak GK, Cowden JM, Nicholas S, Evans HS. (2002):** The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of Campylobacter infection. Epidemiol Infect. 115:15–22.

**2- Asakura, M., W. Samosornsuk, M. Taguchi, K. Kobayashi, N. Misawa, M. Kusumoto, K. Nishimura, A. Matsuhisa, and S. Yamasaki. (2008):** Comparative analysis of cytolethal distending toxin (cdt) genes among *Campylobacter jejuni*, *C. coli* and *C. fetus* strains. Microb. Pathog. 42:174-183.

**3- Asmaa G. Youssef; A.I. Ibrahim; Amal, S.M. Sayed and Mona M. Sobhy (2017):** OCCURRENCE OF *CAMPYLOBACTER* SPECIES IN CHICKENS BY MULTIPLEX POLYMERASE CHAIN REACTION. Assiut Vet. Med. J. Vol. 63No. 152, 1-7.

**4- Atanassova, V. and Ring, C. (1998):** *Campylobacter* species in the surroundings of poultry meat production. Incidence and chinolone resistance, Zentralbl. Hyg. Umweltmed, 200 (5-6): 542-552.

.

**5- Barakat A. M. A. ; Mona, M. Sobhy ; El Fadaly H. A. A.; Nagwa, S. Rabaei ;, Nashwa, M. Othman; Eman, S. Ramadan, Kotb, M. H. R. and Mona S. Zaki (2015):** Zoonotic Hazards of Campylobacteriosis in some areas in Egypt. Life Science Journal 2015; 12(7) p: 9-14.

**6- Bai, Y.; Cui, S.; Xu, X. and Li, F. (2014):** Enumeration and characterization of *Campylobacter* species from retail chicken carcasses in Beijing, China. Key Lab of Food Safety Risk Assessment, Ministry of Health, China National Centre for Food Safety Risk Assessment, Beijing, China. Foodborne Pathog Dis.; 11(11): 861-7.

**7- Collins, E., Glennon, M., Hanley, S., Murray, A.M., Cormican, M., Smith, T., and Maher, M. (2001):** Evaluation of a PCR DNA probe colorimetric membrane assay for the identification of *campylobacter spp.* In human stool specimens. J. Clin. Microbiol. 39, 4163-4165.

**8- Datta, S.; Niwa, H. and Itoh, K. (2003):** Prevalence of 11 pathogenic genes of Campylobacter jejuni by PCR in strains isolated from humans, poultry meat broiler and bovine faeces. Journal of Medical Microbiology, 52, 345–348.

**9- Engberg, J., S.L. On, C.S. Harrington and P. Gerner-** **Smidt, 2000.** Prevalence of Campylobacter, Arcobacter, Helicobacter and Sutterella spp. in human fecal samples as estimated by a reevaluation of isolation methods for Campylobacters. J. Clin. Microbiol. 38: 286-291.

**10- Fitzmaurice J, Duffy G, Kilbride B, Sheridan JJ, Carroll C. and Maher M (2004**): Comparison of a membrane surface adhesion recovery method with an ims method for use in a polymerase chain reaction method to detect Escherichia coli O157:H7 in minced beef. J. Microbiol. Methods 59: 243-252.

**11- Guo B., Lin J., Reynolds D.L. & Zhang Q. (2010):** Contribution of the multidrug efflux transporter CmeABC to antibiotic resistance in different Campylobacter species. Foodborne Path. Dis. 7:77-83.

**12- Goossens, H., and J.-P. Butzler. (1992):** Isolation and identification of *Campylobacter* spp., p. 93-109. *In*I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni.* Current status and future trends. ASM Press, Washington, D.C.

**13- Gormley FJ, Macrae M, Forbes KJ, Ogden ID, Dallas JF, Strachan NJ. (2008):** Has retail chicken played a role in the decline of human campylobacteriosis? Appl Environ Microbiol. ; 74:383–390.

**14- Hermans, D., D. K. Van, A. Martel, I. F. Van, W. Messens, M. Heyndrickx, F. Haesebrouck, and F. Pasmans. (2011):** Colonization factors of *Campylobacter jejuni* in the chicken gut. Vet. Res. 42:82.

**15- Holt, J.H.; Krieg, N.R. and Shneath, P.H.A. (1994):** Bergy's Manual of Determinative Bacteriology 9th Ed. Humphrey, T., O.S. Brien and M. Madsen, (2007): Campylobacter as zoonotic pathogens: A food production perspective. Int. J. Food Microbiol 117: 237-257.

**16- Iroala G, Hernández M, Calleros L, Paolicchi F, Silveyra S, Velilla A, Carretto L, Rodríguez E, Pérez R. (2012)*:*** Application of a multiplex PCR assay for Campylobacter fetus detection and subspecies differentiation in uncultured samples of aborted bovine fetuses. J Vet Sci. 13(4):371-6.

**17- Lin, J. (2009):** Novel Approaches for Campylobacter Control in Poultry. Foodborne Pathog Dis. 2009 Sep; 6(7): 755–765.

**19- Linton, D., A.J. Lawson, R.J. Owen and J. Stanley, 1997**. PCR detection, identification to species level and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples J. Clin. Microbiol. 35: 2568-2572.

**18- Maher, M., Finnegan, C., Collins, E., Ward, B., Carroll, C. &Cormican, and M. (2003**): Evaluation of culture methods and a DNA probe-based PCR assay for detection of Campylobacter species in clinical specimens of feces. J Clin Microbiol 41, 2980–2986.

**19- Moore J. E., Corcoran D., Dooley J. S. G., Fanning S., Lucey B., Matsuda M., McDowell D. A., Mégraud F., Millar B. C., O’Mahony R., O’Riordan L., O’Rourke M., Rao J. R., Rooney J., Sails A., Whyte P. (2005).** Campylobacter – Article review. Vet. Res. 36, 351–38210.1051.

**20- Morris J G (2003):** Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clin. Infect. Dis. 37:272-280.

**21- Nayak M., van der Fels-Klerx, I. & Havelaar, A. (2005).** A poultry-processing model for quantitative microbiological risk assessment. Risk Analysis, 25(1), 85-98***.***

**22- Nicholson, M.A. and Patton, C.M. (1993):** Application of Lior biotyping by use of genetically identified Campylobacter strains. *Journal of Clinical Microbiology* 31, 3348-3350.

**23- - O'connor L, Joy J, Kane M, Smith T and Maher M (2002):** Rapid polymerase chain reaction / DNA probe membrane – based assay for the detection of listeria and *listeria monocytogenes* in food. J. Food Prot. 63:337-342.

**24- O’Sullivan, N.A., Fallon, R., Carroll, C., Smith, T. and Maher, M. (2000):** Detection and differentiation of Campylobacter jejuni and Campylobacter coli in broiler chickens using a PCR/DNA probe membrane based colormetric assay. Molecular and Cellular Probes 14, 7–16.

**25- Reddington K, O'Grady J, Dorai-Raj S, Niemann S, van SD, Barry T (2011):** A novel multiplex real-time PCR for the identification of mycobacteria associated with zoonotic tuberculosis. PLoS One. 6: e23481.

**26- Rosenquist H. Nielsen NL. Sommer HM. Norrung B. Christensen BB. (2003):** Quantitative risk assessment of human campylobacteriosis associated with thermophilic Campylobacter species in chickens. Int J Food Microbiol. 2003; 83:87–103.

**27- Sahin O, Luo N. Huang S, Zhang Q (2003):** Effect of Campylobacter- specific maternal antibodies on Campylobacter jejuni colonization in young chickens. Appl. Environ. Microbiol 69 537 2-5379.

**28- Sheppard, S.K., J.F. Dallas, N.J. Strachan, M. MacRae, D.N. McCarthy, D.J. Wilson, F.J. Gormley, D. Falush, I.D. Ogden, M.C. Maiden and K.J. Forbes, 2009.** Campylobacter genotyping to determine the source of human infection. Clin. Infect. Dis., 48: 1072-1078.

**29- Sincinschi, L. (1995)** the comparative identification of Campylobacter Strains by traditional enzymatic tests and the gene amplification reaction. *Bacteriologia, Virusologia, Parazitologia, Epidemiologia (Bucuresti)* 40, 221–226.

**30- Skirrow MB (1994):** Diseases due to Campylobacter, Helicobacter and related bacteria. J Comp Pathol 111:113–149.

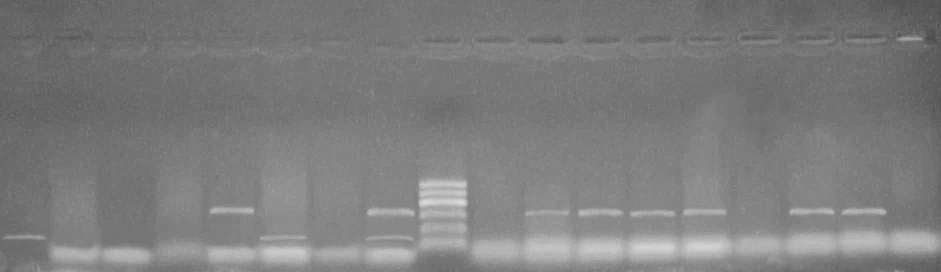
**31- Smibert, R.M (1984):** Genus *Campylobacter* in Berge’s Manual of system bacteriology. Vol. 1 Edited by N.R. Krieg, Williams and Wilkins, Baltimore, *pp*. 111-117.

**32- Wassenaar, T. M. and Newell, D. G. (2007)** the Genus *Campylobacter*. In The Prokaryotes ed. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H. and Stackebrandt, E. pp.119- 138.

**33- Woz´niak, A. and Wieliczko, A. (2011)** Tetracycline, erythromycin, and gentamicin resistance of Campylobacter jejuni and Campylobacter coli isolated from poultry in Poland. Bulletin of the Veterinary Institute in Pulawy 55, 51–54.

**34- Zanoni RG, Debruyne L, Rossi M, Revez J, Vandamme P (2009):** Campylobacter cuniculorum sp. nov., from rabbits. Int J Syst Evol Microbiol 59:1666–1671.

**35- Zilbauer M., Dorrell N., Wren B. W., Bajaj-Elliott M. (2008).** Campylobacter jejuni mediated disease pathogenesis: an update. Trans. R. Soc. Trop. Med. Hyg. 120, 123–12910.1016.



**+ve**

**100**

**M**

**600**

**323 bp**

**126 bp**

**-ve**

**Fig. 1: Multiplex PCR on 1.5% agarose gels. +ve= positive -ve= negative**

**All the 58 *Campylobacter* isolates yielded the genus specific (16S *rRNA*)**

***C. jejuni* 323 bp, *C. coli* 126 bp. LaneM: 100-600 bp ladders; Lane: 10, 15 *C. coli* at 126 bp; Lane: 1, 2, 4, 5, 6,7,11 *C. jejuni at 323 bp.***



**100**

**M**

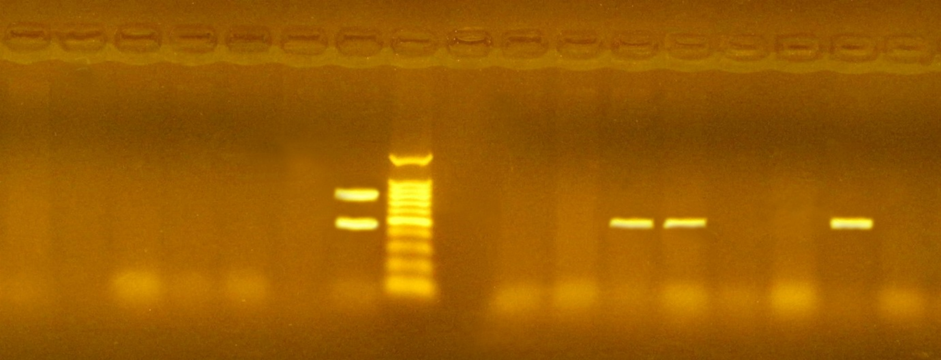
**600**

**495 bp**

**555 bp**

**Fig. 2: *C.jejuni* toxins showed at 555 bp for *CdtC* and at 495 bp for *CdtB*. LaneM: 100 bp – 600 bp ladder; Lane: 2, 4, 7 *C. jejuni CdtC at 555bp* Lane: 1, 5, 6, and 11**

***C.jejuni CdtB at 495b.***



**+ve**

**855 bp**

**494 bp**

**494 bp**

**100**

**M**

**1000**

**Fig. 3: The virulence strains of *C. jejuni* *virB11* showed at 494 bp and *FlaA* at 855 bp**

**LaneM: 100 bp –1000 bp ladder; Lane: 1, 4, 5 *C. jejuni virB11 at 494bp.***

**Table (1): Samples from diseased and apparently healthy rabbits.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Type of samples | | | | | No. of rabbit samples | Rabbit cases |
| Ration | **Water** | **Intestinal**  **samples** | **Liver** | **Cloacal swabs** |
| 7 | **7** | **15** | **13** | **75** | **127** | **Apparently health samples** |
| 3 | **3** | **25** | **27** | **55** | **103** | **Diseased Samples** |
| 10 | **10** | **40** | **40** | **130** | **230** | **Total** |

**Table (2): Oligonucleotide primers sequences.**

|  |  |  |  |
| --- | --- | --- | --- |
| Reference | Length of amplified product | Primer sequence  (5'-3') | Target gene |
| Wang *et al*., 2002 | 323 bp | ACTTCTTTATTGCTTGCTGC | *C. jejuni hipO* |
| GCCACAACAAGTAAAGAAGC |
| 126 bp | GTAAAACCAAAGCTTATCGTG | *C. coli glyA* |
| TCCAGCAATGTGTGCAATG |
| Datta *et al*., 2003 | 855 bp | AATAAAAATGCTGATAAAACAGGTG | *FlaA* |
| TACCGAACCAATGTCTGCTCTGATT |
| 494 bp | TCTTGTGAGTTGCCTTACCCCTTTT | *virB11* |
| CCTGCGTGTCCTGTGTTATTTACCC |
| Bang *et al*., 2003 | 495 bp | GTTAAAATCCCCTGCTATCAACCA | *CdtB* |
| GTTGGCACTTGGAATTTGCAAGGC |
| 555 bp | TGGATGATAGCAGGGGATTTTAAC | *cdtC* |
| TTGCACATAACCAAAAGGAAG |

**Table (3): Cycling conditions of the different primers during PCR**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | Primary denaturation | Secondary denaturation | Annealing | Extension | No. of cycles | Final extension | Reference |
| *HipO* and *glyA* | 94˚C  6 min. | 95˚C  30 sec. | 55˚C  30 sec. | 72˚C  30 sec. | 35 | 72˚C  10 min. | Wang *et al*., 2002 |
| *FlaA and virB11* | 94˚C  5 min. | 94˚C  30 sec. | 53˚C  45 sec. | 72˚C  45 sec. | 35 | 72˚C  10 min. | Datta *et al*., 2003 |
| *cdtB* and *cdtC* | 94˚C  5 min. | 94˚C  30 sec. | 42˚C  45 sec. | 72˚C  30 sec. | 35 | 72˚C  10 min. | Bang *et al*., 2003 |

**Table (4): Detection of *Campylobacter* species in the examined rabbits by using conventional methods.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Campylobacter* isolates | | | | Positive Campylobacter spp. | Diseased Samples | Apparent  health samples | No. of samples | Type of Samples |
| *C. hyointestinalis* | ***C. Lari*** | ***C. coli*** | ***C. jejuni*** |
| 3  2.31% | **8**  **6.15%** | **10**  **7.69%** | **14**  **10.77%** | **35**  **26.92%** | **55** | **75** | **130** | **Cloacal swabs** |
| - | **-** | **-** | **4**  **10%** | **4**  **10%** | **27** | **13** | **40** | **Liver samples** |
| 2  5% | **4**  **10%** | **4**  **10%** | **6**  **15%** | **16**  **40%** | **25** | **15** | **40** | **Intestinal samples** |
| - | **-** | **1**  **10%** | **2**  **20%** | **3**  **30%** | **3** | **7** | **10** | **Water** |
| - | **-** | **-** | **-** | **-** | **3** | **7** | **10** | **Rations** |
| 5  2.17% | **12**  **5.22%** | **15**  **6.52%** | **26**  **11.30%** | **58**  **25.22%** | **103** | **127** | **230** | **Total** |

**Table (5): Biochemical tests to differentiate between Campylobacter species isolated from rabbit samples.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *C. hyointestinalis* | *C. Lari* | *C. coli* | *C. jejuni* | Characteristics |
| + | **+** | **+** | **+** | **Oxidase** |
| + | **+** | **+** | **+** | **Catalase** |
| + | **+** | **+** | **+** | **Nitrate reduction** |
| -- | **--** | **--** | **--** | **Urease** |
| -- | **--** | **--** | **+** | **Hippurate hydrolysis** |
| D  +  + | **--**  **+**  **+** | **--**  **+**  **+** | **--**  **+**  **+** | **Growth at:**  **25◦C**  **37◦C**  **43◦C** |
| + | **+** | **+** | **+** | **Growth at 1% glycine** |
| -- | **--** | **--** | **--** | **%3.5 NaCl** |
| +  + | **+**  **--** | **+**  **D** | **+**  **--** | **H2S, lead acetate strip**  **H2S, TSI** |
| R  S | **R**  **R** | **S**  **R** | **S**  **R** | **Susceptibility to:**  **Nalidixic acid**  **Cephalothin** |

**Table 6: Detection of *Campylobacter* by multiplex PCR in rabbit samples.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Other Campylobacter species.* | *C. coli* | *C. jejuni* | Positive Campylobacter spp. by PCR | No. of examined samples | Type of Samples |
| 4  12.5% | **12**  **37.5%** | **16**  **50%** | **32**  **24.62%** | **130** | **Cloacal swabs** |
| -- | **--** | **4**  **100%** | **4**  **10%** | **40** | **Liver samples** |
| 3  21.43% | **5**  **35.71%** | **6**  **42.86%** | **14**  **35%** | **40** | **Intestinal samples** |
| -- | **--** | **1**  **100%** | **1**  **10%** | **10** | **Water** |
| -- | **--** | **--** | **--** | **10** | **ration** |
| 7  13.73% | **17**  **33.33%** | **27**  **52.94%** | **51**  **(22.17%)** | **230** | **Total** |