INTRODUCTION

Poultry houses can be contaminated by Campylobacter in many different methods from various environmental sources, making the control of flock colonization by Campylobacter a very challenging mission. Nowadays, no single measure is completely effective in minimizing Campylobacter infections on poultry farms. Campylobacter can break through biosecurity barriers and attack poultry houses, colonizing the chicken gut and rapidly multiplying in the intestinal mucosa. However, Campylobacter does not induce health or welfare troubles in chickens. Campylobacter spreads fast within broiler flocks and almost all birds will be infected within seven days [1].

Poultry flocks can be infected by multiple sources; it is difficult to eliminate Campylobacter from poultry farms by biosecurity measures alone. The use of fly screens on poultry farms has been found to be an effective method to decrease the introduction of Campylobacter into broiler farms especially during summer period. The exact mechanism by which C. jejuni is introduced into the poultry farm still unclear, although a variety of important markers for C. jejuni [9] associated markers (iam) genes which considered the genetic habitat, which invading enterocytes in addition to its production of toxin [8] C. jejuni pathogenicity depend on presence of specific genes which is responsible such as flaA, virB11 and invasion associated markers (iam) genes which considered the genetic markers for C. jejuni [9].

The main objectives of this study were to investigate the presence of Campylobacter jejuni in broiler chickens in the area of study and detection of their genes that have been recognized as responsible for the expression of pathogenicity.

MATERIALS AND METHODS

Area of study

The study was conducted in Assiut and New Valley provinces of Egypt.
governorates, that represent about 50% of the total landscape area of Egypt. Samples were obtained from 5 broiler farms located in Assiut City and 7 broiler farms in El-Kharga villages in New Valley governorate. The capacity of these farms were 2000 – 10000 broiler birds. Age of birds was 3 weeks (dead broilers) and 6 weeks (dead and slaughtered birds suffering from diarrhea).

**PM examination**

was performed for all samples and characteristic features were recorded.

**Samples collection**

A total of 200 samples were collected from 100 broiler chickens (dead and live birds suffering from diarrhea). The samples were divided into 100 intestinal (caecal) samples and 100 liver samples. This study was performed during the period between March and September 2016. Samples were sent to the laboratory and prepared for microbiological and molecular examinations.

**Samples preparation**

Samples were prepared as the following: 10 gm collected from each sample were collected aseptically, then transferred into sterile polyethylene bag containing 90 ml of sterile 0.1% peptone water (Oxoid). Samples were blended in a stomacher (Lab-blender, 400) for one minute to provide 10-1 dilution.

**Microbiological examination**

Total bacterial count performed as following: 0.1ml of each dilution to Standard plate count agar (Oxoid), incubated at 35°C/24 hours, count colonies between 30-300 colony (CFU). The isolation of C. jejuni was performed by streaking the dilutions on blood agar medium (Preston agar) (Oxoid), then incubated microaerobically at 42°C/24-48 hours. All the isolates preserved on nutrient agar (Oxoid) and examined microscopically by Gram’s stain and biochemically [10]. Ten pure positive isolates of C. jejuni were selected for further virulence genes identification by PCR.

**PCR protocol**

DNA was extracted from C. jejuni isolates from chicken samples using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications. In brief, 200 µl of the sample suspension incubated with 10 µl of proteinase K and 200 µl of lysis at 56°C/10 minutes. After incubation, 200 µl of 10% ethanol was added to the lysate, washed samples and centrifuged. Nucleic acid was eluted with 100 µl of elution buffer kit. Primers (Biobasic, Canada) showed in Table (1). Primers were added in a 25µl PCR reaction containing 12.5 µl of 2X DreamTaq Green Mastermix kit (Fermentas, Germany), 1 µl/each primer of 10 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reactions were conducted in biosystem 2720 thermal cycler. The thermal cycle condition was as following: initial denaturation at 94°C/4 minutes, followed by 35 cycles of denaturation at 94°C/30 seconds, annealing (listed in Table (1), for 30 seconds, and extension at 72°C/45 seconds, extension at 72°C/5 minutes.

Electrophoresis of PCR products performed on 1.2% agarose gel (Applichem, Germany, GmbH) in 1X TBE buffer at 37°C using gradients of 5V/cm. For gel analysis, 10 µl of PCR products were loaded in each gel slot and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) which detect the fragment sizes. Control negative and positive control were used in each reaction. Photographing the gel by a gel documentation system (Alpha Innotech, Biometra).

**RESULTS**

**PM lesions**

Of dead broiler chickens were accumulation of fluid and gas or mucus in the intestines. Distention of intestines including ceca with watery fluid.

**Total prevalence of C. jejuni among broiler chicken**

Results showed that the total prevalence of C. jejuni among chicken samples was 25.5%. The higher C. jejuni prevalence was in intestinal samples 26 (26%) followed by liver samples 25 (25%).

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primers sequences, target genes, amplicon sizes and annealing temperature of PCR reactions.</th>
<th>Amplified segment (bp)</th>
<th>Annealing</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>flaA</td>
<td>AATAAAAATGCTGATAAAACAGGGTG&lt;br&gt; TACCGAACCAATGCTGCTGCTGATT</td>
<td>855</td>
<td>53˚C</td>
<td>Datta et al., 2003</td>
</tr>
<tr>
<td>VirB11</td>
<td>TCTTGTGAGTGTGCCCTACGCTTT&lt;br&gt; CCTGCTGTCTGTTGTTATTTACC</td>
<td>494</td>
<td>53˚C</td>
<td></td>
</tr>
<tr>
<td>iam</td>
<td>GCGCAAATATTATACCACCC&lt;br&gt; TTCAGACTACTACTATGCGG</td>
<td>518</td>
<td>46˚C</td>
<td>Wieczorek et al., 2012</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Virulence C. jejuni genes</th>
<th>flaA No (%)</th>
<th>VirB11 No (%)</th>
<th>iam No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal samples</td>
<td>2 (20%)&lt;br&gt; 1 (10%)&lt;br&gt; 5 (50%)</td>
<td>-&lt;br&gt; -&lt;br&gt; 3 (30%)</td>
<td>2 (10%)&lt;br&gt; 1 (5%)&lt;br&gt; 8 (40%)</td>
<td></td>
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</table>
Presence of VirB11, flaA, and iam virulence genes among C. jejuni isolates from chicken

The prevalence of three main virulence genes C. jejuni from chicken intestinal (10 samples) and liver samples (10 samples) was reported in table (2) as following: the total prevalence of VirB11, flaA, and iam genes was 10%, 5%, and 40%, respectively. VirB11 gene was found in 2 (20%) in intestinal samples. flaA gene was detected in 1 (10%) only in intestinal samples. The highest rate was detected in iam gene by a percentage of 5 (50%) in intestinal samples and in a lower rate in liver samples 3 (30%).

PCR amplification of the enterotoxins (VirB11) was 494 bp products of DNA extracted from C. jejuni, enterotoxins (iam): 518 bp molecular size ladder. Enterotoxins (flaA): 855 bp of C. jejuni isolates from chicken samples respectively.

DISCUSSION

Chickens are susceptible to many bacterial agents and may be spread by a shortage of hygiene, frequent exposure to the bacterial agents which considered as carriers of these microorganisms due to their genetic immunity. C. jejuni is one of these agents that can be isolated from poultry. [11]. On commercial poultry farms, Campylobacter is rarely detected in birds less than 3 weeks of age. The reason for this lack of infection in young birds is unclear and may be related to various factors including the presence of maternally derived antibodies or differences in environmental or host-related conditions. Typically, the prevalence of Campylobacter rises as the birds grow and reaches the slaughter age for boiler chickens.

Campylobacter infections in poultry usually produce no clinical symptoms of illness under natural conditions. Although, some studies recorded that experimental challenge of young chickens with Campylobacter can produce clinical signs in the form of watery/mucoid/bloody diarrhea, weight loss, or even mortality.

C. jejuni live in the poultry intestinal tract which be more dangerous in chicks which called “enter-invasive transient diarrhea” and appear as; hepatic focal necrosis, watery droppings, jejunal distention, focal hemorrhage or completely absence of clinical signs. Infected chicken shed C. jejuni within 2-3 weeks after infection [12]. However, [13], demonstrated that 100% of birds infected asymptomatically by C. jejuni in their intestine, shedding the C. jejuni through feces.

The prevalence of Campylobacter in commercial broilers ranges from 20-90% [14] In Canada and South America, the prevalence of Campylobacter in commercial broilers ranges from 45-48% and 20-96%, respectively [12,15]. In Europe, the proportion of commercial broiler flocks colonized with Campylobacter varies from 2.9% to more than 92% [16-18], with the lowest flock prevalence (2.9%) observed in Finland [19] In other regions, the prevalence of Campylobacter within commercial broiler flocks ranges from 13.6% to 87% in Africa [20] 24% to 54% in Asia [21]. The obtained results of this study lay approximately in the middle area of the previous ratios. While, it is lower than the results of [22] (42% in Australia) and the prevalence rate of infected flocks is 44-59% [23].

As well as, the average Campylobacter prevalence rate on chicken at retail is 57% [24]. Other study on the prevalence of Campylobacter in commercial broilers has shown that 85-98% of commercial broiler flocks were colonized by C. jejuni [25]. The differences in the prevalence of C. jejuni could be attributed to the geographical area in this study. This study was conducted in a desert area and very hot and dry weather might be a determinant of the prevalence rate. A variety of PCR assays targeting genus- or species-specific sequences have been developed to detect and identify Campylobacter from poultry.

Some important genetic markers of C. jejuni virulence have been detected. FlaA gene, virB11 gene are pathogenic genes and perform the expression of invasion and iam gene is performed the invasiveness and transmission of Campylobacter and its adaptation to other hosts [26,27]. This research, prevalence of VirB11 gene, flaA, and iam genes was 10%, 5%, and 40%, respectively in chicken intestine and liver. Results of VirB11 gene in chicken was higher than that recorded by many authors; 6.1%
REFERENCES


