Prevalence of Clostridium perfringens type A isolates in commercial broiler chickens and parent broiler breeder hens in Egypt

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Summary
The aim of this study was to determine the presence of genes coding for alpha (cpα), beta (cpβ), epsilon (εtx), iota (ιA), enterotoxin (cpe) and beta2 (cpβ2) toxins in Clostridium perfringens isolates from broiler chickens and parent broiler breeder hens, using multiplex polymerase chain reaction (PCR) assay. The prevalence of C. perfringens in the intestinal segments and the effects of age were also investigated. The highest isolation rate was from the duodenum, at 41.7% in broiler chickens and 58.4% in parent broiler breeder hens; the lowest isolation rates came from the ileum, at 15.6% and 27.1%, respectively. Chickens harboured C. perfringens in the intestine and this increased with age. Clostridium perfringens was detected in 35.4% (17/48) of asymptomatic broiler chickens and 22.1% (17/77) of asymptomatic parent broiler breeder hens. The bacterium was detected in 100% of the broiler chickens and parent broiler breeder hens with clinical signs (31/31 and 60/60, respectively). The multiplex PCR assay indicated that in 99 (79.2%) of the 125 samples that tested positive for C. perfringens the strains isolated were type A and were shown to carry the cpα gene (99/99, or 100%). The gene encoding cpβ2-toxin was present in 62.6% (62/99) of the isolates. A significant association was found between C. perfringens possessing the β2-toxin gene and necrotic enteritis in broiler chickens and parent broiler breeder hens, suggesting that this gene might play a key role in the pathogenesis of the disease in Egypt.

The authors suggest that the presence of the cpβ2-toxin gene in C. perfringens isolates found in broiler chickens and parent broiler breeder hens during this study poses a risk of transmission to humans through the food chain.

Keywords

Introduction
Clostridium perfringens is widespread in the environment and is commonly found among normal gut flora. It is often involved in diseases in humans, most domestic animals and some wildlife, including horses, poultry, birds, rabbits, sheep, goats, cattle, mink, ostriches, dogs and cats (42). It is certainly the most serious cause of clostridial enteric disease in domestic animals (27). Avian necrotic enteritis is caused by specific strains of C. perfringens and costs the
world poultry industry an estimated $2 billion annually, largely due to the costs of antimicrobial prophylaxis and inefficient feed conversion (9, 52).

The virulence of this Gram-positive bacterium is largely attributable to prolific toxin production; ~15 different C. perfringens toxins have been reported in the literature (39, 40). Although all C. perfringens types produce α-toxin (23), individual C. perfringens isolates do not produce this entire arsenal of toxins. The pathogenic versatility of this bacterium causes a spectrum of enteric and histotoxic diseases in animals and humans. Variability of toxin production among isolates also provides the basis for a commonly used classification scheme that assigns C. perfringens isolates to one of five toxintypes (types A to E), based on the presence of four major lethal toxins (alpha-, beta-, epsilon-, and iota-toxins). Various strains of C. perfringens can also produce a range of other toxins: enterotoxin (cpα), β2 toxin (cpβ2), perfringolysin O (β-toxin), collagenase (φ-toxin), and necrotic enteritis β-like toxin (netβ) (28, 34). A number of C. perfringens toxin genes such as beta toxin (cpβ), epsilon toxin (εtx) and beta2-toxin (cpβ2) are associated with large plasmids (34). The β2 toxin has been demonstrated in avian C. perfringens type A strains (1, 5).

In addition to the economic importance of C. perfringens in poultry, there is also a risk to public health through the food chain (26, 51). As implied by the name, C. perfringens food poisoning is almost always associated with type A isolates (18, 31, 55). Clostridium perfringens is one of the most frequently isolated bacterial pathogens in foodborne disease outbreaks in humans (6, 18, 58). It is reported to be the third most common cause of foodborne illness in the world (53) and annually ranks as one of the most common causes of food poisoning in the industrialised world (17, 37, 40, 43, 44). Different meats, including poultry meat, have frequently been reported as the most common food vehicles (8, 25, 43). The common occurrence of C. perfringens in raw meat and retail foods may be due to the contamination of carcasses and meat with the intestinal contents of the animals during the slaughtering process (55).

The classification of C. perfringens isolates into toxigenic types has traditionally been based on seroneutralisation in mice or guinea-pigs, but this has largely been replaced by polymerase chain reaction (PCR)-based detection (4, 57). Various PCR protocols, including multiplex PCR assays, have been established to determine the genotype of C. perfringens isolates with respect to the genes cpα, cpβ, etx, εtx, δtx, cpε and cpβ2, encoding the α-, β-, ε-, δ-, entero- and β2-toxin, respectively (4, 22, 57).

Since different C. perfringens types cause different diseases in animals and humans, determining the presence of these toxins is paramount when diagnosing a disease or investigating the epidemiology of C. perfringens. In this study, the multiplex PCR protocol of Yoo et al. (57) was used for detection of alpha (cpα), beta (cpβ), epsilon (εtx), iota (ια), enterotoxin (cpε) and beta2 (cpβ2) genes in a total of 72 C. perfringens isolates from both symptomatic and asymptomatic broiler chickens and parent broiler breeder hens.

Materials and methods

Isolation, identification, typing and enrichment of field isolates of Clostridium perfringens in the gastro-intestinal tract

A total of 216 gastro-intestinal tract samples were collected from broiler chickens (n = 79; 48 asymptomatic and 31 symptomatic) and parent broiler breeder hens (n = 137; 77 asymptomatic and 60 symptomatic). They were acquired from 17 separate commercial poultry-breeding facilities in Lower Egypt in which birds had recently experienced clinical signs of necrotic enteritis associated with C. perfringens, including depression, ruffled feathers, diarrhoea and macroscopically evident lesions in the small intestines. These facilities originally housed birds reared without growth-promoting antibiotics, but the birds were being treated therapeutically with antibiotics at the time of sampling. An additional 144 intestinal samples were taken from the asymptomatic broiler chickens for PCR testing. Similarly, PCR testing was carried out on an additional 231 intestinal samples from the asymptomatic parent broiler breeder hens.

Chickens of various ages (3.2 to 5.2 weeks, n = 256; 10 to 21 weeks, n = 105; 26 to 53 weeks, n = 156) were randomly selected for sampling and sacrificed by cervical dislocation.

To isolate sporulating strains, luminal material from the intestines was enriched for 24 h in cooked meat broth (Oxoid) under anaerobic conditions at 37°C, and subjected to heat and alcohol shock. A loopful of culture was plated onto a blood agar base (Oxoid) with 10% sheep blood and 70 µg/ml neomycin sulphate. The plates were incubated at 37°C for 24 h under anaerobic conditions (gas-generating kit, B 36, Oxoid). Typical colonies showing a double zone of beta haemolysis were picked up and sub-cultured. The sub-cultured colonies were identified by colony morphology, Gram staining, Nagler reaction, urease test, lecinthase test, aerotolerance in chocolate agar plate (at 37°C in 5–10% CO2 for 24 h), reverse CAMP (Christie Atkins and Munch-Peterson) reaction, catalase test, lactose fermentation, gelatinase production, nitrate reduction, motility test, acid phosphatase reaction, and other biochemical tests. The identity of these isolates was further
confirmed using multiplex PCR, as previously described (57), using oligonucleotide gene primers (Table I).

**Bacterial reference strains**

*Clostridium perfringens* NCTC 8239 (for *cpa*, *cpb* genes), ATCC 3626 (for *stx*, *ctp* genes) and CCUG 44727 (for the *tA* gene) were used as positive controls in this study. *Staphylococcus aureus* (ATCC 29737) was used as a negative control.

**Extraction of DNA**

The boiling technique was used to extract DNA from all the isolates, as previously described in the protocol of Croci et al. (13). All strains were streaked onto blood agar plates and incubated under anaerobic conditions at 37°C for 24 h, then picked and suspended in 100 µl of distilled water in a microcentrifuge tube. The cell suspension was centrifuged for 10 min at 14,000 × g. The pellet was re-suspended in 300 µl of DNase-RNase-free sterile distilled water by vortexing. The microcentrifuge tube was incubated for 15 min at 100°C and immediately chilled on ice. An aliquot of 5 µl of the supernatant was used as the template DNA in the PCR.

**Primers**

The sequences of the oligonucleotide primers, based on published DNA sequences of the *cpa*, *cpb* and *stx* genes (57), that were used to amplify portions of the major toxin genes of *C. perfringens* are outlined in Table I.

<table>
<thead>
<tr>
<th>Table I</th>
<th>The DNA sequences of polymerase chain reaction primers for the toxin genes of <em>Clostridium perfringens</em>, their position relative to the published DNA sequences and product size (base pairs) (57)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha toxin gene cpa</strong> (402 bp)</td>
<td><strong>Forward</strong> 5’-GTT GAT GTC GCA GGA CAT GTT AAG-3’  <strong>Reverse</strong> 5’-CAT GTA GTC ATC TGT TCC AGC ATC-3’</td>
</tr>
<tr>
<td><strong>Beta toxin gene cpb</strong> (236 bp)</td>
<td><strong>Forward</strong> 5’-ACT ATACACGACAGATCAAACC-3’  <strong>Reverse</strong> 5’-TTAGGACGATTGAACGTACACGAC-3’</td>
</tr>
<tr>
<td>**Epsilon toxin gene ctX (541 bp)</td>
<td><strong>Forward</strong> 5’-ACT GCA ACT ACT CAT ACT GTG-3’  <strong>Reverse</strong> 5’-CTG GTG CCT TAA TAG AAA GAC TCC-3’</td>
</tr>
<tr>
<td>**Iota toxin gene tA (317 bp)</td>
<td><strong>Forward</strong> 5’-GGGATGAAAACGTCACCCACTAC-3’  <strong>Reverse</strong> 5’-GGTATATCCTCCAGCATAATGTC-3’</td>
</tr>
<tr>
<td>**Enterotoxin gene cpc (233 bp)</td>
<td><strong>Forward</strong> 5’-GGGATGGAATGGATATAGGG-3’  <strong>Reverse</strong> 5’-GGACCAGCAAGTGTGATAA-3’</td>
</tr>
</tbody>
</table>

**Multiplex polymerase chain reaction**

Polymerase chain reaction amplification was used to detect the various toxin genes of each *C. perfringens* isolate (*n* = 72). This included toxin typing (alpha, beta and epsilon toxin genes), which was performed as a multiplex reaction, as described by Yoo et al. (57). Each multiplex PCR reaction mix contained 5 µl *C. perfringens* template DNA, 1 µl (10 pmol/µl) each of *cpa*, *cpb* and *stx* primer sequences, as described by Yoo et al. (57), 2.5 µl of 2 mM deoxyribonucleotide triphosphates (dNTPs), 2.5 µl of 10 × PCR buffer (50 mM potassium chloride [KCl], 10 mM Tris-HCl, pH 8.3, 2 mM MgCl2, 0.1% TritonX-100), 0.25 µl of 5 U/µl of Taq DNA polymerase (Roche) and water to 25 µl. The DNA was initially denatured for 5 min at 95°C then amplified for 30 cycles (1 min at 94°C, 1 min at 55°C, 1 min at 72°C for denaturation, annealing and extension phases, respectively), and followed by an additional period of extension for 3 min at 72°C (Biometra Personal Cycler, Goettingen, Germany). Samples (10 µl) of PCR products were separated by electrophoresis for 45 to 60 min at 80 V in a 2% (weight/volume) agarose gel with ethidium bromide 0.5 µg/ml. Amplified bands were visualised and photographed under ultra-violet (UV) illumination.

**Plasmid-encoded gene cplβ2-specific PCR analysis**

A primer pair was designed for amplification of the *cpβ2* gene, based on published DNA sequences (54). The size of the amplified product was adjusted to be similar to those previously published, to allow the amplified product to be incorporated into the PCR protocol (54). The previously prepared DNA pellets were thawed and plasmid DNA was isolated using the Bio-spin Mini-Prep plasmid extraction kit (BioFlux-Bioer Technology Co. Ltd, Japan) according to the manufacturer's protocol. The plasmid DNA was then subjected to screening in a *cpβ2*-specific PCR with two sets of primers designed in the authors’ laboratory using the Primer Select 3.1 software package of LaserGeneTM version 8.1 (DNASTAR Inc., Madison, Wisconsin, USA) and custom synthesised by Biometra, Germany. The primer set 5’-TTA ATA ACA ATA ACC CTC ACC AAA-3’ (primer 3F) and 5’-TGA AAA ATG GTA-3’ (primer 4R) was used to amplify a 1,300 base pair (bp) DNA fragment carrying the entire *cpβ2*. The PCR samples comprised 100 ng of template DNA, 50 pM of each primer, 200 µM of dNTPs (Roche), 50 mM KCl, 3.0 mM MgCl2, and 2.5 U of Taq DNA polymerase (Fermentas) in a total volume of 50 µl. The reaction mixture was placed in a thermal cycler (Biometra, Germany) for an initial period of 3 min at 95°C (denaturation) for one cycle. It was then subjected to 40 cycles, each consisting of 1 min at 95°C, 45 s at 60°C (annealing), and 1 min at 72°C (extension), followed by an additional period of extension for 10 min at 72°C. After PCR, samples of amplified product...
were electrophoresed at 100 V in 1.5% agarose gels, followed by ethidium bromide staining and visualisation under UV illumination. For confirmation, accuracy and validation, the authors’ prepared plasmid DNA was also subjected to screening with the previously published cpβ2 primers (54). The PCR was performed under the same amplification conditions, with the same reagents and volumes of template.

The statistical software packages Epicalc 2000 version 1.02 and MedCalc version 11.4.4 (MedCalc Software Inc, Mariakerke, Belgium) were used for data analysis. The difference between the proportions of the groups was determined using the Z test. A p-value <0.05 was considered to be statistically significant. Data analysis was performed using the chi-square test or Fisher’s exact probability test, as appropriate.

### Table IIa

<table>
<thead>
<tr>
<th>Broiler chickens</th>
<th>Parent broiler breeder hens</th>
<th>p-value</th>
<th>95% CI</th>
<th>OR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>48/79 (60.8%)a</td>
<td>77/137</td>
<td>0.51</td>
<td>0.687–2.12</td>
<td>1.25</td>
<td>125/216</td>
</tr>
<tr>
<td>(56.2%)b</td>
<td></td>
<td></td>
<td></td>
<td>(57.9%)</td>
<td></td>
</tr>
</tbody>
</table>

CI : confidence interval
OR: odds ratio

a) Percentage calculated from the total number of asymptomatic and symptomatic birds (n = 79)

b) Percentage calculated from the total number of asymptomatic and symptomatic birds (n = 137)

### Table IIb

<table>
<thead>
<tr>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>p-value</th>
<th>95% CI</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>34/125 (27.2%)a</td>
<td>91/91 (100%)b</td>
<td>0.001</td>
<td>0.204–0.362</td>
<td>0.272</td>
</tr>
<tr>
<td>34/216 (15.7%)c</td>
<td>91/216 (42.1%)d</td>
<td>0.001</td>
<td>0.163–0.404</td>
<td>0.257</td>
</tr>
</tbody>
</table>

CI : confidence interval
OR: odds ratio

a) Percentage calculated from the total number (n = 125) of asymptomatic birds (broiler chickens, n = 48, and parent broiler breeder hens, n = 77)
b) Percentage calculated from the total number (n = 91) of symptomatic birds (broiler chickens, n = 31, and parent broiler breeder hens, n = 60)
c) Percentage calculated from the total number (n = 216) of asymptomatic and symptomatic birds (broiler chickens, n = 79, and parent broiler breeder hens, n = 137)
d) Percentage calculated from the total number (n = 216) of asymptomatic and symptomatic birds (broiler chickens, n = 79, and parent broiler breeder hens, n = 137)

### Results

Total prevalence of Clostridium perfringens among asymptomatic and symptomatic broiler chickens and parent broiler breeder hens

A total of 216 samples from broiler chickens and parent broiler breeder hens were tested for the presence of

\[ C.\ perfringens \] detected positive, a prevalence of 57.9% (Table Ia). The prevalence among the broiler chickens (asymptomatic and symptomatic) was 60.8% (48/79) but this figure dropped slightly to 56.2% in the parent broiler breeder hens (77/137). Among the 216 samples, \[ C.\ perfringens \] was isolated from 34 asymptomatic birds (15.7%) and 91 symptomatic birds (42.1%). \[ C.\ perfringens \] was found to prevail significantly (p < 0.001) in the asymptomatic (27.2%; 34/125) and symptomatic birds (100%; 91/91) (Table Iib). In Table III, the results indicate that the percentage of samples that tested positive from asymptomatic and symptomatic broiler chickens reached 35.4% (17/48) and 100% (31/31), respectively. In the asymptomatic and symptomatic parent broiler breeder hens, the percentage of positive samples was 22.1% (17/77) and 100% (60/60), respectively.

### Polymerase chain reaction to detect Clostridium perfringens in intestinal segments from broiler chickens and parent broiler hens

A total of 48 samples were taken from segments of bird-gut from the broiler chickens (20 from the duodenum, 15 from the jejunum and 13 from the ileum). A total of 77 bird-gut segment samples were taken from the parent broiler hens (45 from the duodenum, 20 from the jejunum and 12 from the ileum). These intestinal samples were then examined by PCR to detect \[ C.\ perfringens \] (Table IV). In the broiler chickens, 48/144 (33.3%) of the intestinal segments tested positive for naturally occurring \[ C.\ perfringens \]. This value represented a prevalence of 41.7% (20/48), 31.3% (15/48) and 27.1% (13/48) from the duodenum, jejunum and ileum, respectively. In the parent broiler hens, 77/231 (33.3%) of the intestinal segments tested positive for naturally occurring \[ C.\ perfringens \]. This value represented a significant prevalence (p < 0.001) of 58.4% (45/77), 26.0% (20/77) and 15.6% (12/77) in the duodenum, jejunum and ileum, respectively.

### Toxinotyping of Clostridium perfringens recovered from birds from different sources

In 99/125 (79.2%) of the samples that tested positive for \[ C.\ perfringens \], the strains isolated possessed the \[ cpa \] gene and lacked the \[ cpβ, stx, A \] and \[ cpε \] genes, indicating that these isolates were type A (Table III). Amplification of the 402 bp fragment of the alpha toxin gene \[ cpa \] extracted from
the DNA of the local isolates of *C. perfringens* (99/99; 100%) was observed (Table III).

### Results of *cpβ2* toxin gene amplification in polymerase chain reaction

The extracted DNA of *C. perfringens* isolates was tested with the forward and reverse primers for *cpβ2* toxin. The results revealed positive amplification of 1,300 bp fragments of the *cpβ2* toxin gene (62/99; 62.6%).

### Discussion

*Clostridium perfringens* plays a significant role in foodborne human disease, as well as in human, animal and poultry diseases that are not foodborne (36, 39). *Clostridium perfringens* enteritis has been reported in most areas of the world and adversely affects the integrated system of poultry production, resulting in gastro-intestinal dysbacteriosis and necrotic enteritis (24, 35). The *C. perfringens* isolates used in this study were all obtained from healthy, conventionally reared poultry (i.e. those fed with non-therapeutic, growth-promoting antibiotics).

In the present study, the isolates from the broiler chickens and parent broiler breeder hens were obtained from conventionally cultivated samples by picking a couple of colonies from each sample. Thus, the isolates represented the dominant *C. perfringens* population in the intestines of the broiler chickens and parent broiler breeder hens. The

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### Table III

**Relationship between the genotype obtained by polymerase chain reaction and the phenotype of the 125 *Clostridium perfringens* isolates**

<table>
<thead>
<tr>
<th>Birds</th>
<th>Bird health</th>
<th><em>C. perfringens</em> strains</th>
<th>Toxinotype</th>
<th>Genotype</th>
<th>Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler chickens</td>
<td>Asymptomatic</td>
<td>17 (35.4%)</td>
<td>Type A</td>
<td>cpα</td>
<td>β2</td>
</tr>
<tr>
<td></td>
<td>Symptomatic</td>
<td>31 (100%)</td>
<td>Type A</td>
<td>cpα</td>
<td>β2</td>
</tr>
<tr>
<td></td>
<td>cpα</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parent broiler breeder hens</td>
<td>Asymptomatic</td>
<td>17 (22.1%)</td>
<td>Type A</td>
<td>cpα</td>
<td>β2</td>
</tr>
<tr>
<td></td>
<td>Symptomatic</td>
<td>60 (100%)</td>
<td>Type A</td>
<td>cpα</td>
<td>β2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>216</td>
<td>125/216 (57.9%)</td>
<td>99/125 (79.2%)</td>
<td>99/99 (100%)</td>
</tr>
</tbody>
</table>
A key risk factor for the development of necrotic enteritis is an intestinal environment that favours the growth of *C. perfringens*. Prevalence of *C. perfringens* in the intestinal tract has previously been shown to be high in processed broiler chickens (10, 11). When the intestinal contents of broiler chickens were analysed for the presence of *C. perfringens*, approximately 75% to 95% of the birds were found to have been colonised by *C. perfringens*, with only a small proportion of these ever showing signs of the disease (11, 35). The present authors were able to detect differences among the *C. perfringens* populations. The lowest prevalence was found in the ileum. This contradicts previous findings on the frequency of *C. perfringens* isolations from the intestinal commensals (3, 38, 45). The varying prevalences of *C. perfringens* in the three segments (duodenum, jejunum and ileum) probably result from differences in the pH of the intestinal contents in each segment. Like most microorganisms, *C. perfringens* grows most readily under neutral pH conditions, although excellent growth also occurs at values between pH 6.0 and pH 7.0, a range similar to that found in most meat and poultry products (30). In the intestine, *C. perfringens* growth, bacterial colonisation, numbers of lesions and production of β2 toxin by *C. perfringens* might be regulated by decreasing the intestinal pH (2, 46). This could explain the significantly increased percentage, recorded in this study, of *C. perfringens* in the duodenum where the pH is acidic. There is further evidence that different dietary protein sources and the dietary amino acid balance may influence the proliferation of *C. perfringens* in the ileum (56). Dietary soy oil has been shown to significantly reduce the population of *C. perfringens* in the ileal microflora of broiler chickens (29).

The five toxinotypes of *C. perfringens* cannot be reliably differentiated on the basis of cellular or colonial morphology, biochemical reactions or gas liquid chromatographic analyses of fatty and organic acids and products of metabolism. Nowadays, PCR is used to detect the presence of toxin genes for the typing of isolates and to identify the specific strains of *C. perfringens* associated with the particular disease (20).

The authors of the present study chose the PCR protocol published by Yoo et al. (57) to determine the presence of major toxin genes (cpa, cpb, εtx, αA, cpa and cpb2) in *C. perfringens* isolates obtained from broiler chickens and parent broiler breeder hens in Egypt. The three primer pairs revealed the expected amplification products. This assay was shown to simultaneously and effectively amplify products with all primers used, revealing visible and distinguishable PCR products in gel electrophoresis.

The primer combination used to investigate a total of 125 *C. perfringens* isolates from the broiler and breeder chickens indicated that all the isolates carried the cpa gene but not the other toxin genes detected in the PCR assay. This is not surprising, as type A has been reported to be the dominant type of *C. perfringens* in the intestines of broilers worldwide (16, 41, 57). The colonisation of the intestinal tract of broilers by *C. perfringens* is thought to be an early event, with soil, dust, contaminated feed and litter being implicated as sources of the organism (11).

In this investigation, 72 *C. perfringens* isolates were obtained from the broiler chickens and parent broiler breeder hens that were suffering from necrotic enteritis. Multiplex PCR toxin typing showed that all 72 isolates were *C. perfringens* toxin type A, which is in agreement with surveys carried out in Iran (49), Europe (15, 32, 47), Korea (57), India (14), Australia (28), the USA (12, 50) and Canada (7). In the present investigation, 62/99 isolates tested positive for the cpb2 gene, which encodes the β2-toxin. This toxin has been implicated in necrotic enteritis in other animals; for example, in pigs (54), Asiatic black bears (*Selenarctos thibetanus*) (21), lambs, foals, alpaca, an elk, a swan, a llama, a horse and calves (19, 33). Surprisingly, cpb2-positive *C. perfringens* strains have been reported in a large number of healthy birds, animals and humans, without any sign of intestinal disease (4, 5, 6, 7, 8, 12, 36, 48). The difference in the prevalences of cpb2-positive *C. perfringens* isolates from in-contact asymptomatic and symptomatic broiler chickens and parent broiler breeder hens in this study and those in findings reported by other workers may be due to geographical location (12, 49).

Acute necrotic enteritis may come and go like a storm, but it is ongoing sub-clinical necrotic enteritis that is responsible for the real economic damage. Necrotic enteritis is the most common and financially devastating bacterial disease in broilers, and the sub-clinical form is by far the most damaging for producers.

This study is the first published research to examine the prevalence of *C. perfringens* in parent birds. Studies to understand the association of *C. perfringens* (and other foodborne enteropathogens) with the intestinal tract of the broiler breeder are ongoing. Such research will assist in the development of effective intervention strategies to reduce *C. perfringens* in poultry, thus also reducing human exposure to this important microorganism.

**Conclusions**

1. The prevalence of *C. perfringens* in broiler chickens and parent broiler breeder hens was substantial.

2. The prevalence of *C. perfringens* was slightly higher in broiler chickens than in parent broiler breeder hens.
3. These results provide further circumstantial evidence that breeder-to-broiler transmission of *C. perfringens* should not be overlooked, and indicate that intervention strategies should aggressively target critical control points previously excluded for *C. perfringens*, such as breeder flocks, hatching cabinets and hatchery environments.

4. The results presented here clearly suggest that the normal intestinal flora are of great importance in preventing and silencing toxin production by *C. perfringens* in the host. This mechanism might lead to new ways of preventing disease without the use of antibiotics.

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**Prévalence de *Clostridium perfringens* de type A chez les poulets de chair d’élevages commerciaux et les poules reproductrices en Égypte**

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**Résumé**

La présente étude avait pour but de rechercher la présence des gènes codant pour les toxines alpha (*cpα*), beta (*cpβ*), epsilon (*εtx*), iota (*ι*A), entérotoxine (*cpε*) et beta2 (*cpβ2*) dans des isolats de *Clostridium perfringens* issus de poulets de chair et de poules reproductrices d’élevages commerciaux. L’épreuve utilisée était une amplification en chaîne par polymérase (PCR) multiplexe. L’étude visait également à déterminer quelles étaient les parties de l’intestin privilégiées par *C. perfringens* ainsi que les effets de l’âge des volailles sur l’infection. Le pourcentage le plus élevé d’échantillons positifs provenait du duodénum (avec un taux de 41,7 % chez les poulets de chair et de 58,4 % chez les poules reproductrices), et le plus faible de l’iléon (taux de 15,6 % et de 31,3 % respectivement). La présence de *C. perfringens* dans l’intestin des poulets augmentait avec l’âge. *Clostridium perfringens* a été détecté chez 35,4 % (17/48) des poulets de chair sains et chez 22,1 % (17/77) des poules reproductrices saines. La bactérie a également été détectée chez la totalité des poulets de chair et des poules reproductrices présentant des signes cliniques (31/31 et 60/60, respectivement). Les souches isolées dans 99 des 125 échantillons positifs (79,2%) ont été caractérisées au moyen d’une PCR multiplexe comme appartenant au type A et portant le gène *cpα*. Le gène codant pour la toxine *cpβ2* était présent dans 62,6% de ces 99 isolats. Une corrélation significative a été mise en évidence entre la présence de *C. perfringens* portant le gène codant para la toxine β2 et l’entérite nécrosante chez les poulets de chair et les poules reproductrices, ce qui indique le rôle majeur que ce gène semble jouer dans la pathogénèse de la maladie en Égypte.

Les auteurs considèrent que la présence du gène codant pour la toxine *cpβ2* dans les isolats de *C. perfringens* constitue un risque pour la santé publique, en raison de la possibilité de transmission à l’homme par la chaîne alimentaire.

**Mots-clés**

Prevalencia de *Clostridium perfringens* tipo A en pollos asaderos industriales y sus gallinas progenitoras en Egipto

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**Resumen**

Los autores describen un estudio encaminado a determinar la presencia de los genes codificadores de las toxinas alfa (cpα), beta (cpβ), épsilon (εtx), iota (ιA), la enterotoxina (cpε) y la toxina beta2 (cpβ2) en los microorganismos *Clostridium perfringens* aislados en muestras de pollos asaderos y de las gallinas progenitoras de esos pollos, utilizando para tal fin un ensayo de reacción en cadena de la polimerasa (PCR) múltiple. También se estudiaron la incidencia de *C. perfringens* en los segmentos intestinales y los efectos de la edad. El porcentaje más elevado de muestras positivas en pollos y gallinas se encontró en el duodeno, con una incidencia del 41,7% y el 58,4%, respectivamente, y el más bajo lo arrojaron las muestras tomadas del ileo, con una incidencia del 15,6% y el 31,3% respectivamente. Los pollos albergaban microorganismos *C. perfringens* en el intestino, y esta presencia aumentaba con la edad. Se detectó *C. perfringens* en un 35,4% (17/48) de los pollos asaderos asintomáticos y un 22,1% (17/77) de las gallinas progenitoras asintomáticas. En cuanto a los pollos y gallinas con signos clínicos, se observó la presencia de la bacteria en la totalidad de los casos (31/31 y 60/60 respectivamente). La aplicación de la PCR múltiple reveló que, en 99 (un 79,2%) de las 125 muestras positivas para *C. perfringens*, los microorganismos aislados eran del tipo A, y se comprobó que estos poseían el gen *cpα* en todos los casos (99/99). El gen codificador de la cpβ2 estaba presente en un 62,6% (62/99) de los microorganismos aislados. Tanto en los pollos como en las gallinas progenitoras se observó una relación significativa entre los *C. perfringens* dotados del gen de la toxina cpβ2 y la enteritis necrótica, lo que lleva a pensar que este gen podría tener una función básica en la patogénesis de la enfermedad en Egipto.

Atendiendo a estos resultados, los autores postulan que la presencia del gen de la toxina cpβ2 en los *C. perfringens* descubiertos en este estudio en pollos asaderos y sus gallinas progenitoras entraña riesgo de transmisión al ser humano a través de la cadena alimentaria.

**Palabras clave**

References


