ABSTRACT: *Clostridium perfringens* is a Gram-positive, endospore-forming, anaerobic rod, ubiquitous in nature. *C. perfringens* strains can produce about 17 toxins. Many of them can lead to miscellaneous diseases, among which the enteric ailment may be the most common and is of utmost importance. In the present work 34 strains of *C. perfringens* isolated from feed and one from a cow suspected to have died of clostridial infection were subjected to molecular analysis. In order to detect the genotypes, the following genes coding for toxins were targetted: *cpa, cpb, cpb2, cpe, etx* and *iap*. The multiplex PCR assay revealed that all *C. perfringens* isolates from animal feed were of type A and β2-toxinogenic type A strains, possessing only the *cpa* (n=21), or both the *cpa* and the *cpb2* genes (n=13). The importance of *C. perfringens* toxins α and β-2 in the pathogenesis of enterotoxemia is discussed and the regulation on the detection of this bacteria in animal feed questioned. The use of PCR in practise could enable the toxin-genotyping of *C. perfringens* isolates and, thus, provide a real basis for the establishment of maximum acceptable limits of this bacteria in feed.

Key words: *Clostridium perfringens*, toxin genotyping, animal feed, multiplex PCR assay, *cpa* gen, *cpb2* gen

INTRODUCTION

*Clostridium perfringens* is a Gram-positive, non-motile, endospore-forming, anaerobic (relatively oxygen-tolerant) bacteria, ubiquitous in nature (found in soil, dust, sewage, and fresh and marine water) and a common inhabitant of the intestinal tract in both humans and homeothermic animals (Songer, 1996; Brynestad and Granum, 2002). The species represents a very heterogeneous group with respect to metabolic bioproducts, toxins and pathogenicity. *C. perfringens* strains can produce about 17 toxins (Uzal et al., 2015; Freedman et al., 2016), four of which (α, β, ε and i) are major factors of virulence. Based on the capability to produce them, *C. perfringens* is classified into five toxotypes: A, B, C, D and E (Hatheway, 1990; Sawires and Songer, 2006). Two other major toxins are enterotoxin (CPE) and β-2 toxin, both possibly produced by all types of *C. perfringens* (Ata et al., 2013). Each toxotype may cause different diseases in animals, including acute enteritis and fatal
enterotoxemia (Baums et al., 2004; Ata et al., 2013; Lyhs et al., 2013). Some variet-
ties within types A, B and C produce certain combinations of antigens or toxins
associated with defined diseases or syn-
dromes (Ata et al., 2013; Uzal et al., 2010,
2014). Their quantities vary greatly be-
tween individual strains (Niilo, 1980).
C. perfringens may enter feed via conta-
miminated raw ingredients, or by secondary
contamination during processing, storage
and dispersal of final products. Feed con-
tamination depends on its composition, the
hygiene during production and the storage
conditions, and is directly proportional to
the levels of soil and faecal contamination
(Wojdat et al., 2006).

In the Republic of Serbia, in compliance
with regulations (Regulations on the
quality of animal feed), feed samples of 50
g must not contain any C. perfringens. For
food it is stated in The EFSA Journal
(EFSA, 2005) that "C. perfringens is com-
monly present in foods and ingredients,
ocasionally at hundreds per gram, and
microbiological testing for C. perfringens
has limited value in ensuring food safety,
because the organism is so common in or
on foods that a positive result means little,
unless very high numbers are present". In
spite of being notorious for extremely
toxigenic potential, not all of C. perfringens
strains are capable of producing illness in
neither animals nor humans.

Experimental research has confirmed that
the ingestion of C. perfringens will not lead
to sickness per se, because the majority of
bacteria consumed with food are de-
stroyed by hydrochloric acid in the stomach.
Moreover, C. perfringens does not exhibit
adherence and invasive properties to-
wards healthy intestinal mucosa. The
development of subclinical or clinical clostri-
diosis in domestic animals always results from
complicated interactions between the
virulence of the bacteria (which is highly
variable within this species), immune sta-
tus of the host and the influence of a va-
riety of non-specific factors associated
with the farm management.

Gut microbiota disturbances (most fre-
quently resulting from antibiotic therapy),
management-related stress, sudden chan-
ges in diet, and overeating and/or vorac-
iousness, especially on high-protein and
energy-rich foods, are predisposing factors
for the development of enteritis and ene-
trotoxemia (Collier et al., 2008; Timber-
mont et al., 2011; Uzal et al., 2015).

For all these reasons, without the iden-
tification of toxin genotypes and the levels
of feed contamination it is difficult to as-
sess the health risk C. perfringens in feed
poses to animals. Nonetheless, toxin ge-
netyping of C. perfringens strains isolated
from food stuffs and animal feed is not part
of routine laboratory practice in Serbia.

In this work C. perfringens isolates (n=34)
from food intended for animals were
subjected to multiplex PCR essay in order
in order to detect genes: cpa (α toxin), cpb
(β), cpb2 (β2), etx (ε), iap (i) and cpe
(enterotoxin). Further, the importance of
those findings are discussed from the
standpoint of health risk to food-producing
animals and, indirectly, to humans, con-
sumers of animal products. The results of
such investigations can be the basis for
risk assessment and determination of cri-
tical limits of the presence of C. perfrin-
gen in animal feed.

MATERIALS AND METHODS

C. perfringens isolates. C. perfringens
was isolated from feed samples according
to the standard SRPS ISO 7937:2010. The
species identification was based on: (1)
the characteristics of the colonies grown
on agar with the addition of 5% sheep
blood and incubated in anaerobic con-
ditions (GasPak EZ, Becton Dickinson and
Company, USA), (2) synergistic haemo-
lysis with Streptococcus agalactiae (re-
verse CAMP test), and (3) biochemical
properties: catalase test (negative), fer-
m entation of glucose and lactose and pro-
duction of lecithinase. Thirty-four isolates
of C. perfringens were subjects of ge-
notyping. Their origin is presented in Table
2. In addition, one isolate from the organs
of a cow which was suspected to have
died of enterotoxemia was assessed: the
organs were sent to the laboratory with the
feed that was allegedly the source of the
infection.
DNA extraction and PCR amplification

From *C. perfringens* isolates incubated overnight on blood agar suspensions in PBS were made and turbidity adjusted to 3.5 McFarland. One milliliter was transferred to an Eppendorf tube and boiled in water for 15 minutes. The tubes were cooled on ice and centrifuged for 8 min at 11,000 x g. The supernatant obtained (100 µL) was preserved in a freezer at -20 °C until being processed further. The multiplex PCR assay (mPCR) was performed according to the protocol described by Baums et al. (2004). Six sets of oligonucleotide primers were used (Table 1). The mPCR method was carried out using the "HotStar Taq Master Mix Kit" (Qiagen, Hilden, Germany), with small modification of manufacturer’s instructions. Briefly, the amplification reaction was carried out in a volume of 25 µl containing 3 µl of DNA sample, 12.5 µl of master mix and 25 pmol of each primer. *C. perfringens* ATCC 13124 was used as the positive and *C. sporogenes* ATCC 19404 as the negative control.

The cycling conditions (Thermocycler Gradient, Eppendorf, Germany) were the following: initial denaturation at 95 °C for 2 min 30s, 40 cycles of 1 min at 95 °C, 1 min at 55 °C and 1 min at 72 °C, which was followed by the final extension for 10 min at 72 °C. Ten microlitres of the PCR products were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide (0.25 g/ml) and documented with a gel documentation system (GelDoc-It² Imager, UVP, Cambridge, UK).

RESULTS

All of the *C. perfringens* isolates formed small translucent colonies surrounded by the zone of double haemolysis on blood agar (Figure 1A). Clear zone of haemolysis results from the theta (θ) toxin, whilst the α-toxin is responsible for incomplete haemolysis. The production of α-toxin in all isolates was confirmed with the positive reverse CAMP test (Figure 1B).

The multiplex PCR assay revealed the presence of specific amplicons: 900-bp long, which are characteristic of *cpa* gene, 396 bp of *etx*, and 200 bp of the *cpb2* gene encoding for *C. perfringens* toxins (Figures 2, 3 and Table 2). All *C. perfringens* isolates derived from feed were identified as type A and β2-toxinogenic type A strains, since they possessed only the *cpa* (n=21), or the *cpa* and the *cpb2* genes (n=13). In neither isolate from feed genes coding for beta (*cpb*), epsilon (*etx*) and iota toxin (*iap*) were detected.

![Figure 1. Characteristic colonies of *C. perfringens* on blood agar surrounded by zone of double haemolysis (A); Positive reverse CAMP test: Str. agalactiae ATCC 13813 (vertical line), Staphylococcus aureus ATCC 25923 (positive control, upper left horizontal line) and *C. perfringens* isolates (B)](image_url)
Table 1. 
Target toxin genes, primer sequences and length of amplification products of *C. perfringens* in multiplex PCR (Baums et al., 2004).

<table>
<thead>
<tr>
<th>Toxin gene</th>
<th>Primers</th>
<th>Sequence (5’-3’)</th>
<th>Length of amplification products (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cpa</em></td>
<td>CPA5L</td>
<td>AGTCTACGCTTTGGGATGGAA</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>CPA5R</td>
<td>TTTCTCTGGTCTTGCCATTTC</td>
<td></td>
</tr>
<tr>
<td><em>cpb</em></td>
<td>CPBL</td>
<td>TCCTTTCTTGAGGGGGAGATAA</td>
<td>611</td>
</tr>
<tr>
<td></td>
<td>CPBR</td>
<td>TGAACCTCAGATTGTTGATCCCA</td>
<td></td>
</tr>
<tr>
<td><em>cpe</em></td>
<td>CPEL</td>
<td>GGGAACCTCATAGTTCAG</td>
<td>506</td>
</tr>
<tr>
<td></td>
<td>CPER</td>
<td>ACCAGCTGATTTGACTTATA</td>
<td></td>
</tr>
<tr>
<td><em>etx</em></td>
<td>CPETXL</td>
<td>TGGGAACTTCAAAAGCA</td>
<td>396</td>
</tr>
<tr>
<td></td>
<td>CPETXR</td>
<td>TTAACTCATTCCATAACTGCAC</td>
<td></td>
</tr>
<tr>
<td><em>iap</em></td>
<td>CPLL</td>
<td>AAACGCATTAAGCTCACCC</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td>CPILR</td>
<td>CGCATAAACCTGGAAAGCT</td>
<td></td>
</tr>
<tr>
<td><em>cpb2</em></td>
<td>CPB2L</td>
<td>CAAGCAATGAGGAGTTEA</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>CPB2R</td>
<td>GCAGAATCGAGATTTCAC</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. 
The sources of *C. perfringens* isolates and the results of their toxo-typing

<table>
<thead>
<tr>
<th>Sources and sample numbers</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>cpa</em>+</td>
<td><em>cpa</em>, <em>cpb2</em>+</td>
</tr>
<tr>
<td>Poultry feed mixture (13)</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Pig feed mixture (8)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Cow feed mixture (7)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sunflower meal (2)</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Premix (1)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Cow’s organs (n=1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 2. Lane 1 *C. perfringens* ATCC 13124; lanes 2, 5, 6, 9 and 10: *C. perfringens* isolates type A strains with α toxin (*cpa*); lanes 3, 4, 7 and 8: *C. perfringens* type A with α (*cpa*) and β2 toxins (*cpb2*)
Gene for the enterotoxin (cpe) was detected in only one strain, which was isolated from the cow’s organs.

Fig. 2. and 3. show agarose gel electrophoresis of the amplicons obtained by multiplex PCR. Lane M – O’Gene Ruler 100 bp DNA marker (Fermentas).

DISCUSSION

*C. perfringens* is notorious for being one of the common contaminants of various foodstuffs, which was also proven in this research conducted on several types of animal feed (Kanakaraj et al., 1998; Casagrande et al., 2013; Kukier et al., 2013; Tessary et al., 2014; Udhayavel et al., 2017). In this study, all *C. perfringens* isolates (n=34) were identified as type A and β2-toxinogenic type A strains, possessing only the *cpa* (n=21), or both the *cpa* and the *cpb2* genes (n=13), respectively. There are numerous data of the widespread presence of these. Type A was the most prevalent toxo-type in feed samples, domestic animal intestines and environmental samples (Wojdat 2006; Kanakaraj et al., 1998; Kukier et al., 2010; Mueller-Spitz et al., 2010).

For example, 174 *C. perfringens* isolates from environ-mental sources and sewage were identified as type A (Mueller-Spitz et al., 2010) and 53.3% of isolates from poultry feed belonged to type A and 42% to type A subtype β-2 (Kukier et al., 2010). In 96% faecal samples from poultry fed on these mixtures *C. perfringens* type A was isolated and in 4% β-2 type A; all samples were taken from chickens and poultry which did not show any signs of diseases.

In Poland 334 *C. perfringens* isolates from animal feed stuffs were processed with multiplex PCR to detect six genes coding for toxins. Most of the isolates, 50%, possessed genes coding for both α and β2 toxins, whilst the gene responsible for α toxin only was detected in 46.4% isolates, which means that the vast majority of the strains were *C. perfringens* type A. *C. perfringens* types E, C, (including the one with a β2 toxin gene) and type D capable of producing β2 toxin made up for less than 4% of the isolates in total (each present in less than 1% of the isolates), whilst *C. perfringens* type B was not detected (Wojdat et al., 2006).

*C. perfringens* type A is the most common of all *C. perfringens* types, and is part of the normal gut microbiome in humans and animals. Of all major lethal toxins, type A produces only the α-toxin, common to all
C. perfringens toxotypes (Uzal et al., 2010). The α-toxin is an enzyme, a phospholipase which hydrolyzes lecithin (Niilo, 1980; Yoo et al., 1997). Because the membranes of the majority mammalian cells are composed of lipoproteins containing lecithin, its destruction leads to necrosis, hemolysis, or even death, depending on the tissue(s) involved. The α-toxin is essential for the pathogenesis of gas gangrene and its production is considered a housekeeping capacity of all C. perfringens strains. However, the role of this microorganism and its major α-toxin in natural intestinal disease remains controversial and poorly documented (Uzal et al., 2010). C. perfringens type A is even considered to be the most confusing organism in respect to its pathogenicity (Niilo, 1980; Ata et al., 2013).

C. perfringens type A has been reported to cause enterotoxemia in sheep and calves, sometimes even leading to sudden deaths. However, most of the data were not proven and based mainly on bacterial isolation and clinical signs, which were unspecific. Alpha toxin is present in the gut content of many clinically healthy animals. Thus, its detection in the intestinal content of affected animals is irrelevant for the diagnosis of the disease (Uzal et al., 2010). In addition, C. perfringens type A can be readily isolated from samples taken soon after death, can be grown easily and may mask other bacteria, implying that it had been the only causative agent. Researchers failed to produce acute enterotoxemia in calves and sheep by intraduodenal administration of C. perfringens type A. Administered intravenously, the toxin led to death only in relatively large doses applied within a short time, whilst slow application resulted in milder effects, probably due to rapid elimination. In sum, it seems unlikely that α-toxin can be produced in the intestines in high quantities and absorbed rapidly enough to result in fatal toxemia (Niilo, 1980).

Alfa-toxin production was believed to be an essential virulence factor in the pathogenesis of both clinical and subclinical necrotic enteritis (NE) in laying hens and turkeys (Lyhs et al., 2013; Tessari et al., 2014). Recently it was established that only strains producing NetB toxin, a β-poreforming toxin, are capable of inducing NE in broiler chickens under specific predisposing conditions (Keyburn et al., 2008; Timbermont et al., 2011; Antonissen et al., 2014; Uzal et al., 2015). High numbers of C. perfringens present in the intestinal tract or inoculated to chickens do not produce NE (Baba et al., 1997; Kukier et al., 2010; Lyhs et al., 2013, Antonissen et al., 2014). Given that C. perfringens does not produce enzymes for the synthesis of 13 aminoacids, its growth is limited in the environment with restricted aminoacid contents (Cooper and Songer, 2009; Brynestad and Granum, 2002; Antonissen et al., 2014). The outbreak of the disease is influenced by various non-specific factors contributing to intestinal environment, which favours the growth of C. perfringens, leading to mucosal damage, increased gut permeability and decreased peristaltic movements. The best-known predisposing factor is mucosal damage caused by coccidia (Baba et al., 1997; Williams, 2005; Collier et al., 2008). A variety of factors can contribute to the outbreak of the disease, such as high wheat, barley or fish meal contents in feed (Lyhs et al., 2013), high levels of indigestible, water-soluble, non-starch polysaccharides (Jia et al., 2009), high concentrations of animal proteins (Gholamian-dehkordi et al., 2007), animal fat (Knarreborg et al., 2002), programmed alterations in the feeding regime and immune-suppressive agents such as the viruses of chick anaemia, Gumboro disease or Marek’s disease (Timbermont et al., 2011), and the Fusarium mycotoxin deoxynivalenol (Antonissen et al., 2014).

One of the difficulties in the understanding of the pathogenesis and diagnosis of enteric disease caused by C. perfringens is based on the fact that most of its types can frequently be found in the intestines of healthy animals. Simple isolation of C. perfringens is therefore of no use in diagnostic of enteric infections (Uzal et al., 2015). The intestines of birds suffering NE contain large numbers of C. perfringens, up to $10^6$ or $10^5$ CFU/g of intestinal content (Cooper and Songer, 2009), whereas in healthy broilers the counts usually range
The answer to the question whether *C. perfringens* is a general foodborne pathogen is immensely complicated. In its toxigenic behaviour type A can be subdivided into two varieties. The "classical" variety, characterized mainly by α-toxin production, is associated with gas gangrene, traumatic infections and the normal intestinal tract. In the beginning, α-toxin was considered to play the main role in human food poisoning and enteritis, but another variety of type A, which is producing the enterotoxin (CPE), was later determined. The enterotoxin was first isolated in 1970 (Brynestad and Granum, 2002). About 1-5% of all *C. perfringens* type A strains can produce it (Miyamoto et al., 2004; Lindström et al., 2011; Mueller-Spitz et al., 2010).

Foodborne diseases caused by *C. perfringens* can only result from strains carrying the cpe gene, which encodes for the enterotoxin (Niilo, 1980; Goldner et al., 1986; Xiao et al., 2012) and is located in the bacterial chromosome or on large plasmids (Brynestad and Granum, 2002; Smedley et al., 2004; Miyamoto et al., 2004; Lindström et al., 2011; Kukier et al., 2010). The enterotoxin is a cytotoxic polypeptide which causes fluid and electrolyte loss from the intestinal mucosa. Type A CPE-positive strains also cause several non-foodborne human gastrointestinal diseases, including about 5–10% of all cases of antibiotic-associated diarrhea (Freeman et al., 2016). CPE is produced in the small intestine after ingestion of at least 10^7 *C. perfringens* cells (Brynestad and Granum, 2002).

Molecular detection of genes encoding for enterotoxins is extremely important in the inspection of animal-derived foodstuffs and is more reliable than conventional enterotoxin detection; they are all intended for the prevention of the entrance of these strains into the food chain (Kanakaraj et al., 1998). In this research, the gene coding for enterotoxin was detected only in one *C. perfringens* strain isolated from a dead cow, but not in the feed mixture which the animal had been fed on. This result implies that animal feed is irrelevant to the entrance of cpe-positive strains of *C. perfringens* to the food chain. Previously, the use of toxin genotyping of *C. perfringens* from swine feed sampled on different farms (n=60) found no isolates positive for cpe (Kanakaraj et al., 1998). In addition, molecular typing of isolates obtained from swine denied that this species was a source of cpe-positive strains of *C. perfringens* (Kanakaraj et al., 1998).

Gene coding for β-2 toxin (cpb2) was confirmed in 13 *C. perfringens* isolates: in those originating from feed intended for poultry (n=4), pigs (n=4) and cows (n=4), and from a praemix (n=1). This toxotype might be important for the development of enteric diseases in pigs and cattle. A significant correlation (82%) between *C. perfringens* possessing the β2-toxin gene and diarrhoea in piglets was identified, suggesting that β2-toxin may play a key role in the pathogenesis of the disease (Garmory et al., 2000). More than 85% of isolates from cases of porcine enteritis and 91.8% isolates of newborn pig enteritis were positive for the cpb2 gene (Bueschel et al., 2003). However, cpb2-positive strains can be found in both healthy and diseased chickens and its presence does not correlate with disease (Keyburn et al., 2008). Pulsed-field gel electrophoresis performed on *C. perfringens* isolated from turkeys with NE and from healthy birds, in all of them detected α-toxin, and in one both α and β2 toxins, which indicates that the β2 toxin is not an important virulence factor in the development of NE (Lyhs et
al., 2013). Beta-2 toxin has been associated with porcine, equine and bovine gastroenteritis (Bueschel et al., 2003; Van Immerseel et al., 2004).

In type A strains isolated from various animals, food products and environment the prevalence of the cpb2 gene is high (Bueschel et al., 2003; Garmory, et al. 2000; Mueller-Spitz et al., 2010). The gene is plasmid-borne, at least in some strains (Shimizu et al., 2002), which implies its mobility and the possibility of transfer among strains of C. perfringens.

In this research all animal feed samples from which C. perfringens was isolated were less than 100 CFU/g. In similar trials the numbers in feeds did not exceed 10^2 CFU/g (Kukier et al., 2010) or 10^3 CFU/g (Wojdat et al., 2006). Thus, it is reasonable to raise the question why the numbers of C. perfringens in feed are to be assessed given that the Regulation orders that feed be condemned if this species is even detected. Estimating the numbers would be sound if maximum allowed numbers per gram had been established. The PCR test ensures the detection of all genes considered to be important virulence factors in C. perfringens-mediated enteritis or enterotoxaemia and thus provides a useful and reliable tool for C. perfringens genotyping in routine veterinary diagnostics (Baums et al., 2004). The mPCR assay is recommended for risk assessment of C. perfringens toxotypes isolated from animal feed. The obtained results can help to establish microbiological criteria for critical limits of the presence of C. perfringens in feed (Wojdat et al., 2006).

ACKNOWLEDGEMENTS
This work was supported by the grant provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia for the Projects Nos. TR 31071 and III 46002.

REFERENCES

The mycotoxin deoxynivalenol predisposes for the development of Clostridium perfringens-induced necrotic enteritis in broiler chickens. PLoS ONE, 9 (9), e108775. DOI:10.1371/journal.pone.0108775.


**Токсин генотипизација сојева Clostridium perfringens**

изолованих из хране за животиње и њихов значај у етиологији ентеротоксемија домаћих животиња

Дубравка С. Миланов1, Тамаш Р. Петровић1, Далибор С. Тодоровић1,
Невенка Р. Алексић2, Ивана С. Чабаркапа3

1Научни институт за ветеринарство „Нови Сад”, 21000 Нови Сад, Руменачки пут 20, Србија
2Универзитет у Београду, Факултет ветеринарске медицине, 11000 Београд, Булевар ослобођења 18, Србија
3Универзитет у Новом Саду, Научни институт за прехрамбене технологије у Новом Саду, 21000 Нови Сад, Булевар цара Лазара 1, Србија

**Сажетак:** Clostridium perfringens је Грам-позитивна, анаеробна, спорулишућа, штапићаста бактерија, убицинатно распрострањена у природи. Код различитих сојева C. Perfringens, до данас, идентификовано је око 17 врста токсина. C. perfringens је узрочни агенс различитих обољења (синдрома), али су цревне инфекције/интоксикације најчешће и од највећег значаја за различитих обољења (синдрома), али су цревне инфекције/интоксикације најчешће и од највећег значаја за различитих обољења (синдрома), али су цревне инфекције/интоксикације најчешће и од највећег значаја за различитих обољења (синдрома), али су цревне инфекције/интоксикације најчешће и од највећег значаја за различитих обољења (синдрома), али су цревне инфекције/интоксикације најчешће и од највећег значаја за различитих обољења (синдрома), али су цревне инфекције/интоксикације најчешће и од највећег значаја за различитних обољења (синдрома). У овом раду приказујемо резултате испитивања сојева C. perfringens пореклом из хране за животиње (n=34) и једног изолата из органа краве утичуле са значима ентеротоксемије на присуство гена: cpa, cpb, cpb2, cpe, etx и iap применом мултиплекс PCR технике. Сви сојеви C. perfringens изоловани из хране за животиње, идентификовани су као тип A који поседује само cpa ген (n=21) или тип A који продукује β-токсин, односно има cpa и cpb2 гене (n=13). У раду дискутујемо о улози алфа (α) и бета-2 (β2) токсина у патогенези ентеротоксемија домаћих животиња, као и актуелном законском пропису по којем ова врста бактерије не сме бити присутна у храни за животиње. Примена PCR технике у свакодневној пракси омогућила би токсин-генотипизацију сојева C. perfringens, а тиме и реалне основе за успостављање границих дозвољених вредности за ову врсту бактерије у храни за животиње.

**Кључне речи:** Clostridium perfringens, токсин генотипизација, храна за животиње, мултиплекс PCR техника, cpa ген, cpb2 ген

Received: 17 May 2018

Received in revised form: 6 June 2018

Accepted: 9 June 2018