OCCURRENCE OF CLOSTRIDIUM PERFRINGENS IN FOOD CHAIN

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Abstract

The occurrence of Clostridium perfringens in particular links of food chain and their toxic potential was studied. Compound feeding stuffs, poultry, swine, and bovine faeces, food of animal origin, and human faeces were examined microbiologically. The isolated anaerobes were analysed for presence of toxin genes (cpa, cph, cpb2, etx, iap, and cpe). The presence of C. perfringens on level higher than 1.0 x 10⁴ cfu/g were detected in 68% of feed samples, 36% of poultry faeces, 92% of swine faeces, 67% of bovine faeces, 4% of food samples, and in 67% of human faeces. The detected levels of type A C. perfringens strains did not exceed the number of these strains physiologically present in the intestines of birds and mammals. Food and feed samples also revealed good microbiological quality. The identification of toxin type and subtype revealed domination of type A strains and among them the percentage of subtype β2 strains varied considerably. The second toxotype detected was toxotype E. There were no cpe-positive strains in the analysed food of animal origin.

Key words: Clostridium perfringens, food chain, toxotype, microbiology.

Clostridium perfringens is a Gram-positive sporulating anaerobe recovered from many sources in the environment and it is a part of vertebrate intestinal microflora, as well as a cause of human and animal diseases (12, 13). The species is classified into five toxotypes (A - E) on the basis of differential production of the four major toxins α (cpa), β (cpb), ε (etx), and ι (iap). All type A strains produce α toxin, type B α, β, and ε toxins, type C α and β toxins, type D α and ε toxins, and type E α and ι toxins. C. perfringens type B – E are recognised as frank pathogens for domestic animals and human, while type A strains are commensals in the intestinal tract of vertebrates, and the ability of higher expression of α toxin decides about lethal properties of these strains (12). Unfortunately, there are currently no well-defining factors, which decide about variable expression of α toxin. C. perfringens type A strains are implicated in numerous diseases, for example necrotic enteritis in broiler chicken, enteritis in piglets, abortions and haemorrhagic enteritis in calves, and gas gangrene, food-poisoning, and gastrointestinal illness in humans (3, 5, 7, 8, 14). C. perfringens is capable of producing many additional toxins or enzymes, inter alia β2 toxin (cpb2) and enterotoxin (cpe), whose part in the pathogenesis is confirmed. Toxin genes are located on a chromosome (cpa) and on plasmids (cpb, etx, iap, cph2) or regarding cpe, they may occur alternatively on both of them. It was proven that acquisition or loss of cph, cpb2, cpe, etx, and iap genes in the nature is associated with change of ecological niche or host (2, 9). However, there are affirmative proofs for loss of toxin genes within the same host species.

Taking into account epidemiologic evidences suggesting a possible feed-to-animal and animal-to-human route of transmission of C. perfringens strains, the occurrence of these anaerobes in particular links of food chain and their toxic potential was studied.

Material and Methods

Compound feeding stuffs were sampled from Polish feed factories, imported feed batches, and farms (n=100). There were feeds for poultry, swine, and cattle. Poultry faeces were sampled from slaughterhouse (n=20), clinical healthy chicken broilers (n=50), turkey broilers (n=20), and reproductive cocks (n=10). Chicken broilers aged 18-63 d, and turkey broilers aged 105 d. Swine faeces were sampled from fatteners, sows, and one-day piglets (n=100). Bovine faeces samples derived from animals (23 males and 77 females) from 2 months to 10 years of age (n=100). Samples of food of animal origin contained raw poultry meat from chicken broilers (n=15) and turkey broilers (n=5), raw pork meat (n=20), raw beef meat (n=20), ready-to-eat smoked sausages and meat products (n=43), poultry boneless meat (n=2), and powdered soups containing meat (n=4). Meat and meat products derived from local retail, boneless meat were produced in meat factory, and soups were from army reserves. Human faeces samples were collected from patients of Pulawy hospital and from employees of the National Veterinary Research Institute in Pulawy. The analysed group of patients comprised 54 females and 46 males, aged from 2 to 84 years.
C. perfringens organisms were enumerated according to standard PN-EN 13401:2000 “Microbiology of food and animal feeding stuffs. Horizontal method for enumeration of Clostridium perfringens”. Taking into account possible low food contamination, C. perfringens was additionally detected in 1 g of food samples, which were inoculated to two tubes of Wrzosek broth. In order to stimulate spore germination and vegetative cell killing, one of the tubes was pasteurised at 80°C for 10 min. After anaerobic incubation at 37°C ± 1°C for 24 h, positive tubes (turbidity of broth and gas in Durham tube) were spread on Willis-Hobbs agar. After parallel anaerobic and aerobic incubation (control for growth under aerobic conditions) at 37°C ± 1°C for 24 h, the plates were examined for the presence of colonies suspected to be C. perfringens species. Afterwards, from one to 13 C. perfringens strains were isolated from positive samples and analysed for the presence of toxin genes. The isolates (n=1,401) were examined for the presence of eps (α toxin), cpb (β), cpb2 (β2), etx (ε), and cpe (enterotoxin) toxin genes by multiplex PCR (mPCR) according to Baum’s et al. (1) with a little own modification. Protocol changes regarded the template DNA preparation, which was obtained from overnight culture of C. perfringens at 37 ± 1°C on Willis-Hobbs agar under anaerobic conditions and then resuspended in 2 ml of PBS to obtain a McFarland turbidity standard equal to 3.5. One milliliter of this solution was transferred to Eppendorf tube and boiled in water for 15 min. After heat lysis, the tube was cooled on ice and centrifuged at 11,000 x g for 8 min. The obtained supernatant (10 μl) were added to master mix as a source of target DNA. Further mPCR conditions were identical to above mentioned protocol.

Results and Discussion

The presence of C. perfringens at the level higher than 1.0 x 10^1 cfu/g was detected in 68% of feed samples, 36% of poultry faeces, 92% of swine faeces, 67% of bovine faeces, 4% of food samples, and 67% of human faeces. Feed contamination level ranged from 1.0 x 10^1 to 9.5 x 10^2 cfu/g (Fig. 1). C. perfringens number ranged from 1.0 x 10^1 cfu/g to 3.6 x 10^2 cfu/g in poultry faeces (Fig. 2), from 1.0 x 10^1 cfu/g to 1.2 x 10^2 cfu/g in swine faeces (Fig. 3), and from 1.0 x 10^1 cfu/g to 7.4 x 10^2 cfu/g in bovine faeces (Fig. 4). Among 109 samples of food of animal origin, the presence of the anaerobes was detected in 19 (17.4%) of them. Contamination level of samples classified as positive ranged from 1.0 x 10^1 cfu/g to 6.2 x 10^3 cfu/g (Fig. 5). C. perfringens occurrence level in human faeces extended from 1.0 x 10^1 cfu/g to 7.3 x 10^7 cfu/g but in nearly half of positive samples ranged from 10^2 to 10^6 cfu/g (Fig. 6). The analysis of C. perfringens occurrence in the studied chain links revealed, that the highest level of these anaerobes were in swine and human faeces (10^7 cfu/g). However, nearly 40% of swine and human faeces samples contained no more than 10^5 cfu/g. The bacteria were seldom noticed in food of animal origin, compound feeds, and poultry faeces samples. Additionally, it was observed that the number of C. perfringens in poultry faeces was strongly depended on farm hygiene (positive correlation). The way of sampling was also important. When faeces were sampled from live birds and transported as quickly as possible to the laboratory in cool temperature, or were frozen till analyses, C. perfringens number did not exceed 10^4 cfu/g, which is typical for healthy birds. In cases, in which the content of the intestines from dead birds was analysed, the obtained results were significantly higher (ranged from 10^6 to 10^9 cfu/g), which is typical for birds affected by necrotic enteritis. On the contrary, the level of C. perfringens in swine faeces was positively correlated with herd size and age of animals. The anaerobes were detected in one-day piglet faeces but their level did not exceed 10 cfu/g. The number of the anaerobes in cow faeces compared with number of these microorganisms in young bull faeces proved that in bulls the number of C. perfringens was 250 times higher than that in cows. The highest number of the anaerobes in samples of food of animal origin was detected in boneless poultry meat (6.0 x 10^6 cfu/g). Additionally, more than 1.0 x 10^5 cfu/g were found in raw poultry meat and raw pork meat. No more than 1.0 x 10^1 cfu/g anaerobes were found in raw pork meat, raw beef meat, raw poultry meat, and ready-to-eat meat products (white sausage, zwyczajna sausage, head cheese, poultry sausage). In contrast to ready-to-eat meat products, which contained only spores, vegetative cells and spores of C. perfringens were isolated from raw meats. Statistical analysis of patient age and number of anaerobes in human faeces did not confirm linear dependence between both parameters. Sex of patient also did not influence the number of anaerobes in human faeces.

It should be mentioned that lower limit of detection for C. perfringens enumeration method was 10 cfu/g, which means that in samples classified as negative, anaerobes might have occurred on level less than 10 cfu/g. The obtained results for C. perfringens number were analysed according to distributive series. Figs 1–6 present bacterial number in logarithm scale (e.g. log_{10} = 3 means range from 10^3 to 9,999 cfu/g; log_{10} = 4 means range from 10^4 to 99,999 cfu/g, etc.).

Table 1
Kind of analysis and number of studied samples

<table>
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<tr>
<th>Kind of analysis</th>
<th>Kind of studied sample</th>
<th>Feeds</th>
<th>Poultry faeces</th>
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<th>Bovine faeces</th>
<th>Food of animal origin</th>
<th>Human faeces</th>
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<td>-</td>
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<td>109</td>
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<tr>
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<td>404</td>
<td>294</td>
<td>50</td>
<td>323</td>
<td>1,401</td>
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Fig. 1. *C. perfringens* number in compound feeding stuffs.

Fig. 2. *C. perfringens* number in poultry faeces.

Fig. 3. *C. perfringens* number in swine faeces.

Fig. 4. *C. perfringens* number in bovine faeces.

Fig. 5. *C. perfringens* number in foods of animal origin.

Fig. 6. *C. perfringens* number in human faeces.
Fig. 7. Occurrence of *C. perfringens* toxotypes in compound feeding stuffs.

Fig. 8. Occurrence of *C. perfringens* toxotypes in poultry faeces.

Fig. 9. Occurrence of *C. perfringens* toxotypes in swine faeces.

Fig. 10. Occurrence of *C. perfringens* toxotypes in bovine faeces.

Fig. 11. Occurrence of *C. perfringens* toxotypes in foods of animal origin.

Fig. 12. Occurrence of *C. perfringens* toxotypes in human faeces.
In the midst of all studied isolates for toxic type and subtype identification, type A strains dominated, and among them the percentage of subtype β2 strains varied considerably. The occurrence frequency of subtype β2 strains was comparable to the occurrence frequency of type A strains among feeds and swine faeces (Figs 7–12). An interesting observation was made regarding Clostridium perfringens strain population (n=294) isolated from bovine faeces, where the number of subtype β2 strains was two times higher than type A isolates. In our past and present studies, it was the first case with so obvious majority of subtype β2 strains, taking into account all analysed food production chain links. Subtype β2 strains in poultry faeces (4%) and food of animal origin (8%) were relatively rarely detected. The presence of enterotoxin gene was demonstrated only in 12 isolates (0.86%) from among 1,401 studied strains. Enterotoxigenic strains occurred in compound feeds and swine, bovine, and human faeces. Additionally, 14 of the isolated strains possessed only β2 toxin-encoding gene, which was observed in feed isolates (16).

The second toxotype detected in our study was toxotype E. This toxotype was found in three isolates (0.2%) and all of them belonged to subtype β2. Besides, there was one isolate, which according to toxotype classification should belong to types E and C simultaneously. It contained α,β, and ι toxin genes and additionally β2 toxin-encoding gene. Similar situation was observed in Clostridium botulinum species (6, 15). All four strains described above were isolated from faeces of clinically healthy cattle, although it is assumed that Clostridium perfringens type B – E are always associated with disease.

Taking into account the low number of detected type B – E strains, considered as the frank pathogens, it is impossible to assess the risk resulting from the occurrence of these strains in cattle, or in the next links of food chain. Detected levels of type A Clostridium perfringens strains did not exceed physiological number of these strains in the intestines of birds and mammals. Additionally, the obtained results and results of earlier study (16) confirm good microbiological quality of feed samples with reference to Clostridium perfringens in Poland. In default of Polish performance standards regarding Clostridium perfringens number in food of animal origin, the obtained results were compared to American criterion in relation to food of animal origin. On February 2001, the United States Department of Agriculture, Food Safety and Inspection Service established performance standards for Clostridium perfringens to a maximum of 1-log_{10} (a factor of 10 cfu/g) to all ready-to-eat and all partially cooked meat and poultry products on a basis of conducted a quantitative risk assessment (4). In regard to these performance standards, the studied samples of food of animal origin revealed good microbiological quality. The 10 cfu/g value was slightly exceeded only in raw meat samples, which are usually subjected to thermal treatment before consumption.

The presented studies confirmed cpe occurrence in 0.86% of isolates from different samples (feeds; swine, bovine, and human faeces). There were no cpe-positive strains in the analysed samples of food of animal origin. However, the panmictic nature of Clostridium perfringens orders to be careful during meal preparation. Taking into account literature data regarding the average enterotoxin-positive strains occurring in food of animal origin (5%), infection dose of Clostridium perfringens for humans (10^{-2}–10^{-9} cfu), and assuming that zwyczajna sausage contamination level amounts to 10 cfu/g, healthy adult man should eat 2 tons of the sausage to induce the disease (10, 11). Supposing more probable scenario, the disease may appear after consuming 200 g portion of sausage contaminated by 10^{5} cfu/g anaerobes. There are many potentialities, which may occur in the reality and the variables may be the percentage of occurring enterotoxin-positive strains or size of the infective dose. Besides, each of the factors mentioned above is occurring, in fact, as a set of data dealing with e.g. various levels of food contamination, or different immunity, which have an influence on the seeking of the final value. Furthermore, above calculations regard only “food-human” food chain fragment and do not concern probability of the diseases occurrence in animals, which is determined by other Clostridium perfringens toxotypes and predisposing factors (3). In spite of classification of toxotypes B – E for frank pathogens, the assessment of the risk in this range was impossible due to the low percentage of detected strains in our study (0.3%) and impediments resulting from the lack of sufficient epizootical data.

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References


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