CHARACTERIZATION OF CLOSTRIDIUM PERFRINGENS ISOLATED FROM MEAT AND BONE MEAL

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Abstract Clostridium perfringens is an important cause of enteric disease in humans and domestic animals. Ninety samples of bone and meat meal used in swine feed from Brazilian producers were evaluated about the presence of C. perfringens. The isolates were characterized by PCR for the presence of genes codifying the enterotoxin, alpha, beta, epsilon, iota and beta-2 toxins. From ninety samples evaluated by bacteriology 29 were positive (32.2%) to C. perfringens. PCR to phospholipase C gene revealed 77 positive samples (85.5%). Fifty-one C. perfringens strains isolated were submitted to PCR detection of toxin genes. All strains were positive to alpha and beta-2 toxin genes; 12 strains were positive to beta toxin gene and were classified as C. perfringens type C. The high number of meals samples positive to C. perfringens type C strains indicates the risk of meat and bone meal in maintaining the agent in swine herds and increasing the carcass contamination at slaughter.

Introduction Clostridium perfringens is an important cause of enteric disease both in humans and domestic animals (Songer, 1996). Type A enterotoxigenic strains are a common cause of food poisoning outbreaks worldwide. C. perfringens type C is generally considered the primary cause of necrotic enteritis in piglets, while type A has been linked to enteric disease in suckling and feeding pigs presenting mild necrotic enterocolitis (Klaasen, 1999). Genetic analysis of isolates from meat and bone meal used in swine feed may lead to a better understanding of agent transmission in herds and will help the recognition of the agent potential in the contamination of carcasses at slaughterhouses.

The objectives of this trial were to isolate and detect C. perfringens from meat and bone meal used in swine and poultry feed and characterize the strains in relation to the presence of enterotoxin, alpha, beta, epsilon, iota and beta-2 toxins genes using polymerase chain reaction (PCR).

Materials and Methods A total of 90 meat and bone meal samples collected in a 3 month period from 83 meal producers were used in this study. Meal samples were obtained from Brazilian States of São Paulo, Santa Catarina, Parana, Minas Gerais e Rio Grande do Sul. Bacteriological examination was conducted with a sample of 5 g of meal. The sample was submitted to enrichment in 25 ml of Thioglycolate liquid medium and cultured overnight under anaerobic condition. Next the culture was streaked in Tryptose Sulphite Cycloserine agar (Oxoid) under anaerobic condition. Isolated strains were confirmed by the use of Gram stains, lecithinase and lipase reaction in egg-yolk agar and presence of storm clout in Litmus milk.

The colonies of C. perfringens were cultured in 10 ml of thioglycolate broth for 24 h at 37°C. The DNA extraction was conducted with 200 µl of bacterial culture treated with Lysozyme (140µl of 100mg/ml) and Proteinase K (40µl of 20 mg/ml) for 1 h at 37°C. The bacterial lysates were submitted to DNA purification with the guanidium thiocyanate method described by Pitcher et al. (1989).

PCR assays were performed using specific primers to phospholipase C gene to identify C. perfringens directly in meal sample and confirm the biochemical characterization. The toxin genes (alpha, beta, beta2, epsilon, iota and enterotoxin) were detected in the isolates by PCR as described elsewhere (Moreno, 2003). Reference strains C. perfringens type A (ATCC 3624), type C (ATCC 3628), type B (ATCC 3626) and type D (ATCC 3629) were kindly supplied by Instituto Biológico de São Paulo, and were used as positive controls.

Results From the 90 meal samples tested for the presence of C. perfringens through bacteriological examination 29 were positive (32.2%). The PCR to phospholipase C gene of C. perfringens revealed 77 positive samples (85.5%). From 29 meal samples positive in bacteriological examination, 51

<table>
<thead>
<tr>
<th>Culture</th>
<th>PCR positive</th>
<th>PCR negative</th>
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<tr>
<td>positive</td>
<td>29 (32.2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>negative</td>
<td>48 (53.3%)</td>
<td>13 (14.4%)</td>
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Table 1- Results from C. perfringens culture and phospholipase C PCR examination of meat and bone meal.
strains of *C. Perfringens* were selected for toxin characterization. Twelve strains were positive for presence of beta toxin gene (23.5%), and were classified as type C. The other 39 strains (76.4%) were type A.

**Discussion** The meals of animal origin are important ingredients in animal nutrition in relation to economical, nutritional and sanitary aspects. The microbiological contamination of these ingredients must be studied, since this fact, can negatively influence the biological efficiency of diets, increase the elimination of pathogenic bacteria through feces and the risk of carcass contamination at abattoir.

The contamination of meat and bone meals by *Salmonella spp.* is described by different authors; thereby literature does not show any study involving the isolation or typing of *C. perfringens* from this feed component. (Bellaver, 2001). *Clostridium perfringens* is a significant causative agent of human food poisoning. Meat, which is often contaminated with *C. perfringens*, is suspected to be a major source of food poisoning due to agent (Miwa *et al*, 1998).

Bacteriological examination presented a lower number of positive results when compared with PCR. This difference observed can be explained on different ways. One of them is the effect of heat treatment of analyzed meals at mills, killing the *C. perfringens* but not destroying their DNA on sample. Another hypothesis is the influence of high sample contamination reducing the success of isolation procedure. The occurrence of twelve *C. perfringens* type C strains (23.5%) indicates the potential of this isolates to cause necrotic enteritis in piglets. The absence of enterotoxin positive strains among the isolates studied suggest a low risk to carcass contamination, but a constant monitoring of this profile must be done, considering the high percentage of detection of the agent in the meat and bone meal samples analyzed.

**Conclusions** *Clostridium perfringens* is very frequent in meat and bone meal samples used to animal nutrition and can play an important role in keeping the agent in swine herds.

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**References**
Bellaver, C., 2001. Ingredientes de origem animal destinados à fabricação de rações. In: Simpósio sobre ingredientes na alimentação animal, Campinas, SP Anais...Campinas: CBNA 167-190


<table>
<thead>
<tr>
<th>Toxin</th>
<th><em>C. perfringens</em> positive</th>
<th><em>C. perfringens</em> negative</th>
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<tr>
<td>Alpha</td>
<td>51 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Beta-2</td>
<td>51 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Beta</td>
<td>12 (23.5%)</td>
<td>39 (76.4%)</td>
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Table 2: Frequency of toxin genes in fifty-one strains of *C. perfringens* isolated in meat and bone meal.