

## PRESENCE OF *Clostridium perfringens* IN BROILER FROM CHICKEN FARMS IN RIBEIRÃO PRETO-SP

PRESENÇA DE *Clostridium perfringens* EM FRANGOS DE CORTE PROVENIENTES DE AVIÁRIOS DA REGIÃO DE RIBEIRÃO PRETO – SP

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### SUMMARY

The aim of the present study was to detect *Clostridium perfringens* in broiler chickens from intensive poultry farms in Ribeirão Preto-SP. We collected 516 samples of caecal content that were cultivated onto selective medium for *Clostridium* and incubated under anaerobic conditions. Gram smears were carried out from the positive samples and identified using biochemical tests. Mice inoculation test was performed in order to confirm pathogenicity. The results showed that 66.78% of the samples were positive for Clostridia, of which 16.78% were *C. perfringens*. It was confirmed that 19 (3.4%) of the samples were positive to other Clostridia pathogenic species such as *Clostridium chauvoei* (3 - 0.54%), *Clostridium sordelli* (9 - 1.61%), *Clostridium bifermentans* (3 - 0.54%), *Clostridium septicum* (3 - 0.54%) and *Clostridium tetani* (1 - 0.18%).

**KEY-WORDS:** Aviculture. Health. Microbiology. Necrotic enteritis.

### RESUMO

Visando estudar a presença de *Clostridium perfringens* em frangos de cortes provenientes de aviários da região de Ribeirão Preto-SP, amostras de conteúdo cecal de aves foram pesquisadas quanto a presença desse microrganismo. As amostras foram semeadas em meios seletivos para clostrídios e incubadas anaerobicamente. As culturas foram identificadas e caracterizadas pelo método de Gram e séries bioquímicas. Posteriormente realizou-se teste de inoculação em camundongos para confirmar patogenicidade. De um total de 560 amostras coletadas, 374 (66,78%) foram positivas para o gênero *Clostridium*, sendo 94 (16,78 %) *Clostridium perfringens*. Verificou-se que 19 (3,4%) amostras foram positivas para outras espécies de clostrídios patogênicos como *Clostridium chauvoei* (3 - 0,54 %), *Clostridium sordelli* (9 - 1,61%), *Clostridium bifermentans* (3 - 0,54%), *Clostridium septicum* (3 - 0,54%) e *Clostridium tetani* (1 - 0,18%).

**PALAVRAS-CHAVE:** Enterite necrótica. Avicultura. Microbiologia. Sanidade.

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## INTRODUCTION

Among the most important pathogens that affect poultry, nosological entities, apparently of minor importance, are highlighted, among which we find the *Clostridium perfringens*. This rod-shaped, Gram-positive, anaerobic bacterium, with central and subterminal spores, is found mainly in young animals and is responsible for necrotic enteritis (CARTER, 1995). This acute, non-contagious, enterotoxemic disease, with sudden onset and confluent necrosis of the small intestine, in subclinical infections causes decreasing nutrient absorption, weight gain, poor feed conversion and increased condemnation of carcasses (colangio hepatitis), resulting in economic losses to poultry production (SCHOCKEN-ITURRINO *et al.*, 2009; LOVLAND & KALDHUSDAL, 2001; VAN DER SLUIS, 2000).

Several papers in literature report on the frequency of this microorganism in the intestines of birds (CRAVEN *et al.*, 2001; DE CESARE *et al.*, 2009; GOMES *et al.*, 2008; MANFREDA *et al.*, 2006; SVOBODOVÁ *et al.*, 2007; TSCHIRDEWAHN *et al.*, 1991), but there are only few studies in Brazil on this subject.

Furthermore, studies which demonstrate the occurrence of this pathogen are of great importance for the national poultry industry; therefore, the aim of this study was to isolate and determine the presence of *C. perfringens* in broilers from poultry farms in Ribeirão Preto, SP.

## MATERIAL AND METHODS

We randomly collected a total of 560 birds alive and healthy, fed diets supplemented with antibiotics, directly from slaughterhouses in Ribeirão Preto, SP, from January to November 2009. After being killed by neck dislocation, their intestines were removed, bound and transported in isothermal boxes filled with ice to the Department of Microbiology, UNESP, Jaboticabal, SP. The samples were processed within 2 hours after collection.

Mucous and stool samples were removed from the intestines, and cultured in test tubes containing Tarozzi medium and Brain Heart Infusion (BHI - Difco). Subsequently, they were incubated in anaerobiosis at 37°C for 48 hours (SCHOCKEN-ITURRINO *et al.*, 1986). After the cultures developed, smears stained by Gram's Method were performed to confirm the presence of Gram positive, with or without spores, characteristic of the genus *Clostridium* (HOLDEMAN *et al.*, 1977).

In order to isolate *Clostridium*, 1 ml of the Gram-positive cultures was sown in previously sterilized Petri dishes containing sulfite-tryptone-neomycin agar (TSN-Difco), and incubated under anaerobic conditions, using GasPak jars at 37°C for 24-48 hours (SCHOCKEN-ITURRINO *et al.*, 1986).

The characteristic colonies were subjected to microscopic observation on Gram stained slides while

suspicious samples were subcultured in BHI tubes (Difco) to be characterized by biochemical tests, according to DOWELL & HAWKINS (1968).

The biochemical series consisted of testing the catalase, gelatinase, motility reaction in Litmus Milk, Indole production, nitrate reduction, acid production resulting from the fermentation of glucose, sucrose, lactose and maltose (SCHOCKEN-ITURRINO *et al.*, 1988).

To confirm the presence of toxins, 2 ml of pure cultures were filtered through Millipore membranes (0.45 micron), of which 0.5 ml was injected intraperitoneally into mice. Subsequently, the mice were observed for seven days for neurological symptoms or death (HOBBS *et al.* 1972).

## RESULTS AND DISCUSSION

Table 1 and Figures 1 and 2 show that from the 560 samples analyzed, 374 (66.78%) developed sporulating anaerobic bacteria of the genus *Clostridium* while the remaining 186 (33.22%) showed the presence of other genera bacteria. Of the total samples, 94 (16.78%) were positive for *C. perfringens*, whereas 280 (50%) had other species of *Clostridium*.

The biochemical identification and mice inoculation demonstrated that from the 280 samples negative for *C. perfringens*, 19 (3.41%) were pathogenic clostridia and the remaining 261 (46.61%) were saprophyte clostridia.

Biochemical and toxicity tests performed on 19 samples positive for *Clostridium* pathogenic bacteria, except for *C. perfringens*, showed the presence of *Clostridium chauvoei* (3 - 0.54%), *Clostridium sordelli* (9 - 1.61%), *Clostridium bifermentans* (3 - 0.54%), *Clostridium septicum* (3 - 0.54%) and *Clostridium tetani* (1- 0.18%) (Table 1).

Svobodová *et al.* (2007) and Bjerrum *et al.* (2006) found values close to those observed in the present study regarding the frequency of *C. perfringens*, isolating the same at 18.64% and 30% of the analyzed samples, respectively. However, Kalender & Ertas (2005) found that only 5% of intestinal contents were positive for *C. perfringens*.

In contrast Gomes *et al.* (2008), in a national study, reported the presence of *C. perfringens* in 68.4% of the samples from a slaughterhouse in Pará de Minas-MG.

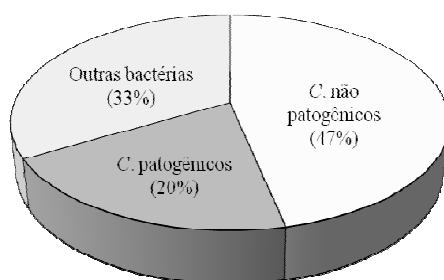
Likewise De Cesare *et al.* (2009) studied the incidence of *C. perfringens* in chickens raised in Italy and the Czech Republic; and the microorganism was detected in 64.7 and 82.9% of the studied samples, respectively. Tschirdewahn *et al.* (1991) reported the presence of *C. perfringens* in 80% of 59 chickens tested.

Corroborating the work cited above, Manfreda *et al.* (2006) while researching the presence of the pathogen in 33 poultry farms found *C. perfringens* in 87 of a total of 149 samples (58.40%).

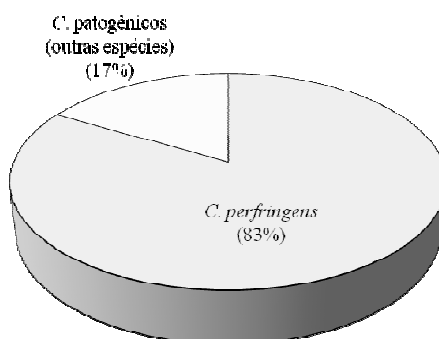
**Table 1** - Distribution of bacteria (*C. perfringens*, *C. pathogenic* and other bacteria) isolated from the intestines of broiler chickens, collected in slaughterhouses in Ribeirão Preto - SP, in 2009.

| Bacterial groups             | (%) * | Positive samples * |
|------------------------------|-------|--------------------|
| <i>Clostridium</i> SSP       | 66.78 | 374                |
| <i>C. Pathogenic</i>         | 20.17 | 113                |
| <i>C. nonpathogenic</i>      | 46.61 | 261                |
| Other bacteria               | 33.22 | 186                |
| <b><i>C. perfringens</i></b> | 16.78 | 94                 |
| <i>C. tetatni</i>            | 0.18  | 1                  |
| <i>C. septicum</i>           | 0.54  | 3                  |
| <i>C. sordelli</i>           | 1.61  | 9                  |
| <i>C. bifermentans</i>       | 0.54  | 3                  |
| <i>C. chauvoei</i>           | 0.54  | 3                  |

\* Total 560 samples



**Figure 1** - Distribution of the percentage of bacteria (*C. pathogenic*, *C. saprophytes* and other bacteria) isolated from 560 samples collected in the intestines of broiler from slaughterhouses in Ribeirão Preto - SP, in 2009.



**Figure 2** - Relationship between the percentage of *C. perfringens* and other clostridia species of pathogens isolated from 374 samples of intestines of chickens from 560 total samples collected in a slaughterhouses in Ribeirão Preto - SP, in 2009.

The data regarding the subject vary widely while the percentage of samples positive for *C. perfringens* in this study is lower than those reported in the literature. It is believed that these differences may result from the different methodologies used primarily for isolating and classifying the microorganisms, as well as the management used in the poultry farms (diet composition, rearing type and time, health status of the birds and the use of growth promoting or additives). *C. perfringens* is commonly found in the intestines of healthy birds at high concentrations (CRAVEN *et al.*, 2001), not necessarily evolving to necrotic enteritis, whether clinical or subclinical. However, we believe that its control is necessary taking into account that an outbreak of necrotic enteritis is also associated with predisposing factors to which the birds are exposed, such as concurrent coccidial infection, as well as diets containing high levels of rice, wheat, and barley, as well as animal origin meals (fish, bone and meat meal) (LILLEHOJ *et al.*, 2007, VAN IMMERSEEL *et al.*, 2004).

In addition to animal health, another important point concerns possible contamination of carcasses if accidentally the crop or bowel opens during slaughtering (FIORENTIN, 2005; ENGSTRÖM *et al.*, 2003), thus becoming also a public health issue.

## CONCLUSION

*C. perfringens* was found in several intestines in this study. It is suggested to control these microorganisms in the entire production chain because they can cause great damage to the national poultry industry as well as create problems for public health.

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