PRESENCE OF VIABLE SPORES OF BACTERIA OF THE GENUS Clostridium IN MUSCLE AND LIVER OF BOVINE SLAUGHTERED FOR CONSUMPTION

(PRESENÇA DE ESPOROS VIÁVEIS DE BACTÉRIAS DO GÊNERO Clostridium EM MÚSCULO E FÍGADO DE BOVINOS ABATIDOS PARA CONSUMO)

R. P. SCHOCKEN-ITURRINO¹, E. B. NEVES², L.F.S.A.M. GAMA², A. C. F. B. CARVALHO³, F.A. ÁVILA¹, P.F. SCHOCKEN⁴, S.C.P. BERCHIELLI¹

SUMMARY

Clostridiosis attack principally bovines causing deep economical loses. Samples of liver muscles of animals slaughtered for consumption were obtained to detect the presence of Clostridium spores. Samples were streaked onto clostridial medium and incubated under anaerobic conditions. The cultures isolated were identified through Gram smears and characterized by biochemical tests. It was demonstrated the presence of Clostridium chauvoei in 7.5% of the muscles samples and in 1.7% of the liver samples; Clostridium perfringens in 7.5%; Clostridium novyi in 5.0%; Clostridium tertium in 2.5%; and Clostridium sp in 15.0% of muscles samples only. These results confirm that Clostridium spores would enter in to the organs of the animals and remain viable in liver and muscles.

KEY-WORDS: Clostridium, viables spores, muscles, liver, bovine

INTRODUCTION

The symptomatic carbuncle is an enzootic illness that it is characterized by producing a gangrenous miositis, due to performance of a latent infection caused by spores of the Clostridium chauvoei in the organism of the host (MICALIZZI & GUZMÁN, 1990)

It is believed that the agent arrives to the muscular...
tissue through the circulatory current where, after finding good conditions of development, it is located and multiples forming toxins and gas, causing the presence of the inflammatory oedema, that in short period can extend, to all the great muscular masses of the body (BEER, 1988).

According to BEER (1988), in 1865 the agent was isolated for the first time in the subcutaneous conjunctive tissue of bovine by Feser, receiving the name of *Bacillus sarcophysematos* and etiologically describe by Bollinger in 1875, being after separate from the bacterial carbuncle by Feser in 1876.

The infections and the intoxications by *Clostridium* appear endemic, being of great importance principally for ruminant, because pathogenic *Clostridium* are dispersed in the environment and they are found in the superficial layers of the soil, rich in organic matter, in water and in the intestinal content of healthy men and animal (BEER, 1988).

According to MICALIZZI & GUZMÁN (1990) the spores of *Clostridium chauvoei* that penetrate in the animal, can lodge in the liver and in the great muscular masses, staying latent by indefinite periods until anaerobic conditions that allow germination and the unchain of the disease, what could happen through “stress”, traumatism, lesions, hematomas, etc.

BALDASSI et al. (1985) accomplished 2082 laboratory samples at São Paulo State, during the decade of 1970-1979, isolated *Clostridium chauvoei* and *Clostridium septicum* in 226 samples, where 6,67% were in fragments of organs. Already in the research of *Clostridium chauvoei* accomplished by MALONE et al. (1986), these demonstrated that of the 29 cases of lesions caused by the agent, 14 (48,3%) they were isolated in muscle, 8 (27,6%) in muscle and pericardium, 6 (20,7%) in pericardium and just 1 (3,4%) in meninge.

In work accomplished by GIORGI & TROISE (1968), in the countryside of São Paulo State, 45 exams were processed for symptomatic carbuncle in organs of animals, and of these 2 (4,4%) they were positive.

HARBOLA & KUMAR (1990) demonstrate the economic importance of the genus *Clostridium*, mainly for the *Clostridium perfringens* and *Clostridium chauvoei* after researching samples of tissues of animals with clostridiosis suspicion in India, and they isolate *Clostridium septicum* and *Clostridium chauvoei* in 15% of the studied samples, while HULLAND et al. (1985) analysing several tissues of healthy animals, demonstrated the presence of latent spores of *Clostridium chauvoei*.

Thus, the present work was delineated with the objective of confirming the hypothesis that, the spores of *Clostridium chauvoei* can penetrate in the animal host, will lodge in the liver and in the great muscular masses, staying latent until anaerobic conditions caused by stress, traumatism, hematomas, lesions, etc, activate the spores, that will provoke blackleg, showing the importance of vaccination of cattle against this disease.

### MATERIAL AND METHODS

During the period of December of 1996 to February of 1997, were picked 60 liver samples and 40 muscle samples (palette) of bovine abated for consumption, in two slaughter houses of the region of Ribeirão Preto-SP. The liver samples correspond to the same animals from where the samples of muscles were obtained plus 20 samples of liver of others animals. The samples were collected with disinfected knives with iodized alcohol, cutting the meat in blocks of approximately 10x10x10 cm from the palette and of 8x8x8 cm from the liver, which were deposited separately in sterilized plastic sacks and transported until the laboratory analysis in isotherm boxes with ice.

Of each sample, were retired pieces of the internal portion of the tissue then, through a special technique, composed by a previously sterilized metal apparatus use to bore. In this way the internal samples were free from possible contamination of the slaughter or transport.

The retired samples, weighing around 10 g, were sowed in screw caps tubes, with media for clostridia growth; Brain heart infusion (BHI-Difco), Tarozzi broth and Cooked meat medium (CMM-Difco) and later on submitted to a thermal shock (80ºC for 10 minutes and cold soon after to 30ºC with water and ice) and incubated at 32-35ºC by 24-48 hours (ANDO, 1953).

Gram stains were done to confirm the presence of small Gram positive sporulated sticks. Cultures were sowed in plates of Clostrisel agar enriched with 5% of defibrinated sheep blood and incubated in anaerobic jars by 24-48 hours to 35º C. Cultures were also sowed in plates of blood agar and incubated in aerobiosis at 37°C for 24-48 hours.

The colonies with typical growth of *Clostridium* according to STERNE & BATTY (1978) were submitted to biochemical identification through the tests of catalase production, Voges Proskauer, H2S, citrate and maltose, manose, sucrose, glucose, inositol, sorbitol, rhamnose, melibiose, arabinose, lactose fermentation, reduction of nitrate, indole and urease production, motility, hemolysis, and gelatin liquefaction (Api 20 E®) (BLAIR et al. 1971).

### RESULTS AND DISCUSSION

From the 60 samples of bovine liver analyzed, just one (1,7%) was positive, being isolated *Clostridium*
Tabela 1 – Strains of *Clostridium* isolated from healthy bovine muscles and liver slaughtered in two slaughter houses of the region of Ribeirão Preto - S.P.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Muscles Samples</th>
<th>%</th>
<th>Liver Samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. chauvoei</em></td>
<td>40/3</td>
<td>7,5</td>
<td>60/1</td>
<td>1,7</td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td>40/3</td>
<td>7,5</td>
<td>60/0</td>
<td>-</td>
</tr>
<tr>
<td><em>C. novyi</em></td>
<td>40/2</td>
<td>5,0</td>
<td>60/0</td>
<td>-</td>
</tr>
<tr>
<td><em>C. tertium</em></td>
<td>40/1</td>
<td>2,5</td>
<td>60/0</td>
<td>-</td>
</tr>
<tr>
<td><em>Clostridium sp</em></td>
<td>40/6</td>
<td>15,0</td>
<td>60/0</td>
<td>-</td>
</tr>
</tbody>
</table>

* Total analyzed samples / positive samples
**Percentage of positive samples

*chauvoei*. Similar data were obtained by SINGH et al. (1993), which also demonstrated the agent’s presence in the inoculated musculature, in sections of the tissue, muscular lesions and small liver samples.

From the 40 samples of bovine muscle analyzed were isolated several types of *Clostridium*, being 3 (7.5%) *Clostridium chauvoei* in the muscles samples. Latent spores of *Clostridium chauvoei* were found in several tissues and in muscles of bovine by HULLAND et al. (1985). This confirms the idea that spore, germination are stimulated by degeneration, intramuscular haemorrhagiae or lowers tension of oxygen.

Other types of *Clostridium* were isolated being 3 (7.5%) *Clostridium perfringens*, 2 (5.0%) *Clostridium novyi*, 1 (2.5%) *Clostridium tertium* and 6 (15.0%) *Clostridium sp*.

It can be observed by the results obtained in this research the importance of the vaccination of cattle against the diseases provoked by *Clostridium*, mainly the symptomatic carbuncle, once we could check the theory that, usually healthy bovine, that is to say, in perfect health condition, they can carry viable spores inside of the tissues, which can be activated when inoculated in culture media, confirming the statements of STERNE & BATTY (1978), that the organism penetrates in the animal and will lodge in the great muscular masses.

This research confirm that spores of *Clostridium* penetrate in the animals, remaining latent in the tissues, demonstrating that animals could develop the disease as soon as there was a decrease of the oxygen tension for the spores germination. Still in agreement with this author, the data found for the muscle, when compared to the liver, it evidences that the predilection place more common of this genus it would be in the great muscular masses.

REFERENCES


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