

## Role of intestinal flora in acute hemorrhagic gastroenteritis (Parvovirus infection) of dogs \*

### Papel da flora intestinal bacteriana na gastroenterite hemorrágica (parvovirose) dos cães

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## SUMMARY

Canine parvovirus infection might be associated to internal parasites, other viral infections or bacterial enteropathogens. In case of severe diarrhea, the clinical picture is worsened by endotoxic shock. In order to evaluate the role of concomitant enteric bacterial infection or bacteremia, 34 dogs with hemorrhagic enteritis due to parvovirus infection were studied. Blood and fecal specimens were cultured, in an attempt to isolate enteropathogens, as well as *Campylobacter jejuni*. Electron microscopy was done for virus detection. Presence of parvovirus-like particles in the stools confirmed the diagnosis of parvovirus infection, but enteropathogens isolation attempts were unsuccessful. Neither *Salmonella* sp, nor enteropathogenic *E. coli* were isolated, while *C. jejuni* was cultured from five samples of feces from diarrheic dogs. Concerning hemoculture, *Pseudomonas* sp, *E. coli*, *Alcaligenes odorans* and a nonidentified labile non fermenter Gram-negative rod were isolated from severe cases of parvovirus. Results allowed the conclusion that concurrent bacterial infection did not play an important role on the evolution of the process, but there was an invasion of normal intestinal bacteria into the blood stream, leading to the installation of endotoxic shock in cases of fatal parvovirus infection.

UNITERMS: Blood; Dogs; Hemorrhagic enteritis; Parvovirus; Bacteria; *Campylobacter jejuni*.

## INTRODUCTION

Parvovirus infection in dogs, specially in the young ones, may result in a severe and fatal disease<sup>8</sup>. The evolution of parvovirus infection depends on the age of the dog its general physical conditions, and on preexisting or concurrent parasitic, bacterial or viral infections. *Toxocara canis*, *Ancylostoma caninum*, *Giardia canis*, *Coccidia* sp, *Campylobacter jejuni*, *Clostridium perfringens* are referred to as predisposing or aggravating factor for parvovirus infection of dogs. Canine coronavirus, or distemper virus are often found as concurrent infectious agents dogs<sup>8, 14, 15, 18, 19</sup>. Neonates, or dogs germ free in which the normal intestinal flora is limited have a less intense epithelial cell turnover and, when infected by parvovirus, they present less severe lesions and mild infection. On the other hand, in cases of severe parvovirus infection, there are evident signs of endotoxemia or septicemia, such as intense leukopenia, rapid worsening of clinical conditions and death of the patient with signs of endotoxemic shock<sup>8</sup>.

This paper presents the results of a bacteriological study made on dogs presenting severe parvovirus infection, in an attempt to determine the role of *Campylobacter jejuni*,

*Salmonella* sp or other enteropathogenic bacteria as concurrent infectious agents, or as cause of septic shock.

## MATERIAL AND METHODS

The thirty-four dogs included in this study were examined at the Department of Medical Clinics, Faculty of Veterinary Medicine and Zootechny, University of São Paulo. They presented an acute hemorrhagic gastroenteritis with a presumptive diagnosis of parvovirus. Leukopenia was found in the majority of cases. Electron microscopy of stools suspension allowed to observe parvovirus-like particles, thus confirming the clinical diagnosis.

Fecal samples were collected in a flask and mixed with transport medium of Cary & Blair (three parts to one part of feces) as previously described<sup>16, 17</sup>, and processed before 12 hours. For isolation of *Campylobacter jejuni*, the method described elsewhere by Pavan *et al.*<sup>13</sup> (1987) and for isolation pathogenic enterobacteria the method described by Ewing<sup>7</sup> (1986) were employed. Immediately after physical examination, blood samples were collected for hematological studies and hemoculture. The latter was performed according to Bartlett *et al.*<sup>2</sup> (1974) with slight modifications: 5 ml of venous

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blood were collected and inoculated into appropriate flasks containing 30 ml of tripticase soy broth added with 0.05% of anticoagulant (sodium polyanetholsulfonate - Roche<sup>®</sup> and 0.05% of bovine hemine - SIGMA). Flasks were incubated at 35°C for seven days. After 48 hours and seven days, a subculture was made in agar chocolate plates and *Campylobacter medium* without antibiotics.

Blood culture was negative when both plates did not show any bacterial growth. Enterobacteria were identified according to Ewing<sup>5</sup> (1986), while non fermenter Gram-negative rod was identified according to Barrishan<sup>1</sup> (1979) and Gilardi<sup>7</sup> (1985).

## RESULTS

Neither *Salmonella* sp, nor enteropathogenic *E. coli* were isolated from feces of dogs that had presented severe gastroenteritis. Concerning *Campylobacter jejuni*, it was found in five samples (14.7%) of dogs studied.

*Escherichia coli* (2 samples), *Pseudomonas* sp (1), *Alcaligenes odorans* and a non fermenter Gram-negative rod (non identified) were isolated from blood stream by means of hemoculture (Tab.1).

## DISCUSSION

Attempts to isolate enteropathogens as *Salmonella* sp or enteropathogenic *E. coli*, from feces of dogs with severe gastroenteritis due to parvovirus infection were unsuccessful, leading to the conclusion that, at least in the present study, these organisms were not involved as concurrent infectious agents in the pathogenesis of the disease. As to *Campylobacter jejuni*, its role as enteric pathogen is well known<sup>6,9,10,12,14</sup> and the possibility of concomitant occurrence of parvovirus and *Campylobacter* has been described elsewhere<sup>9</sup>. Notwithstanding, since the isolation rate observed in the

stools of parvovirus infected dogs is quite similar to that found in healthy dogs as reported in a previous study recently published<sup>11</sup>, the microorganism was not considered as an associated cause of the diarrhea.

Concerning the blood culture, isolation of microorganisms as *Escherichia coli* or *Alcaligenes odorans* was expected, since the dogs included in this study were severely affected and probably had a deep lesion of intestinal mucosa, allowing any indigenous bacteria of the lumen to invade the blood stream. According to Calvert; Greene<sup>1</sup> (1984), isolation of *Escherichia coli*, *Pseudomonas* sp, *Staphylococcus aureus*, *Proteus* sp and *Klebsiella* sp always has a diagnostic meaning of bacteremia, while *Staphylococcus epidermidis*, *Corynebacterium* sp and *Bacillus* sp can be found as a consequence of skin contamination at the site where venipuncture was made. As far as *Campylobacter jejuni* is concerned, although it has not been found in the blood of any patient, it could have contributed to the worsening of physical condition in case of bacteremia, as reported by Guerrant *et al.*<sup>9</sup> (1978) in humans, or by Oswald *et al.*<sup>12</sup> (1994) in dogs.

In two dogs, strains of *E. coli* that showed the same biochemical and antimicrobial susceptibility patterns were isolated from both, blood and fecal specimens of bacteremic dogs. These data support the statement that in severe cases of parvovirus infection, the patient's death might occur due to an endotoxic shock, mainly caused by Gram-negative bacteria<sup>4</sup>. All the dogs from which microorganisms were isolated through hemoculture had presented severe illness, had leukopenia and blood picture resembling that of endotoxic shock due to Gram-negative bacteria, and all, but one, died before 20 hours. From the only one dog that survived, a non-fermentative rod, non-identified, was isolated. Importance of the invasion of blood stream during parvovirus infection of dogs, by usually non-pathogenic bacteria such as those isolated in this study, is highlighted by the fact that, 80% of bacteremic dogs died, in opposition to 20% of deaths in the group of dogs in which blood culture was negative.

Table 1

Isolation of microorganism from feces and blood stream of 34 dogs severely affected with parvovirus infection. São Paulo, 1989.

| Microorganism               | Frequency of isolation |
|-----------------------------|------------------------|
| From feces                  |                        |
| <i>Campylobacter jejuni</i> | 5                      |
| From blood                  |                        |
| <i>Escherichia coli</i>     | 2                      |
| <i>Pseudomonas</i> sp       | 1                      |
| <i>Alcaligenes odorans</i>  | 1                      |
| NFGNR *                     | 1                      |

\* non fermenter Gram-negative rod

## RESUMO

O parvovírus canino pode estar associado a parasitas intestinais, vírus ou bactérias enteropatogênicas, os quais podem predispor ou contribuir para o agravamento do quadro mórbido e, nos casos de diarreia grave, na instalação do choque endotóxico. Com a finalidade de analisar a existência de infecção bacteriana concomitante ou de bacteremia, em cães com enterite hemorrágica aguda e grave, causada por parvovírus, procedeu-se à coprocultura e à hemocultura, em 34 animais atendidos no Hospital Veterinário da FMVZ/USP. O diagnóstico clínico e etiológico de parvovirose baseou-se no perfil hematológico e na presença de partículas virais nas fezes dos cães, observadas por meio de microscopia eletrônica. Não foram isoladas *Salmonella* sp ou *E. coli* enteropatogênica, ao contrário de *Campylobacter jejuni*, que foi isolado das fezes de cinco dos pacientes estudados. Em relação à hemocultura, *Pseudomonas* sp, *E.coli*, *Alcaligenes odorans* e bacilo Gram-negativo não fermentador, não identificado, foram isolados de cinco cães, dos quais quatro sofreram evolução fatal. Aparentemente, a infecção bacteriana concomitante não exerce papel de destaque na evolução do processo, mas existe uma invasão da corrente sanguínea por bactérias pertencentes à flora intestinal normal, o que resulta no agravamento do quadro mórbido, contribuindo para a instalação do choque endotóxico e o óbito do animal.

UNITERMOS: Sangue; Cães; Enterite hemorrágica; Bactérias; Parvovírus; *Campylobacter jejuni*.

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