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CLINICAL ASPECTS OF EXPERIMENTAL PERITONITIS IN HORSES[1]

ASPECTOS CLÍNICOS DA PERITONITE EXPERIMENTAL EM EQÜINOS

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SUMMARY

*Sixteen adult horses were randomly divided into 4 equal groups (GI, GII, GIII and GIV) of 4 animals and each group was injected intraperitoneally with one of the following suspension: GI (100 x 10^7 colony-forming units (CFU) of **Escherichia coli** diluted in 500ml of 0.9% saline); GII (100 x 10^7 CFU of **Bacteroides fragilis** in 500ml of 0.9% saline); GIII (100 x 10^7 CFU of **E. coli** in combination with 100 x 10^7 CFU of **B. fragilis** in 500ml of 0.9% saline); GIV (500ml of 0.9% saline). Abdominal wall sensitivity to external pressure and tension, diarrhea, decreased intestinal sounds and increased of heart rate were the clinical signs more frequently observed in inoculated horses. Horses inoculated with pure cultures of either **E. coli** or **B. fragilis** demonstrated mild and self-limiting peritonitis, while those inoculated with the combination of both bacteria demonstrated clinical signs of higher intensity and duration.*

Key words: *peritonitis, horses, **Escherichia coli**, **Bacteroides fragilis**.*

RESUMO

*Dezesseis eqüinos adultos foram distribuídos aleatoriamente em 4 grupos (GI, GII, GIII e GIV), constituídos por quatro animais, recebendo cada grupo o seguinte inóculo por via intraperitoneal: GI (100 X 10^7 unidades formadoras de colônia (UFC) de **Escherichia coli** diluídos em 500ml de salina 0,9%); GII (100 X 10^7 UFC de **Bacteroides fragilis** diluídos em 500ml de salina 0,9%); GIII (100 X 10^7 UFC de **E. coli** associados a 100 X 10^7 UFC de **B. fragilis** diluídos em 500ml de salina 0,9%); GIV (testemunho - 500ml de salina 0,9%). Aumento da sensibilidade e tensão da parede abdominal, diarréia, diminuição dos sons intestinais e aumento da freqüência cardíaca foram os sinais mais freqüentemente observados nos eqüinos inoculados com cepas bacterianas. Eqüinos inoculados com culturas puras de **E. coli** ou **B. fragilis** apresentaram peritonites brandas e autolimitantes, enquanto que os inoculados com a associação dessas bactérias apresentaram sinais de maior intensidade e duração.*

Palavras-chave: *peritonite, eqüinos, **Escherichia coli, Bacteroides fragilis.***

INTRODUCTION

Peritonitis may be induced by any contaminant or irritating agent. It is a complex disease characterized by multiple organ involvement (HOSGOOD & SALISBURY, 1989). Horses have always been considered to be highly susceptible to peritonitis (SCHNEIDER, 1992) and their susceptibility has been tentatively attributed to the equine omentum being smaller than that of other species (MAIR ***et al.***, 1990). It was considered a potentially fatal or permanently incapacitating condition, and has been identified as a major postoperative complication of surgical colic (RICKETTS, 1987). There have been few detailed studies on peritonitis in adult horses (MAIR ***et al.***, 1990). Much of the knowledge about peritonitis has been determined through studies on laboratory species and humans (TRENT, 1995).

The clinical signs of horses with naturally acquired peritonitis are abdominal pain, ileus, hyperthermia or normothermia, abdominal distention, anorexia, weight loss and diarrhea or constipation (COFFMAN & TRISCHLER, 1972; DYSON, 1983; CLABOUGH & DUCKETT, 1992; MOLL & SCHUMACHER, 1992). Horses with acute diffuse peritonitis also show prolonged capillary refill time, dehydration, increased heart and respiratory rates, pawing, depression, anorexia, red-

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to-purple mucous membranes and muscle fasciculation (MAIR ***et al.***, 1990). Abdominal pain is most evident in the early stages of disease and is characterized by reluctance to move, splinting of the abdominal wall, and sensitivity to external abdominal pressure (SEMRAD, 1990).

E. coli and ***B. fragilis*** are present in the normal flora of gastrointestinal tract and most cases of peritonitis in horse are caused by these agents (COFFMAN & TRISCHLER, 1972; MAIR ***et al.***, 1990; MOLL & SCHUMACHER, 1992). Fifty-six per cent of the positive samples yielded one of these species and 26 per cent yielded the both species (SEMRAD, 1990). The association between obligate anaerobes and facultative organisms enhances the virulence of some bacteria that are relatively nonpathogenic in normal circumstances. Mixed infection by these two bacteria in horse peritonitis has synergic effects (MOORE, 1993).

The principal goal of the present experiment was to study the clinical alterations of equine experimental peritonitis caused by ***E. coli*** or ***B. fragilis*** or by the combination of these two agents.

MATERIAL AND METHODS

Sixteen healthy horses (twelve male and four female not pregnant) of various breeds, ranging from 3 to 10 years were used. The horses were randomized in four groups (GI, GII, GIII and GIV) of four animals each. During the study the horses were housed in individual stalls, fed ration with 12% protein (3 kg/animal/day), coast-cross (***Cynodon dactilon L.***) hay and water ***ad libitum***.

B. fragilis was isolated from a human patient with peritonitis in the University Hospital, Faculty of Medicine of Ribeirão Preto - São Paulo University, and was cultivated at the Anaerobic Laboratory of the Faculty of Agronomic and Veterinary Sciences, Campus of Jaboticabal, São Paulo State University, using JANG & HIRSH (1991) method. ***E. coli*** was isolated from a sample of feces from a healthy horse at the same university, using EDWARDS & EWING (1972) method. The inoculum was standardized as 10 x 10^7 colony-forming units (CFU) per milliliter.

For the inoculations, paracentesis was performed according to the technique described by WHITE II (1990), and the horses were inoculated intraperitoneally as described in table 1. All animals were examined at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 120, 168 and 216 hours after inoculation (HAI). Clinical records included rectal temperature, heart and respiratory rates, color of the mucus membranes, capillary refill time, skin elasticity, tension and sensitivity of the abdominal wall, intestinal sounds and other observations, if necessary.

Table 1 - Treatment of horses by intraperitoneal injection.

Group	Inoculum/dose
I	***E. coli*** (100 x 10^7 CFU) +500 ml of 0.9% saline
II	***B. fragilis*** (100 x 10^7 CFU) + 500 ml of 0.9% saline
III	***E. coli*** (100 x 10^7 CFU) + ***B. fragilis*** (100 x 10^7 CFU) + 500 ml of 0.9% saline
IV	500 ml of 0.9% saline

CFU = Colony-forming units.

Data were analyzed using a randomized design, and Tukey test was used to compare data obtained from the two groups at each observation time and results were considered to be significant at the $P < 0.05$ level.

RESULTS

Physical examination of the animals before inoculation did not show any abnormality in any parameter recorded. Rectal temperature was significantly increased from 6 to 8 HAI when compared to the control group (table 2). Significant tachycardia was detected in groups I, II and III at times between 8 and 120 HAI (table 3). Only the horses of group III showed an increased in respiratory rate at 10 HAI (table 4). All the inoculated animals showed other clinical signs which are listed in tables 5, 6 and 7. It is important to emphasize that the increase in sensitivity and tension of the abdominal wall were more intense in group I when compared to the other groups. Abdominal pain and diarrhea were more intense in the group III. The animals of control group (group IV) did not shown any clinical signs.

DISCUSSION

Sensitivity and tension of the abdominal wall, diarrhea, an increase in intestinal sounds followed by a decrease and hyperthermia were the clinical signs more frequently observed in horses inoculated with ***E. coli, B. fragilis*** or with a combination of ***E. coli*** and ***B. fragilis***. These signs were similar to those occurring in natural peritonitis in horses (COFFMAN & TRISCHLER, 1972; DYSON, 1983; CLABOUGH & DUSKETT, 1992;

Table 2 - F values, coefficient of variation (CV%) and means for the four groups of horses studied -Rectal temperature (°C).

	Hours after inoculation														
	0	2	4	6	8	10	12	24	36	48	60	72	120	168	216
F for groups	4.32^{NS}	1.19^{NS}	2.09^{NS}	7.25^{**}	3.95^{*}	2.84^{NS}	2.26^{NS}	1.12^{NS}	1.89^{NS}	3.64^{*}	2.22^{NS}	2.75^{NS}	2.23^{NS}	1.09^{NS}	2.67^{NS}
CV (%)	1.10	1.38	1.75	1.19	1.51	1.53	1.58	2.09	1.93	1.48	1.63	1.80	1.49	1.09	1.55
Mean GI	37.95^{A}	38.32^{A}	39.10^{A}	39.00^{A}	39.05^{A}	39.10^{A}	39.05^{A}	37.80^{A}	38.97^{A}	38.40^{A}	38.70^{A}	38.27^{A}	38.12^{A}	37.97^{A}	37.87^{A}
Mean GII	37.10^{A}	37.95^{A}	38.65^{A}	39.25^{A}	39.12^{A}	39.17^{A}	39.27^{A}	37.72^{A}	38.05^{A}	37.15^{B}	37.87^{A}	37.52^{A}	37.35^{A}	37.52^{A}	37.15^{A}
Mean GIII	37.62^{A}	38.20^{A}	38.32^{A}	38.97^{A}	39.10^{A}	39.02^{A}	39.05^{A}	37.97^{A}	38.92^{A}	38.10^{AB}	38.90^{A}	38.73^{A}	38.23^{A}	37.43^{A}	38.20^{A}
Mean GIV	37.30^{A}	37.67^{A}	37.95^{A}	37.85^{B}	37.95^{B}	38.10^{A}	38.22^{A}	37.10^{A}	38.22^{A}	37.52^{AB}	38.30^{A}	37.42^{A}	37.17^{A}	37.15^{A}	36.97^{A}

NS -Non significant
* - P< 0.05 compared between groups at the same time.
** - P< 0.01 compared between groups at the same time.

Table 3 - F values, coefficient of variation (CV%) and means for the four groups of horses studied - Heart rate (bpm).

	Hours after inoculations														
	0	2	4	6	8	10	12	24	36	48	60	72	120	168	216
F for groups	0.85^{ns}	2.36^{ns}	2.28^{ns}	2.88^{ns}	8.16^{**}	6.85^{**}	3.45^{ns}	6.54^{**}	4.69^{*}	8.64^{**}	5.69^{*}	7.72^{**}	11.31^{**}	3.20^{ns}	2.54^{ns}
CV (%)	12.06	25.35	25.92	21.34	20.23	25.26	21.57	22.17	24.47	17.60	21.40	13.73	12.45	16.23	18.36
Mean GI	42.00^{A}	61.00^{A}	66.00^{A}	61.00^{A}	66.00^{AB}	64.00^{AB}	67.00^{A}	53.00^{AB}	58.00^{AB}	50.00^{AB}	46.00^{AB}	47.00^{AC}	48.00^{A}	46.00^{A}	45.00^{A}
Mean GII	38.00^{A}	63.00^{A}	69.00^{A}	53.00^{A}	52.00^{B}	49.00^{B}	54.00^{A}	37.00^{B}	38.00^{B}	40.00^{B}	41.00^{B}	38.00^{BC}	36.00^{B}	40.00^{A}	42.00^{A}
Mean GIII	43.00^{A}	72.00^{A}	64.00^{A}	69.00^{A}	81.00^{A}	85.00^{A}	64.50^{A}	65.00^{A}	66.00^{A}	63.50^{A}	65.00^{A}	57.33^{A}	54.67^{A}	53.33^{A}	54.67^{A}
Mean GIV	41.00^{A}	44.00^{A}	43.00^{A}	45.00^{A}	41.00^{B}	40.00^{B}	42.00^{A}	37.00^{B}	41.00^{AB}	36.00^{B}	38.00^{B}	38.00^{BC}	35.00^{B}	38.00^{A}	38.00^{A}

NS-Non significant
* - P< 0.05 compared between groups at the same time
** - P< 0.01 compared between groups at the same time

Table 4 - F values, coefficient of variation (CV%) and means for the four groups of horses studied -Respiratory rate (mpm).

	Hours after inoculations														
	0	2	4	6	8	10	12	24	36	48	60	72	120	168	216
F for groups	0.39^{NS}	1.56^{NS}	2.86^{NS}	0.23^{NS}	2.55^{NS}	4.06^{*}	2.37^{NS}	0.16^{NS}	1.06^{NS}	1.96^{NS}	3.06^{NS}	2.84^{NS}	2.58^{NS}	1.65^{NS}	0.11^{NS}
CV (%)	45.81	42.72	36.00	48.73	35.36	30.34	36.58	30.50	50.83	39.44	33.84	42.54	18.78	31.73	36.47
Mean GI	19.00^{A}	31.00^{A}	38.00^{A}	28.00^{A}	31.00^{A}	23.00^{AB}	19.00^{A}	17.00^{A}	30.00^{A}	25.00^{A}	24.00^{A}	15.00^{A}	16.00^{A}	14.00^{A}	18.00^{A}
Mean GII	17.00^{A}	50.00^{A}	46.00^{A}	34.00^{A}	39.00^{A}	28.00^{AB}	21.00^{A}	17.00^{A}	17.00^{A}	13.00^{A}	16.00^{A}	14.00^{A}	13.00^{A}	17.00^{A}	16.00^{A}
Mean GIII	15.00^{A}	41.00^{A}	28.00^{A}	26.00^{A}	28.00^{A}	37.00^{A}	33.00^{A}	17.00^{A}	31.00^{A}	22.00^{A}	32.50^{A}	30.67^{A}	18.67^{A}	13.33^{A}	16.00^{A}
Mean GIV	21.00^{A}	28.00^{A}	23.00^{A}	30.00^{A}	19.00^{A}	18.00^{B}	20.00^{A}	15.00^{A}	33.00^{A}	17.00^{A}	21.00^{A}	21.00^{A}	18.00^{A}	16.00^{A}	16.00^{A}

NS -Non significant
* - P< 0.05 compared between groups at the same time
** - P< 0.01 compared between groups at the same time

Table 5 - Clinical signs observed in group I*.

Horses	Clinical Signs	Hours after inoculations
1	Sensitivity of abdominal wall	4 , 6 , 8, 10, 12, 24, 36 and 48
	Tension of abdominal wall	4, 6, 8, 10, 12, 24, 36 and 48
	Increased intestinal sounds	2 , 48 and 60
	Decreased intestinal sounds	4, 6, 8, 10, 12 and 24
	Diarrhea	2
2	Sensitivity of abdominal wall	2, 4, 6, 8, 10 and 12
	Tension of abdominal wall	2, 4, 6, 8, 10 and 12
	Increased intestinal sounds	2
	Decreased intestinal sounds	4, 6, 8, 10 and 12
	Diarrhea	2
3	Sensitivity of abdominal wall	2, 4, 6, 8, 10, 12 and 24
	Tension of abdominal wall	2, 4, 6, 8, 10, 12 and 24
	Decreased intestinal sounds	2, 4, 6, 8, 10 and 12
	Diarrhea	2
	Recumbence	2
4	Sensitivity of abdominal wall	2, 4, 6, 8, 10 and 12
	Tension of abdominal wall	4,6, 8, 10 and 12
	Increased intestinal sounds	2
	Decreased intestinal sounds	4, 6, 8, 10, 12, 24, 48, 60 and 72
	Diarrhea	2 and 36

* Horses inoculated intraperitoneally with 100 x 10^7 CFU of ***E. coli***

Table 6 - Clinical signs observed in group II*.

Horses	Clinical Signs	Hours after inoculations
5	Increased intestinal sounds	24
	Decreased intestinal sounds	6, 8, 10 and 12
	Diarrhea	2 and 4
	Recumbence	4
	Turning the head toward the flank	10
	Restlessness	10
6	Sensitivity of abdominal wall	24
	Tension of abdominal wall	4, 6, 8 and 10
	Decreased intestinal sounds	2, 4, 6, 8 and 10
	Diarrhea	2
7	Sensitivity of abdominal wall	24
	Tension of abdominal wall	2, 4, 6, 8, 10, 12 and 24
	Diarrhea	2
8	Decreased intestinal sounds	2, 4, 6, 8, 10 and 12
	Tension of abdominal wall	2, 4 and 6
	Diarrhea	2
	Restlessness	2

*Horses inoculated intraperitoneally with 100 x 10^7 CFU of ***B. Fragilis***.

MAIR ***et al.***, 1990; MOLL & SCHUMACHER, 1992), and when present they are indicative of clinical peritonitis occurrence.

Clinical signs were more intense and more prolonged in animals inoculated with the combination of bacteria when compared with single inoculation of ***E. coli*** or ***B. fragilis***. These findings suggest that synergism occurred in horses experimentally inoculated with ***E. coli*** and ***B. fragilis***, and mortality was only observed in one animal of this group (III).

Statistically significant hyperthermia was detected in all inoculated groups only at times 6 and 8 HAI, although isolated peaks occurred in all inoculated animals until 60 HAI. Previous studies of peritonitis in equine reported normothermia (COFFMAN & TRISCHLER, 1972; MOLL & SCHUMACHER, 1992) and hyperthermia intermittent (DYSON, 1972; MAIR ***et al.***, 1990). Release of exogenous pyrogens occurs in infectious or inflammatory processes stimulating neutrophils and eosinophils to produce interleukin 1 (IL-1) (WHITE II, 1990). Hyperthermia is a central response for IL-1 release. In addition, release of prostaglandin E_2 occurs in inflammation and endotoxemia and is responsible for increases in rectal temperature (WHITE II, 1990). Therefore the increases in rectal temperature observed in the horses with experimental peritonitis were important only until 6 HAI.

Statistically significant tachycardia occurred in all inoculated groups between 8 and 120 HAI. Several investigators have suggested that alterations occur in response to fluid loss, toxin absorption and release of inflammatory mediators (KUNESH, 1984; HOSGOOD & SALISBURY, 1989; MAIR ***et al.***, 1990; BONOUS, 1993; HAWKINS ***et al.***, 1993).

Only animals in group III, inoculated with ***E. coli*** and ***B. fragilis*** presented a significant increase of respiratory rate. Tachypnea occurs as a consequence of toxin absorption by peritoneum (BONOUS, 1993). This finding reinforces the hypothesis that the combination of these bacteria produces a greater pathogenicity than when each is injected alone. ***B. fragilis*** in pure culture, when injected intraperitoneally into rats, presents low pathogenicity (WHITE, 1990). Likewise, ***E. coli*** is also more pathogenic for rats when combined with adjuvant factors that favour bacterial growth, such as hemoglobin (HAU ***et al.***, 1978).

We conclude that the prompt clinical examination is important for the evaluation and prognosis of peritonitis, since some clinical signs will only be observed in the early phase of this

Table 7 - Clinical signs observed in group III*.

Horses	Clinical Signs	Hours after inoculations
	Depression	2
	Tension of abdominal wall	2, 4, 6, 8, 10, 12, 24, 36 and 48
9	Recumbence	2 e 4
	Decreased intestinal sounds	4, 6, 8, 10, 12 and 24
	Diarrhea	2
	Cyanotic mucous membranes	8, 10 and 12
	Death	60
	Sensitivity of abdominal wall	60, 72, 96 and 168
	Tension of abdominal wall	2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 96, 120 and 168
10	Depression	2 and 60
	Decreased intestinal sounds	2, 4, 6, 8, 10, 12, 60, 72 and 168
	Diarrhea	72
	Inappetence	72
	Sensitivity of abdominal wall	2, 4, 8, 10, 12, 24, 48 and 72
	Tension of abdominal wall	2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 96 and 120
11	Recumbence	2
	Decreased intestinal sounds	2, 4, 6, 8, 10, 12, 24, 36, 48 and 60
	Diarrhea	2 and 4
	Cyanotic mucous membranes	6
	Sensitivity of abdominal wall	8, 10, 12, 24, 36, 48, 60, 72, 96 and 120
	Tension of abdominal wall	2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72, 96, 120 and 168
12	Pale mucous membranes	2
	Decreased intestinal sounds	2, 4, 6, 8, 10, 12, 24, 36, 48 and 60
	Diarrhea	2

*Horses inoculated intraperitoneally with 100 x 10^7 CFU of ***E. coli*** + 100 x 10^7 CFU of ***B. fragilis***.

condition. Horses inoculated with pure cultures of either ***E. coli*** or ***B. fragilis*** demonstrated mild and self-limiting peritonitis, while those inoculated with the combination of both bacteria demonstrated clinical signs of higher intensity and duration.

REFERENCES

BONOUS, D.I. Test your diagnostic skill. **Comp Cont Edu Pract Vet**, v. 15, p. 611-613, 1993.

CLABOUGH, D.L., DUCKETT, W. Septic cholangitis and peritonitis in a gelding. **J Am Vet Med Assoc**, v. 200, p. 1521-1524, 1992.

COFFMAN, J.R., TRISCHLER, L.G. Exudative peritonitis in two horses. **J Am Vet Med Assoc**, v. 160, p. 871-872, 1972.

DYSON, S. Review of 30 cases of peritonitis in the horse. **Eq Vet J**, v. 15, p. 25-30, 1983.

EDWARDS, P.R., EWING, W.H. **Identification of *Enterobacteriaceae***. Minneapolis:Burges Publishing, 1972. p. 82-105.

HAU, T., HOFFMAN, R., SIMMONS, R.L. Mechanisms of the adjuvant effect of hemoglobin in experimental peritonitis I. In vivo inhibition of peritoneal leukocytosis. **Surgery**, v. 83, p. 223-229, 1978.

HAWKINS, J.P., BOWMAN, K.F., ROBERTS, M.C., ***et al.*** Peritonitis in horses: 67 cases (1985-1990). **J Am Vet Med Assoc**, v. 203, p. 284-288, 1993.

HOSGOOD, G.L., SALISBURY, S.K. Pathophysiology and pathogenesis of generalized peritonitis. **Problems in Vet Med**, v. 1, p. 159-167, 1989.

JANG, S.S., HIRSH, D.C. Identity of ***Bacteroides*** isolates and previously named ***Bacteroides spp*** in clinical specimens of animal origin. **Am J Vet Res**, v. 52, p. 738-741, 1991.

KUNESH, J.P. Therapeutic strategies involving antimicrobial treatment of large animal peritonitis. **J Am Vet Med Assoc**, v. 185, p. 1222-1225, 1984.

MAIR, T.S., HILLYER, M.H., TAYLOR, F.G.R. Peritonitis in adult horses: A review of 21 cases. **Vet Rec**, v. 126, p. 567-570, 1990.

MOLL, D.H., SCHUMACHER, J. Septic peritonitis associated with caudal myotomy in a tennessee walking horse. **J Am Vet Med Assoc**, v. 201, p. 458-459,1992.

MOORE, R.M. Pathogenesis of obligate anaerobic bacterial infections in horses. **Comp Cont Edu Pract Vet**, v.15, p. 278-287, 1993 .

RICKETTS, S.N. Peritonitis. In: ROBINSON, N.E. **Current therapy in equine medicine**. 2. ed. Philadelphia: Saunders, 1987. p. 79-81.

SCHNEIDER, R.K. Peritonitis. In: MANSMAN, R.A., MCALLISTER, E. S. **Equine Medicine and Surgery**. 3. ed. Santa Barbara: Am. Vet., 1982. p. 620-632.

SEMRAD, S.D. Peritonitis In: SMITH, B.P. **Large animal internal medicine**. Saint Louis: Mosby, 1990. p. 674-679.

TRENT, A.M. The peritoneum and peritoneal cavity. In: KOBLUK. (ed) **The Horse diseases & clinical management**. Philadelphia: Sauders, 1995. p. 373-401.

WHITE II, N.A. **The equine acute abdomen**. Philadelphia: Lea & Febiger, 1990. 443 p.

WHITE, S.L. Alterations in body temperature. In: SMITH, B. P. **Large animal internal medicine**. Saint Louis: Mosby, 1990. p. 35-46.