Evaluation of liver function in horses with duodenitis-proximal jejunitis

Avaliação da função hepática em equinos com duodeno-jejunite proximal

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Abstract

Previous reports of duodenitis-proximal jejunitis (DPJ) have noted histopathologic changes in the liver. However, there have been few studies evaluating the hepatic function during the process of DPJ in horses. The objective of this study was to investigate the hypotheses that there is a correlation between DPJ and hepatic dysfunction. Blood samples from 8 horses with DPJ were collected for aspartate aminotransferase (AST), gama-glutamyl transferase (GGT), total (TB), direct (DB) and indirect bilirubin (IB), total serum protein and albumin analysis. The blood samples were collected from horses that survived on the moment of admission at the veterinary hospital, at the time of nasogastric reflux (NGR) end, and at the time the animals were allowed to eat. At the same time, heart rate, respiratory rate, rectal temperature and packed cell volume (PCV) were performed and the volume and duration of NGR were evaluated. Blood samples and parameters of horses that have died were collected at the moment of admission at the hospital. The values of respiratory rate, rectal temperature, volume of NGR, AST, GGT, total protein and albumin were not statistically significant during the trial. By the time of admission at the hospital, the values of heart rate and PCV were significantly greater in horses that have died compared to the survivors. In the survivors there were significant values for heart rate, PCV and for bilirubins. This study did not confirm the hypothesis that DPJ causes hepatic dysfunction in horses when serum biochemical analyses were performed.

Keywords: Horses. Duodenitis-proximal jejunitis. Liver function.
Resumo
Relatos sobre a duodeno-jejunitis proximal (DJP) indicam alterações histopatológicas no fígado dos animais. Porém, existem poucos estudos avaliando a função hepática durante o processo de DJP em cavalos. O objetivo deste estudo foi investigar a hipótese de correlação entre a DJP e disfunção hepática nos animais. Foram colhidas amostras de sangue de oito cavalos com DJP para a determinação de aspartato-aminotransferase (AST), gama-glutamiltransferase (GGT), bilirrubina total (BT), direta (BD) e indireta (BI), proteína total e albumina. As amostras de sangue dos animais foram colhidas na admissão ao hospital veterinário, no momento que o refluxo nasogástrico (RNG) cessou e no momento em que os animais voltaram a se alimentar. Nos mesmos momentos foram aferidas a frequência cardíaca (FC), respiratória (FR) e a temperatura dos animais, além da determinação do hematócrito, do volume e da duração do RNG. As amostras de sangue e os parâmetros vitais dos animais que vieram a óbito foram colhidos no momento de admissão ao hospital. Os valores de FR, temperatura retal, volume de RNG, AST, GGT, proteína total e albumina não mostraram diferença significativa durante a pesquisa. No momento de admissão ao hospital, os valores de FC e hematócrito foram significante-mente maiores nos animais que vieram a óbito em relação aos que sobreviveram. Para os animais que sobreviveram os valores de FC, hematócrito e bilirrubinas diferiram estatisticamente pelo aumento. Não foi possível confirmar a hipótese que a DJP causa alteração hepática em cavalos por meio da análise bioquímica realizada na presente pesquisa.


Introduction
The duodenitis-proximal jejunitis (DPJ) is an acute disease, characterized by inflammation and edema of the duodenum and the proximal portion of jejunum, with accumulation of fluid in the small intestine (ARROYO, 2006), profuse nasogastric reflux (NGR), adynamic ileus, abdominal pain (DAVIS et al., 2003a), leucocytosis, pyrexia and high peritoneal fluid total protein concentrations (Underwood et al., 2008). The accumulation of fluid in the small intestine is secondary to the paralytic ileus, and the increase of the luminal pressure can result ascending bacterial infection to the bile duct (DAVIS; JONES, 2003b). The initiating cause of DPJ is unknown, however, some trials suggested that clostridia, Salmonella spp, mycotoxins and verminous arteritis can be implicated with that disease (ARROYO et al., 2006; JOHNSTON; MORRIS, 1987). The prevalence of DPJ is around 3 to 22% of all the colic cases in the state of Illinois-United States (UNDERWOOD et al, 2008) with survival rates ranging from 25 to 94% (SEAHORN et al., 1992). Fernandez et al. (2003) made a retrospective study of DPJ and concluded that the prevalence of the disease was around 12,7% of all colic cases in Veterinary Hospital at the University of São Paulo-Brazil. Complications such as cardiac arrhythmias, laminitis, and hepatic injuries have been reported (CORNICK; SEAHORN, 1990; COHEN et al, 1994; DAVIS et al, 2003a). Processes of intestinal strangulation and ischemia followed by shock, could have further effects on the neighbouring organs of the splanchnic area, such as the liver and the pancreas (GRULKE et al., 2002).

Intestinal injuries from a variety of causes can increase the permeability of the mucosal barrier. The absorption of bacteria and its toxins promotes intestinal inflammation. The inflammatory response in the intestine itself can be associated with the sprouting of inflammatory mediators in the portal blood. The action of these mediators on the endotelial cells and neutrophils in the portal circulation can induce a periportal inflammation and eventually the suppurative cholangiohepatitis. There is an association between suppurative cholangiohepatitis and inflammatory gastrointestinal disease in horses, emphasizing the importance of monitoring the serum hepatic enzymes and the liver function in horses with that pathological disturbance (DAVIS; JONES, 2003b). Some studies relate inflammatory gastrointestinal disease
with hepatic dysfunction in horses (BERGERO; NERY, 2008; DAVIS et al., 2003a; DAVIS; JONES, 2003b; ETTLINGER et al., 1990; HANAU; STEIGBIGEL, 2000; JOHNSTON et al., 1989).

Davis et al. (2003a) reviewed the medical records of all horses admitted for evaluation of DPJ in an 18 year period, to investigate if the horses with DPJ are predisposed to hepatic injury. As a result, they observed that the animals developed hepatic disease, and concluded that the mechanism of hepatic injury may involve ascending infection from the common bile duct, absorption of endotoxin, or hepatic hypoxia resulting from systemic inflammation and endotoxemic shock.

The aim of this study was to further investigate the hypothesis that there is a correlation between DPJ and hepatic dysfunction.

Materials and methods

Eight horses admitted to the Veterinary Hospital of the University of São Paulo with DPJ were included in the trial. That was the number of enteritis received at the hospital in a total of 3 years. The diagnosis was made on the basis of history (age, gender, previous colic history, duration of colic prior to admission, attitude and pain level at admission), physical examination (heart rate, respiratory rate, rectal temperature, gut sounds, oral and ocular membrane color), volume and color of NGR and laboratory abnormalities consistent with the disease. The horse’s age ranged from 4 years to 20 years. Of the 8 horses in this study, 6 were male (2 stallions, 4 geldings) and 2 were female; with 1 Thoroughbred, 2 Mangalarga Paulista, 1 American Trotter, 2 Brazilian Sport Horse, 1 Pampa horse and 1 Andalusian. All horses had haematology (white blood cell count, red blood cell count), serum biochemistry (venous pH, pCO2, bicarbonate concentration and anion gap [(Na++K+) – (HCO3–+CL–)], serum urea and creatinine concentrations) and peritoneal fluid (color, total protein, nucleated cells count analysis and lactate concentration) profiles performed on admission to the hospital.

All animals of the present study were treated and received the same treatment. The treatment was based on fluid replacement, correction of acid-base and electrolyte abnormalities, non-steroidal anti-inflammatory agents, antibiotics, gastrointestinal motility stimulating drugs, gastrointestinal mucosal protective agents and gastric decompression.

The time spent of the disease and the severity of it was different between horses. Both animals with DPJ that survived and that were subjected to euthanasia were included in the trial. The experiment was performed according to the Ethical Principles in Animal Research adopted by the Bioethic Comission of the School of Veterinary Medicine and Zootechny of University of São Paulo, protocol number 1117/2007, and by the Ethic Commission of the Veterinary Hospital. Blood samples were taken via jugular vein by blood collection needle (BD Vacutainer® Eclipse™) collected into serum tubes (glass tube BD366431). Samples were analysed for determination of serum biochemistry including AST (AST/GOT 11531), GGT (Gamma-GTFS 128019910021), total, direct and indirect bilirubin (Bilirubin 11515), total serum protein (STRUFDALDI, 1987) and albumin (DOUMAS; BIGGES, 1972). Biochemical analyses were performed using a wet chemistry analyser at 37 °C ± 1 °C (Labmax model 240). The parameters collected for horses with DPJ included physical examination (heart rate, respiratory rate, rectal temperature), volume of NGR and hematocrit test. The blood samples were collected from horses that survived on the moment of admission at the hospital, at the time of NGR end, and at the time the animals were allowed to eat. Blood samples and parameters of horses that were subjected to euthanasia were collected on the moment of admission at the hospital.

Statistical analysis

Data were analyzed by descriptive statistics and as all data were parametric, comparison was made by parametric analysis tests (MINITAB, Statistical Software, Release 14 for Windows”). The variables analyzed were heart rate, respiratory rate, rectal temperature, volume of NGR, PCV, AST, GGT, TB, DB, IB, total serum protein and albumin concentrations. Nonpaired t-test was employed to compare the variables between survived horses and horses subjected to euthanasia at the moment of admission at the hospital. One-way ANOVA was used to compare the results...
within the survivors, at the time of admission at the hospital, at the time of nasogastric reflux end and at the time they were allowed to eat. The level of significance was \( p < 0.05 \). Data are presented as mean ± s.d. Only significant data are reported.

**Results**

**Biochemical and physical analysis**

From the 8 horses in this study, 3 were euthanized due to persistent NGR, and 5 recovered completely. Only 2 horses were necropsied, as there was an ethical impairment of the third one. By the time of admission at the hospital, the values of heart rate and PCV were significantly greater in the non-survivors compared to the survivors (\( p = 0.029 \) and \( p = 0.01 \), respectively). The values of respiratory rate, rectal temperature, volume of NGR, AST, GGT, bilirubins, total protein and albumin concentrations were not statistically different between these groups (Table 1).

**Table 1 - Clinical signs and laboratory findings at the time of admission at the hospital, of survived horses vs. euthanized horses (mean ± s.d.)**

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Survived</th>
<th>Euthanized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>48.8 ± 13.00</td>
<td>69.33 ± 6.11</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>13.80 ± 7.29</td>
<td>15.33 ± 9.02</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.00 ± 0.62</td>
<td>38.53 ± 0.38</td>
</tr>
<tr>
<td>Nasogastric reflux (l)</td>
<td>5.60 ± 2.88</td>
<td>7.33 ± 0.38</td>
</tr>
</tbody>
</table>

**Laboratory findings**

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Survived</th>
<th>Euthanized</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT (U/l)</td>
<td>10.74 ± 4.91</td>
<td>9.91 ± 2.84</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>249.0 ± 121.0</td>
<td>219.1 ± 93.6</td>
</tr>
<tr>
<td>TB (mg/dl)</td>
<td>4.62 ± 2.00</td>
<td>3.91 ± 0.78</td>
</tr>
<tr>
<td>DB (mg/dl)</td>
<td>0.40 ± 0.06</td>
<td>0.48 ± 0.22</td>
</tr>
<tr>
<td>IB (mg/dl)</td>
<td>4.21 ± 1.95</td>
<td>3.42 ± 0.85</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>69.00 ± 18.10</td>
<td>59.90 ± 2.12</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>34.88 ± 5.06</td>
<td>32.3 ± 1.63</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>41.00 ± 6.04</td>
<td>51.33 ± 6.51</td>
</tr>
</tbody>
</table>

Legend: GGT = gama-glutamyl transferase; AST = aspartate aminotransferase; TB = total bilirubin; DB = direct bilirubin; IB = indirect bilirubin; PCV = packed cell volume.
Source: Research data.
Note: Significant difference between the survived and euthanized horses: \( p < 0.05 \).

The statistical analysis showed that in the survivors (between the time of admission at the hospital, the time of NGR end and at the time horses were allowed to eat) there were different values for heart rate, PCV and for bilirubin. The heart rate was significantly greater at the time of admission at the hospital compared to the time animals were allowed to eat (\( p = 0.005 \)). Related to PCV, the value was greater at the time of admission compared to the time of NGR end and to the time animals were allowed to eat (\( p = 0.003 \) and \( p = 0.008 \), respectively). And there were greater values at the time of NGR end when compared to the time animals were allowed to eat for TB, DB and IB (\( p = 0.036 \), \( p = 0.042 \), \( p = 0.041 \), respectively) (Table 2). The values of respiratory rate, rectal temperature, NGR volume, AST, GGT, total protein and albumin concentrations were not statistically different in the survivors (Table 2).

**Table 2 - Clinical signs and laboratory findings from survived horses at the time of admission at the hospital, at the time of NGR end and at the time they were allowed to eat (mean ± s.d.)**

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Time of admission</th>
<th>Time of NGR end</th>
<th>Time allowed to eat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>48.80 ± 13.00</td>
<td>40.80 ± 7.56</td>
<td>37.60 ± 6.07</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>13.80 ± 7.29</td>
<td>11.20 ± 1.79</td>
<td>11.80 ± 3.19</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.00 ± 0.61</td>
<td>37.74 ± 0.29</td>
<td>37.50 ± 0.70</td>
</tr>
</tbody>
</table>

**Laboratory findings**

| GGT (U/l) | 10.74 ± 4.91 | 13.70 ± 1.49 | 11.43 ± 4.09 |
| AST (U/l) | 249.0 +121.0 | 256.8 ± 59.1 | 380.0 ± 289.0 |
| TB (mg/dl) | 4.62 ±2.00 | 4.77 ± 1.64 | 5.97 ± 1.79 |
| DB (mg/dl) | 0.40 ± 0.06 | 0.38 ± 0.09 | 0.45 ± 0.08 |
| IB (mg/dl) | 4.21 ± 1.95 | 4.39 ± 1.63 | 5.52 ± 1.73 |
| Total protein (g/l) | 69.00 ± 18.00 | 73.38 ± 6.34 | 76.56 ± 6.09 |
| Albumin (g/l) | 34.88 ± 5.06 | 37.34 ± 2.32 | 37.46 ± 2.15 |
| PCV (%) | 41.00 ± 6.04 | 34.20 ± 3.19 | 35.20 ± 1.92 |

Legend: GGT = gama-glutamyl transferase; AST = aspartate aminotransferase; TB = total bilirubin; DB = direct bilirubin; IB = indirect bilirubin; PCV = packed cell volume.
Source: Research data.
Note: Significant difference between the time of admission, the time of NGR end and the time allowed to eat: different characters in the same line indicate statistical difference (\( p < 0.05 \)).
Complications

Among the 5 horses that survived, one had colitis and another one had difficulty in deglutition after the resolution of DPJ. They were treated and both animals had completed recovered and were discharge from the hospital.

Histopathologic analysis

The histopathologic analysis of the liver of the 2 horses that were necropsied indicated mild hepatocellular vacuolization; cholestasis; and cellular infiltration of monocytes in biliary duct in one horse, and vascular congestion and mild hepatic degeneration were observed on the other one.

The histopathologic analysis of the stomach, duodenum and proximal jejunum of both horses showed inflammation and hemorrhagic focus in gastric and intestinal mucosa. The histopathologic analysis of the duodenum and proximal jejunum indicated neutrophilic infiltration in the mucosa and submucosa and extensive hemorrhages in the muscularis layers and serosa.

Discussion

The hypothesis that DPJ causes hepatic dysfunction in horses was not accepted, in contrast to the study of Davis et al. (2003) who concluded that the majority of animals with DPJ had biochemical evidence of hepatic injury. In the present study, although one of the horses that had died presented GGT mean value 17,94 U/L above the reference range (4,3-13,4 U/L) and another horse that had survived presented AST mean 422.25 U/L above the reference range (226-366 U/L) (KANEKO et al., 1997), the values of AST, GGT, total serum protein and albumin were statistically analyzed at the time of admission at the hospital, at the time end of nasogastric reflux and at the time animals were allowed to eat, and they were not statistically different. According to this, the horses with DPJ were not predisposed to hepatic injury when serum biochemical analysis was performed. only the bilirubin values were different during the study probably due to the fact that those animals were fasted and, in general, this condition increases total and indirect bilirubin. Those animals had been subjected to a long fasting period in result of the DPJ.

The liver function tests have some limitations including: a) that an extensive damage is required to impair function because of the great metabolic power of the liver; b) that the tests are lacking in sensitivity or are too sensitive; c) that are so many functions of the liver that testing one does not indicate the functional status of the entire organ (BERGERO; NERY, 2008; COLES, 1967). The results of the present study may be so different in comparison of the trial of Davis et al. (2003) due to those limitations. However, the histopathologic analysis of the liver of the 2 horses that died indicates hepatic injury. It was found mild hepatocellular vacuolization; cholestasis; and cellular infiltration of monocytes in biliary duct in one horse, and vascular congestion and mild hepatic degeneration were observed on the other one. The result is in agreement with the trial of Davis et al. (2003) and the tutorial article of Freeman (2000) that found pathologic evidence of liver disease in horses with DPJ. According to Freeman (2000), the most common hepatic changes in horses with DPJ are fine changes in the cytoplasm of the hepatocytes and moderately to markedly congestion in the liver. Less common hepatic changes are bile duct hyperplasia, mild mixed mononuclear cell infiltrate in the portal triad and even small foci of coagulative necrosis.

Davis et al. (2003) and the tutorial article of Freeman (2000) believed that the mechanism of hepatic injury in horses with DPJ may involve increased luminal pressure in the proximal small intestine that results in ascending biliary infection or regurgitation of intestinal contents into the bile duct and decreased blood flow or macrophage activation from endotoxaemia.

During the trial, the survival rate was 62.5%, consonant with other studies with survival rates ranging from 25 to 94% (SEAHORN et al., 1992). According to Freeman (2000), the pathological changes in DPJ suggest an inflammatory process that causes gastric and intestinal stasis, with fluid accumulation and distention in the proximal part of the gastrointestinal tract. This process could have deleterious effects on intestinal motility and on water and electrolyte transport mechanisms inducing a vicious cycle events. In the present study,
the volume of NGR seemed not to influence the hepatic function. Davis et al. (2003a) evaluated the duration of the NGR and concluded that it wasn't significantly associated with the likelihood of hepatic injury, suggesting that it is the severity of the increase in luminal pressure or the degree of inflammation present that determines the likelihood of hepatic injury rather than the duration.

By the time of admission at the hospital, the values of heart rate and PVC were significantly greater in non-survivors horses when compared to survivors. The increased heart rate can be associated with pain, anxiety, dehydration and fever; and the higher PCV can be associated with dehydration, hypoxia and polycythemia (ZUCKERMAN, 2007). The animals of the trial were dehydrated, with electrolyte abnormalities and pain that varied from mild to severe. These characteristics had interfered in heart rate and in PCV and although they are non-specific parameters the results suggested that they are very sensitive exams to perform in animals during the process of DPJ to evaluate the improvement of the disease.

PCV is an easy, rapid and inexpensive test that can be widely done to follow up on the patient's condition. Trials that are similar with the current study, did not discuss about heart rate and PCV. These parameters can indicate pain and dehydration that indirect reflect the inflammation process in the small intestine which is the basis of DPJ. According to Freeman (2000), many of the changes seen in DIP could be the result of intestinal inflammation and endotoxaemia superimposed on a functional obstruction.

Conclusion

In conclusion, the horses with a favorable evolution of DPJ evaluated in the present study did not show predisposition to hepatic injury, according to the serum biochemical analyses that were performed.

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References


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