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Lactate dehydrogenase activity in abdominal fluid from horses with colic

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Abstract:

The purpose of this study was to determine whether lactate dehydrogenase (LDH) activity in abdominal fluid could be used as a prognostic indicator in horses with colic. LDH activity was measured in 27 abdominal effusions from horses with colic presented to Murdoch University veterinary teaching hospital using three different LDH test methods. Lactate in effusions was also measured in 11 of the horses. LDH activity was significantly different for each test methods used - the ratio of Randox wet chemistry LDH lactate to pyruvate (L-P): Randox wet LDH P-L:IDEXX dry chemistry P-L was approximately 1:2:4. LDH activity in the abdominal effusions was significantly higher with all methods in the horses that died or were euthanased due to abdominal sepsis or advanced neoplasia than in those that survived following treatment for colic signs due to mechanical obstructions or non-septic abdominal inflammation. LDH activity showed moderate to good correlation (r=0.73-0.86) with lactate concentration of the fluid. In conclusion, LDH activity in abdominal fluid may be a useful prognostic test in horses with colic. The test method for LDH measurement must be known and remain constant for meaningful interpretation. Significantly higher levels of LDH activity may be present in horses with colic due to sepsis or advanced neoplasia than in those with colic due to non-septic inflammation or mechanical obstructions, that may respond to treatment.

Keywords

Equine colic, abdominal lactate dehydrogenase activity, LDH, prognosis
1. Introduction

Although LDH activity measurement is used extensively in human medicine to
differentiate mainly between transudative and exudative pleural effusions [1-4],
few studies have been carried out on the body cavity effusions of veterinary
patients [5-7].

Lactate dehydrogenase (LDH) is an enzyme that is present in the cytoplasm of
almost all cells, including leukocytes [8] and red blood cells, and is an end
enzyme in the glycolysis pathway. It acts as a hydrogen transfer enzyme,
catalysing the oxidation of L-lactate to pyruvate, which is the final step in the
metabolic chain of anaerobic glycolysis. The reaction is reversible and the
reaction equilibrium favours the reduction of pyruvate to lactate [9, 10]. LDH
can be measured using both the forward and reverse reactions, using both wet
chemistry (conventional) and dry (solid phase) chemistry methods.

On average human tissues have about 500 times the total LDH levels found in the
serum, with very high levels in the liver (9000 IU/g), heart (25000 IU/g), skeletal
muscle (9000 IU/g) and lung (9500 IU/g) [11]. Enzymes such as LDH appear to
serve no function in body cavity effusions, but can serve as indicators of
disturbed cellular integrity induced by pathological conditions [11]. Even a
small amount of tissue damage can result in significant elevation of LDH activity
and its extracellular appearance can therefore be used to detect cell injury or
death [10]. Increased activities of LDH in peritoneal fluid may indicate
inflammation and tissue damage including ischaemia [12]. Lactate can also be
measured in abdominal fluid on lab-based and point-of care monitors and is frequently used for evaluating abdominal fluid in horses with colic [13].

This study explores the use of lactate dehydrogenase activity of abdominal effusions as an aid in determining prognosis in horses with colic.

2. Materials and methods

Abdominal fluid was collected from 27 horses presented to the Murdoch University Veterinary Hospital and submitted to the MUVH clinical pathology laboratory for evaluation. Samples submitted to the laboratory were often from referral cases and in cases wherein the clinicians required more information than their in-house analysis provided, e.g. prolonged colic, complicated medical or surgical cases or insurance cases. Samples were excluded if they were analysed later than 24 hours after collection. Use of samples complied with the Murdoch University animal ethics protocols for excess samples obtained for diagnostic purposes.

Lactate was also measured in the abdominal samples from 11 of the horses on a radiometer ABL 700 blood gas analyzer (Radiometer Medical ApS, Denmark) using samples collected into EDTA anticoagulant and analysed within 15-30 minutes after collection.

Routine analysis was performed on all submitted abdominal effusions. This included obtaining a total nucleated cell count and red cell count by flow cytometry (Advia® 120 haematology analyser, Siemens Health Care Diagnostics, USA) on the effusion sample. Packed red cell volume (PCV) was measured in haematocrit capillary tubes following centrifugation. Total protein was
measured on the supernatant of the effusion using a temperature compensated
refractometer (Reichert Vet 360, NY, USA).

A direct smear and a cytocentrifuged preparation (50 and 200μL of abdominal
fluid, depending on visual density of the sample) were air dried and stained with
Wright-Giemsa (HEMA-TEK modified Wright-Giemsa stain pack, Bayer,
Germany) and evaluated microscopically by a clinical pathologist. Culture and
sensitivity were performed by an independent laboratory (Vetpath, Perth,
Western Australia) on samples with a high neutrophil percentage (>70%),
presence of micro-organisms observed visually on microscopy and/or high
clinical suspicion of infection.

An aliquot of each abdominal effusion sample was spun at 3000rpm (1500 G) in
a Jouan CR3i multifunction centrifuge (Thermo Fisher Scientific, USA) at 4°C for
10 minutes, the supernatant placed in a 1ml plastic tube and the sediment
discarded. The supernatant was kept at 4°C and analysed for lactate
dehydrogenase (LDH) activity within 24 hours of sample submission. LDH was
measured on each sample using three different methods. Firstly LDH was
measured via two wet chemistry (traditional) methods using the pyruvate to
lactate (LDH P-L) and lactate to pyruvate reactions (LDH L-P) using a Randox
Daytona biochemistry analyser (Northern Ireland, UK) and two commercial
Randox reagents (catalogue numbers 3818 and 3842, respectively). If results
obtained from samples were out of analytic range, the samples were diluted 1 in
20 in distilled water (50μL of sample in 950μL of distilled water) and the
analysis repeated.
LDH activity was measured using dry-slide technology on a VetTest® Chemistry analyser (IDEXX laboratories, USA). This test was run on undiluted samples if the wet chemistry results did not require dilution, or the diluted sample if dilution had been required for the Randox Daytona analyser.

Bland-Altman plots, regression analysis and one-way analysis of variance (ANOVA) functions in Excel 2011 for Mac (Microsoft, USA) and StatPlus:mac LE (v5.9.20, AnalystSoft Inc) were used to determine agreement and statistical differences between different methods of LDH measurement. The final clinical diagnosis and outcome were recorded for each horse, noting whether it survived (>1 month) or succumbed to the condition causing colic. Independent sample t-tests were used to compare LDH activity between horses that died due to sepsis or neoplasia and those that survived following treatment with or without surgery (mostly with non-septic inflammation or mechanical intestinal obstruction). Receiver operating characteristic (ROC) curves for determining cut-off values between survival and death were produced using Medcalc (Version 12.3.0.0, MedCalc Software, Mariakerke, Belgium).

3. Results

LDH activity was measured in the abdominal fluid of 27 horses with colic, 19 of which survived and 8 that died or were euthanased due to abdominal sepsis or neoplasia (Table 1).

LDH activity was significantly higher in the latter group (Randox wet LDH L-P: p=0.039; Randox wet LDH P-L: p=0.040; IDEXX dry LDH P-L: p=0.039) (figure 1).
The different methods of testing LDH activity provided significantly different results from each other (ANOVA, \( p=0.028 \)), however, there was good agreement between the different methods (figure 1). The ratio of Randox wet LDH L-P:Randox wet LDH P-L:IDEXX dry LDH P-L was almost 1:2:4 from all samples.

LDH was significantly increased using all three methods of LDH measurement in horses that died compared to those that survived \((p<0.05\), figure 2\). There was no significant difference in LDH activity between colic horses that survived without surgery \((n=11)\) compared to the 8 horses that survived but where surgery was considered necessary \((p=0.37-0.52\) for all three methods of measuring LDH activity) or between horses that died due to neoplasia \((n=3)\) or sepsis \((n=5, p>0.71)\).

A cut-off value for all methods was attempted using ROC curves to aid in determining prognosis for horses with colic in our study population based on LDH activity (Table 2).

Lactate concentration was also measured in abdominal effusions from 11 horses, 10 of which recovered and 1 that died with a septic peritonitis. There was moderate correlation of lactate concentration with wet LDH L-P and wet LDH P-L activity \((r = 0.73)\) and good correlation with IDEXX dry chemistry LDH P-L \((r=0.86)\).

4. Discussion

In this small study, we found that LDH activity in abdominal effusions may provide information regarding the prognosis in horses with colic, and may be useful for identifying sepsis or neoplasia, although further comparison with
abdominal fluid lactic acid levels is required to check for its added value both from a practical and scientific point of view. Very high LDH activity was usually associated with sepsis (due to intestinal leakage or rupture) or advanced intra-abdominal neoplasia, with all but one septic/neoplastic effusion (1 of 9) resulting in death or euthanasia, and the higher the activity the more likely it was that sepsis or neoplasia was present.

Three methods of LDH measurement were compared in this study, and they resulted in significantly different values, although they did follow a similar trend. The cut-off values mentioned in this study depend on the test method used. It is therefore essential to know which methods for measuring LDH activity was used and to use one method consistently. For practitioners an in-house dry chemistry test may be more relevant (the IDEXX dry chemistry is an in-house method of rapid analysis), though larger laboratories usually use the wet chemistry (traditional) analysis. LDH activity can be measured on samples immediately after centrifugation to remove particulate matter. Only two analysers and three test kits were used in this study, and further investigation should be done into other available test kits and analysers.

We found moderate to good correlation between LDH activity and lactate concentration in abdominal fluid from horses with colic. A disadvantage of lactate is the lability of the molecule, therefore its concentration must be measured soon after sample collection for accurate results (point-of-care devices are available) [14]. We found LDH to be stable for up to 3-7 days in a refrigerator, as reported by others [9, 10], which allows a little more flexibility between sample collection and testing. Measuring LDH activity may therefore be
a useful adjunct to routine laboratory and in-house analysis of effusion fluid, clinical signs and effusion lactate concentration [15, 16], especially where an on-site lactate meter is not available, or possibly to evaluate retrospectively how a condition has progressed, though more investigation of ongoing disease is required for this.

The reason for the high proportion of horses with gastro-intestinal rupture and neoplasia in this group may be that they were drawn largely from a referral population. One of the shortcomings of this study is that there are few if any horses with uncomplicated colic that could be relatively easily treated. In addition, a relatively small number of samples were tested and further investigation of LDH activity in abdominal fluid in horses with both complicated and uncomplicated colic would be useful.

Another limitation of this study is the absence of a normal control population of equine abdominal fluid. A previous study found that lactate dehydrogenase activity in the peritoneal fluid of 20 normal horses was 143.0 ± 106.1 IU/L [17], though surprisingly the method of LDH measurement was not mentioned. In our study of 27 colic horses we calculated a mean of >550 (wet LDH L-P), >1000 (wet LDH P-L) or >2500 (dry chemistry LDH P-L) IU/L LDH with 3 methods. Even the lowest mean value is twice as high as LDH previously reported in peritoneal effusions of healthy horses [17].

The effect of hemolysis and blood contamination on LDH concentration were not analysed in this study, however, cytological analysis of the equine effusions in
the non-survivors did not show PCVs or RBC content significantly different to the survivors. Effects of hemolysis could be further investigated in larger study.

To our knowledge this is the first time that LDH activity in abdominal fluid from horses with colic has been evaluated to differentiate between horses with colic that survived the disease process for which they were admitted to the hospital and those that did not survive (usually due to abdominal sepsis or neoplasia).

LDH activity in this population was not useful for differentiating between whether horses required surgery or not (although LDH activity may be an indicator of ischemia in humans [12]), nor between abdominal sepsis versus neoplasia. LDH activity might therefore be a helpful test to indicate septic peritonitis and neoplasia, whereas lactic acid is considered an important indicator to assess bowel ischemia, though more research is needed.

5. Conclusions

LDH activity in abdominal effusion fluid may provide additional useful prognostic information in horses with colic, with high values suggesting possible neoplasia or sepsis as a cause for colic. LDH activity could not be used to determine whether surgery was required or not and could not be used to distinguish between sepsis and neoplasia as in both cases large number of LDH containing, dead cells/bacteria are present in the effusion. LDH results varied depending on the method of measurement used, therefore use of a consistent method is highly recommended. There was moderate to good correlation between lactate concentration and LDH activity in abdominal effusions from colic horses and, although more research is needed, LDH activity might be an
interesting method to indicate septic peritonitis and neoplasia, whereas lactic acid is an important indicator to assess intestinal ischemia [16, 18]. LDH might have potential to function as an add on prognostic indicator in colic horses, however, further comparison with peritoneal fluid lactic acid levels is required to check for its added value both from a practical and scientific point of view.

Acknowledgements

Thanks to the MUVH equine clinical staff and the clinical pathology laboratory staff for their help and support, and to Murdoch University who provided the funding to make this project possible.

References


<table>
<thead>
<tr>
<th>Final Diagnosis</th>
<th>TCC (x10^9/L)</th>
<th>TP (g/L)</th>
<th>PCV</th>
<th>Lactate (mmol/L)</th>
<th>Wet LDH L-P (IU/L)</th>
<th>Wet LDH P-L (IU/L)</th>
<th>Dry LDH P-L (IU/L)</th>
<th>Survival &gt;1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Colic, non-surgical</td>
<td>1.2</td>
<td>28</td>
<td>&lt;0.01</td>
<td>1.15</td>
<td>124</td>
<td>220</td>
<td>660</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>2 Colic, non-surgical</td>
<td>2.1</td>
<td>&lt;25</td>
<td>&lt;0.03</td>
<td>ND</td>
<td>147</td>
<td>252</td>
<td>532</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>3 Colic, non-surgical</td>
<td>2.7</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>71</td>
<td>129</td>
<td>528</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>4 Diarrhoea, hepatic fibrosis</td>
<td>0.9</td>
<td>32</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>132</td>
<td>267</td>
<td>571</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>5 Mild abdominal inflammation</td>
<td>1.4</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>35</td>
<td>66</td>
<td>ND</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>6 Peritonitis (no bacterial growth)</td>
<td>4.2</td>
<td>31</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>188</td>
<td>377</td>
<td>619</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>7 Peritonitis (no bacterial growth)</td>
<td>4.9</td>
<td>31</td>
<td>0.01</td>
<td>ND</td>
<td>197</td>
<td>392</td>
<td>601</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>8 Peritonitis (no bacterial growth)</td>
<td>3.2</td>
<td>30</td>
<td>0.01</td>
<td>ND</td>
<td>231</td>
<td>452</td>
<td>701</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>9 Septic peritonitis</td>
<td>8.5</td>
<td>35</td>
<td>0.02</td>
<td>3.6</td>
<td>281</td>
<td>497</td>
<td>771</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>10 Uterine haematoma/inflammation</td>
<td>39.1</td>
<td>39</td>
<td>0.01</td>
<td>ND</td>
<td>279</td>
<td>542</td>
<td>877</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>11 Colic, non-surgical, abdominal inflammation</td>
<td>60.2</td>
<td>41</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>1080</td>
<td>2815</td>
<td>6490</td>
<td>Survived without surgery</td>
</tr>
<tr>
<td>12 Cecal impaction, non-strangulating 180 degree cecal torsion</td>
<td>0.61</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>3</td>
<td>118</td>
<td>227</td>
<td>179</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>13 Colic (torsion, viable)</td>
<td>0.4</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>3.3</td>
<td>178</td>
<td>338</td>
<td>664</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>14 Colic strangulated colon, viable</td>
<td>2.6</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>5.1</td>
<td>264</td>
<td>476</td>
<td>899</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>15 Colon volvulus (black, viable)</td>
<td>0.2</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>2.1</td>
<td>153</td>
<td>281</td>
<td>ND</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>16 Small colon feed impaction and a subacute grade 1 rectal tear</td>
<td>1.1</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>3.3</td>
<td>315</td>
<td>557</td>
<td>962</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>17 Splenic entrapment colic, no necrosis</td>
<td>1.1</td>
<td>26</td>
<td>&lt;0.01</td>
<td>2.7</td>
<td>349</td>
<td>674</td>
<td>1092</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>18 Thromboembolic colic, ischemia of the large colon, resection</td>
<td>0.9</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>1.6</td>
<td>117</td>
<td>215</td>
<td>653</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>19 Uroperitoneum</td>
<td>0.1</td>
<td>&lt;25</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>14</td>
<td>35</td>
<td>281</td>
<td>Survived with surgery</td>
</tr>
<tr>
<td>20 Duodenal rupture and sepsis</td>
<td>0.3</td>
<td>ND</td>
<td>0.03</td>
<td>13.6</td>
<td>416</td>
<td>773</td>
<td>2000</td>
<td>Died</td>
</tr>
<tr>
<td>21 Gastric squamous cell carcinoma</td>
<td>41.6</td>
<td>65</td>
<td>0.01</td>
<td>ND</td>
<td>2800</td>
<td>5380</td>
<td>15340</td>
<td>Died</td>
</tr>
<tr>
<td>22 Gastric squamous cell carcinoma</td>
<td>26.1</td>
<td>41</td>
<td>0.01</td>
<td>ND</td>
<td>371</td>
<td>658</td>
<td>995</td>
<td>Died</td>
</tr>
<tr>
<td>23 GIT necrosis and sepsis</td>
<td>0.65</td>
<td>54</td>
<td>0.04</td>
<td>ND</td>
<td>573</td>
<td>1092</td>
<td>1553</td>
<td>Died</td>
</tr>
<tr>
<td>24 GIT rupture and sepsis</td>
<td>31</td>
<td>35</td>
<td>0.02</td>
<td>ND</td>
<td>258</td>
<td>479</td>
<td>815</td>
<td>Died</td>
</tr>
<tr>
<td>25 Hepatic squamous cell carcinoma</td>
<td>32.3</td>
<td>33</td>
<td>0.01</td>
<td>ND</td>
<td>287</td>
<td>539</td>
<td>910</td>
<td>Died</td>
</tr>
<tr>
<td>26 Septic peritonitis</td>
<td>65.8</td>
<td>46</td>
<td>0.01</td>
<td>ND</td>
<td>1920</td>
<td>3380</td>
<td>11060</td>
<td>Died</td>
</tr>
<tr>
<td>27 Septic peritonitis (Actinobacillus sp.)</td>
<td>500</td>
<td>42</td>
<td>&lt;0.01</td>
<td>ND</td>
<td>4920</td>
<td>8360</td>
<td>12300</td>
<td>Died</td>
</tr>
</tbody>
</table>

Table 1: Table of 27 effusions from horses presenting with colic. TCC = total nucleated cell count, TP = total protein measured by refractometry, PCV = packed cell volume, GIT = gastro-intestinal tract, ND = not done
TABLE 2: TABLE SHOWING CUT OFF VALUES OF LDH ACTIVITY IN ABDOMINAL EFFUSIONS ABOVE WHICH THE PROGNOSIS IS POOR FOR SURVIVAL OF COLIC HORSES IN THIS STUDY

<table>
<thead>
<tr>
<th>Methods of measurement</th>
<th>LDH activity cut off</th>
<th>Survival likely</th>
<th>Survival unlikely</th>
<th>Accuracy/area under curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randox Wet</td>
<td>280 IU/L</td>
<td>Sensitivity 84%, Specificity 87%</td>
<td>Sensitivity 87%, Specificity 94%</td>
<td>0.85</td>
</tr>
<tr>
<td>LDH L-P</td>
<td>500 IU/L</td>
<td>Sensitivity 79%, Specificity 87%</td>
<td>Sensitivity 87%, Specificity 94%</td>
<td>0.81</td>
</tr>
<tr>
<td>IDEXX Dry</td>
<td>900 IU/L</td>
<td>Sensitivity 82%, Specificity 87%</td>
<td>Sensitivity 87%, Specificity 93%</td>
<td>0.84</td>
</tr>
</tbody>
</table>
(b) Wet LDH L-P vs Dry LDH P-L

\[ y = 0.6036x - 17.705 \]
\[ r = 0.97 \ (p<0.01) \]

(a) Wet LDH L-P vs Wet LDH P-L

\[ y = 0.2792x + 34.531 \]
\[ r = 0.98 \ (p<0.01) \]

(c) Wet LDH P-L vs Dry LDH P-L

\[ y = 0.3777x - 60.381 \]
\[ r = 0.91 \ (p<0.01) \]
FIGURE 1: BLAND AND ALTMAN PLOTS OF THE DATA OBTAINED FROM 27 EQUINE ABDOMINAL EFFUSION SAMPLES ANALYSED COMPARING (A) WET LDH L-P TO WET LDH P-L (B) WET LDH L-P TO DRY LDH P-L AND (C) WET LDH P-L TO DRY CHEMISTRY LDH P-L
FIGURE 2: COMPARISON OF LDH ACTIVITY MEANS (±SE) IN ABDOMINAL EFFUSIONS FROM HORSES WITH NON-SURGICAL AND SURGICAL COLIC THAT SURVIVED AND THOSE THAT DIED WITH COLIC SIGNS AND EFFUSIONS ASSOCIATED WITH SEPSIS OR NEOPLASIA THAT DIED, MEASURED BY 3 DIFFERENT METHODS
Highlights
LDH activity in abdominal fluid may aid in determining prognosis in horses with colic. It has moderate to good correlation with lactate concentration and is stable in refrigerated samples for at least 3 days. Different methods of measuring LDH activity give markedly different values, and method of evaluation must be known and consistent to provide useful information. Marked increases in LDH activity in abdominal fluid are most consistent with a poor prognosis due to advanced abdominal neoplasia and sepsis.