Biochemical and Pathological profiles in streptococci infection in imported horses

By

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SUMMARY

The present work is dealing with a field problem arise from introducing a new transported horses to farm. The horses were suffered from respiratory manifestations and fever associated with death of some horses. Out of twenty two imported horses were diseased, fifteen horses recovered after 5 weeks of treatments (12 adult + 3 foals), three horses were incompletely recovered and 4 horses died during the same period. In the quarantine, Microbial, biochemical as well as histopathological investigations were carried out to diagnosis the causative agent responsible for this problem.

Streptococcus equi was the main cause of this problem in the infected horses. Several biochemical changes associated with different symptomatic stages together with clinical observations either in sick, treated and cure or unresponsive to the treatment horses were recorded. There were statistical significant increase of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Total protein, urea, uric acid and creatinine in diseased horses. In treated horses, ALP was statistically increased where both LDH and urea were significantly decreased. ALT, ALP, LDH, urea, uric acid and creatinine remained significantly increased in unresponsive to the treatment lived and dead horses.

The predominant histopathological lesions in the lung were hyperplasia of bronchial epithelium, atlectasis of some alveoli, emphysema of others with rupture of some alveolar wall. The bronchial lymph node showed lymphocytic depletion, subcapsular edema, thickening in the wall of the blood vessels and severe hemorrhage with hemosedrosis. It was concluded that the biochemical profile associated with development and trajectory and clinical consequences of streptococci infections in horses was powerful tools and effectives. It is useful tools to detect efficiency of information’s obtained to follow up, dissection and itemization during the courses of streptococci infections.
INTRODUCTION

Strangles is one of the most widespread horses diseases world wide (May et al., 2004). It's caused by bacteria Streptococcus equi (S. equi. subspecies equi) (Bailey et al., 2003). Mixed infections could be happened (Galant and Timoney, 1988; Carol et al., 1978 and Timoney, 2004). In reverse climatic conditions the morbidity rate reached 100% with mortality rate ranged from 0 to 10% (Timoney et al., 2008). An intermittent shedding period of S. equi is up to 15 weeks (Gron back et al., 2006). Normally it is not disease to humans as or other domestic species (Equine facts, 2006). Virulence of the S. equi is believed to be as a result of M like protein (Sponsiller et al., 2005.)

The Arabian horses (Equidi Arabia) are famous among horses for beauty, due to transportation and shipping process, either through overseas or due to airplanes, veterinary Quarantine and Food scheme changes, all these stress factors are predisposing to develop the typical signs of strangles as respiratory profile manifestations. Unsteady gate and lameness may be observed in sporadic cases. Milinovich et al., (2006) suggested that streptococci within the streptococcus bovis/sequines complex may be involved in the serious of events which precede the onset of laminitis in horses.

Streptococcus equi, pneumonia is danger to adult and young horses (Rose et al., 1981). Brain abscesses as a manifestations of strangles were diagnose (Spoormakers et al., 2003), Also meningitis (Elsayed et al., 2003 and Pepe-scru et al., 2006), genital infections and mastitis (Thomas et al., 1989). It is normally not present on the mucosa until 24 to 48 h after the onset of fever (Galant and Timoney, 1988). The antibodies protect against group C streptococcus but in horses correlated poorly with protection against S. equi (Marshall et al., 1981 and Timoney and Eggers, 1985). Several antibiotics are used in treatment of diseased horses (Thomas et al., 2006).

The present work aimed to: 1. Isolate the bacterial microorganism causes this problem. 2. Follow up and identify the biochemical profiles either in diseased, treated and un-responded to the treatment horses. 3. Determine the pathological changes in dead animals.
MATERIAL AND METHODS

22 imported horses from Germany were under Veterinary Guaranty control (Cairo airport). It reserved in closed cages in authorized military academy. Respiratory manifestations were observed with nasal discharge and rise in temperature reach to 39.5°C. The horses received several symptomatic treatments including antibiotic, with antipyretic drugs. With treatment, all cases return to its normal pattern with case settlements, except that four horses died. The horses set under hygiene condition received food and water in programmed order.

1. Animals:

The animals were classified to 5 groups. A: Control horses-normal adult horses (number of horses 10) from associated healthy horses. Group B: Diseased horses before start of the symptomatic treatment (number of horses equal 22). Group C: Treated horses—the horses that received treatment and followed up (number of horses equal 15). Group D: Treated horse with no response of the treatment (number of horses equal 4). Group E: Treated for long period at the end of the treatment (number of horses equal 3).

2. Samples:

Blood smears, blood and fecal samples and tissue specimens from lungs and the bronchial lymph nodes were taken. Blood samples were withdrawal from both normal and symptomatic horses (Group A, B), with treatment start, weekly blood samples were taken for 3 weeks (Group C, D and E).

3. Isolation and Identification:

The swabs the diseased horses were inoculated into to add-Hewitt broth overnight at 37°C, they were streaked onto sheep blood agar and Edward’s media for 24 hours at 37°C, the suspected β haemoltic were examined for gram staining and biochemical identification according to Quinn et al. (2002). The isolate were serogically identified by using slide strepto kit (Biomerieux).

4. Extraction of S. equi M. like protein:

S. equi isolates, according to Galan and Timoney (1987a, b and 1988) were grown overnight in THB and centrifuged at 8000 rpm/15 min then washed twice with 20 mM phosphate buffer (pH 7.0). Cells were suspended in 3 ml of 0.5 M phosphate buffer containing 10 mg of ml and 0.5 M sucrose, and incubated at 37°C/ 1 hour. The mixture centrifuged at 9000 rpm and supernatant contain M. like protein were saved.

5. Enzyme linked immunosorbent assay (ELISA):

Detection of S. equi anti-sera in horse serum were against M like
protein (Voller et al., 1978 and Wallace et al., 1995). Coating the immuno-plates was made by 100 µl / well with M. like protein in buffer carbonate. Blocking was preformed by bovine serum albumin. Adding the serum samples (diluted with B.S.A.). Conjugation with peroxidase labeled conjugate. Washing after each one of the previous steps, the adding the ABTS peroxidase substrate then read the heamoplate in Elisa reader at 405 nm and the present were recorded. Positive results are indicated by positive control in the test.

6. Biochemical analysis:
The collected blood samples were subdivided into three parts: 1-2 ml was mixed with sodium fluoride and further separated to get plasma where glucose was estimated. 1-2 ml of blood used directly for ammonia determination. The reminder of blood sample was let to complete clot, serum obtained for further biochemical variables.

Biochemical parameters were estimated according to the methods described in the following references including LDH, (E.D.G. K.C, 1972); ALP, (Belfield and Goldberg, 1971); AST and ALT, (Reitman and Frankel, 1957), total protein, (Kato and Physik, 1960) urea, (Hallett and Cook, 1971); creatinine, (Bartels, 1972); and uric acid, (Caraway, 1955).

Hyaluronidase (Joyce and Mack, 1986); acid phosphatase, (Babson and Read, 1959); glucose, (Tinder, 1969); albumin, (Drupt, 1974); lactic acid, (Neville and Gelder, 1971) and ammonia; (Fenton, 1962).

6. Statistical analysis: The student T-test were used to compare between the group A and different groups according to Snedecor and Cochran (1980) and Farver, (1989) using SPSS statistical computer based programs version 3.1.

7. Post-mortem examination:
Careful gross examination of the lungs and bronchial lymph nodes of the freshly dead horse was carried out and gross lesions were recorded.

8. Histopathological examination:
The collected specimens from the lungs and bronchial lymph nodes of the freshly dead horses were fixed in 10% neutral buffered formalin solution. The specimens were dehydrated in different graded of ethyl alcohol, cleared by xylene, embedded in paraffin wax, sectioned at 4-5 µ and routinely stained with Hematoxylin and Eosin stain. The stained slides were examined microscopically and the histopathological lesions were recorded (Bancroft and Stevens, 1996).
RESULTS

1. Bacteriological result:

Twenty two isolates from horses (stallions, mares and foals) (table 1) which were suspected strangles were identified as B. haemolyte streptococci. The isolates identified by using culture, biochemical testes and slidex strepto kit as lancifield group C. (Streptococcus equi subspecies equi). Morbidity and mortality rate as shown in table 1. The mortality rate in stallion was 13.64%, mares were 4.6% but in foals was zero. The result of ELISA test confirmed the traditional culture method of the 18 cases (live cases) but with different optical density reading.

2. Biochemical results

No statistical differences, in biochemical over view were observed according to sex or age between, stallion, mares and foals. The main difference was associated between healthy horses and diseased horses before start of the symptomatic treatment, horses received treatment, treated horse with no response of the treatment and treated for long period at the end of the treatment.

Tables 2 showed high statistical significant increase on most measured parameter in diseased horses (group B) (P<0.001). In treated horses (group C), ALP was significantly increased (P<0.05) where both LDH and urea were significant statistical decreased (P<0.05) and (P<0.001). ALT, ALP, LDH, urea, uric acid and creatinine were statistically significantly increased in last recorded parameter in dead horses (group D). ALT, AST, ALP, urea and creatinine were significantly increased in non-respond to treatment (group E) compared to control horses (group A) (P<0.001) and (P<0.05).

Statistical significant increase in hyaluronidase and acid phosphatase activity and lactic acid (lactate), albumin, globulin and ammonia in diseased horses (group B) (P<0.001) with exception of glucose. In treated horses (group C), hyaluronidase and acid phosphatase remained significantly increased (P<0.001), although glucose level decreased but not significantly decreased, lactic acid level was significantly increased (P<0.001), albumin was significantly increased (P<0.05), globulin level was significantly decreased (P<0.001) and ammonia level was significantly increased compared with control group (group A) (P<0.001).

Hyaluronidase and acid phosphatase activity were significantly increased (P<0.001), glucose was not changed, lactic acid level was significantly increased (P<0.001), albumin level was significantly increased (P<0.001), globulin level
was significantly decreased (P<0.001) and ammonia level was significantly increased (P<0.01) (group D) compared with control (group A).

Hyaluronidase and acid phosphatase level were significantly increased (P<0.001), glucose and albumin level were not changed statistically, two fold increase in lactic acid level (P<0.001), globulin was significantly increased (P<0.001) and ammonia level was significantly increased in non-responde to treatment (group E) compared to control horses (group A) (P<0.001).

3. Pathological results:

Macroscopically: Most of the examined lungs and bronchial lymph nodes of the four dead horses were congested and enlarged, some parts of the lungs appeared dark red in color. In two cases the pleura covered the lung was thick and cloudy. An abscess in the lung was observed in one case.

Microscopically: The lungs revealed congestion of Bronchial blood vessels and preialveolar capillaries accompanied with areas of Hemorrhages (Fig. 1). The bronchial epithelium showed hyperplasia of its epithelial lining forming figure like projection in the lumen (Fig. 2), some cases showed peri-bronchial edema (Fig. 3). The alveoli were filled with transudate and some alveoli showed emphysema with rupture of alveolar wall (Fig. 4). Moreover the bronchial lymph node showed sub-capsular edema (Fig. 5). Some cases showed hemorrhage and haemosedrosis (Fig. 6).

List of figures:

Fig. (1): Lung of horse showing congestion of peri-alveolar capillaries with transudate in lumen of alveoli. H&E stain. X 400.

Fig. (2): Lung of horse showing hyperplasia of bronchial epithelium with rupture of wall of some alveoli. H&E stain. X 100.

Fig. (3): Lung of horse showing pre-bronchial edema. H&E stain. X 250.

Fig. (4): Lymph node of horse showing lymphocytic depletion. H&E stain. X 250.

Fig. (5): Lymph node of horse showing subcapsular edema. H&E stain. X 250.

Fig. (6): Lymph node of horse showing severe hemorrhage and haemosedrosis. H&E stain X 400.
Table 1: Morbidity and Mortality rates of S. equi infected horses:

<table>
<thead>
<tr>
<th>Total N of Animals</th>
<th>Statues</th>
<th>No of infected animals</th>
<th>Stallion</th>
<th>Mares</th>
<th>Foals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases</td>
<td>Rate(%)</td>
<td>Cases</td>
<td>Rate (%)</td>
</tr>
<tr>
<td>18</td>
<td>Diseased</td>
<td>4</td>
<td>22.23</td>
<td>10</td>
<td>55.56</td>
</tr>
<tr>
<td>4</td>
<td>Died</td>
<td>3</td>
<td>75.00</td>
<td>1</td>
<td>25.00</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>7</td>
<td>31.82</td>
<td>11</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Table 2: Biochemical parameters in serum of diseased, treated and control horses.

<table>
<thead>
<tr>
<th>Item</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>LDH (IU/L)</th>
<th>Total Protein (gm/L)</th>
<th>Urea (mg/dl)</th>
<th>Uric Acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>143.54 ± 12.09</td>
<td>226.98 ± 19.52</td>
<td>23.43 ± 5.15</td>
<td>162.43 ± 15.91</td>
<td>52.00 ± 3.42</td>
<td>0.90 ± 0.06</td>
<td>1.10 ± 0.08</td>
<td>1.27 ± 0.04</td>
</tr>
<tr>
<td>Group B</td>
<td>300.12 ± 29.11</td>
<td>400.77 ± 30.17</td>
<td>35.11 ± 2.73</td>
<td>212.54 ± 25.87</td>
<td>79.65 ± 4.32</td>
<td>1.83 ± 0.18</td>
<td>2.67 ± 0.20</td>
<td>1.54 ± 0.12</td>
</tr>
<tr>
<td>Group C</td>
<td>134.34 ± 11.82</td>
<td>220.76 ± 4.48</td>
<td>30.23 ± 4.99</td>
<td>145.56 ± 7.54</td>
<td>54.98 ± 3.62</td>
<td>0.70 ± 0.08</td>
<td>1.08 ± 0.07</td>
<td>1.23 ± 0.04</td>
</tr>
<tr>
<td>Group D</td>
<td>215.65 ± 26.34</td>
<td>250.56 ± 22.04</td>
<td>45.12 ± 3.89</td>
<td>225.65 ± 10.45</td>
<td>55.80 ± 2.55</td>
<td>1.35 ± 0.08</td>
<td>1.54 ± 0.07</td>
<td>1.65 ± 0.16</td>
</tr>
<tr>
<td>Group E</td>
<td>220.56 ± 17.42</td>
<td>370.34 ± 32.56</td>
<td>36.45 ± 5.25</td>
<td>200.76 ± 22.57</td>
<td>56.98 ± 3.37</td>
<td>1.25 ± 0.09</td>
<td>1.35 ± 0.18</td>
<td>1.54 ± 0.05</td>
</tr>
</tbody>
</table>

(N) = number of horses per group. Mean ± standard Error.
*, **, *** significant at different level of probability P< 0.05, 0.01 and 0.001.
Table 2 continued: Biochemical parameters in serum of diseased treated and control horses.

<table>
<thead>
<tr>
<th>Item</th>
<th>Hyaluronidase (mol NAG/min/l)</th>
<th>Glucose (mg/dl)</th>
<th>Lactic acid (lactate) (.mg/l)</th>
<th>Albumin (Gm/L)</th>
<th>Globulin (Gm/L)</th>
<th>Acid phosphatase (U/L)</th>
<th>Ammonia (.μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (10)</td>
<td>43.54±5.09</td>
<td>96.56±19.52</td>
<td>50.33±2.16</td>
<td>38.43±5.15</td>
<td>13.57±0.74</td>
<td>5.00±0.16</td>
<td>11.88±0.52</td>
</tr>
<tr>
<td>Group B (22)</td>
<td>57.12±9.11</td>
<td>115.77±30.17</td>
<td>65.45±2.81</td>
<td>51.11±2.73</td>
<td>28.54±1.56</td>
<td>8.65±0.28</td>
<td>15.96±2.41</td>
</tr>
<tr>
<td>Group C (15)</td>
<td>58.34±7.82</td>
<td>88.76±4.48</td>
<td>75.40±3.24</td>
<td>45.23±4.99</td>
<td>9.57±0.52</td>
<td>5.98±0.19</td>
<td>18.34±1.06</td>
</tr>
<tr>
<td>Group D (4)</td>
<td>66.65±6.34</td>
<td>100.56±22.04</td>
<td>110.34±4.74</td>
<td>48.12±3.89</td>
<td>7.68±0.42</td>
<td>5.80±0.19</td>
<td>18.56±2.92</td>
</tr>
<tr>
<td>Group E (3)</td>
<td>67.56±7.42</td>
<td>99.34±32.56</td>
<td>114.55±6.21</td>
<td>30.45±5.33</td>
<td>26.53±1.49</td>
<td>6.80±0.22</td>
<td>17.56±3.32</td>
</tr>
</tbody>
</table>

(N) = number of horses per group. Mean ± standard Error.
*, **, *** significant at different level of probability P< 0.05, 0.01 and 0.001.
DISCUSSION

Transportation stress as predisposing factor to develop streptococci infection was not documented here in Egypt. *S. equi* subspecies *equi* is a patent horse pathogenic bacteria causing a strangles (*Lannergurd et al.*, 2003). It is highly host-adapted to Equidae (*Timoney, 1993*). Also, it may be of zoonotic importance and cause disease in human (*El-Sayed et al.*, 2003).

Respiratory symptoms and further fever were pointed (*Wannamaker and Matsen, 1972*). Our results agreed with *Timoney et al.*, (1997) who said that *S. equi* subspecies *equi* a lancifield group’s C streptococcus has anti-phagocytic molecular M like protein which is a major virulence and protective antigen.

In the present article, ELISA used to detect antibodies against strangles because it is so fast and sensitive, also many authors recommend Elisa in horse to determine serum titers of antibiotic to *S. Equi* M like protein (*Heath et al.*, 1991). *Galan and Timoney*, (1988) recorded that M protein of *S. equi* known to be important in stimulating mucosal nasopharyngeal immune response. Our result, therefore confirm that M protein of *S. equi*, is recorded.

Our result in 4 out of 22 death were recorded agreed with *Sweeney et al.*, (1987) who recorded that complications associated with *S. Equi* infections developed and death was attributed to respiratory lesions as pneumonia.

In the farm, the clinical cases showed that it could not differentiate those that are found streptococci to be belong can be (C group) to further classified into group four other species (*Duma et al.*, 1969). However, vaccines based on acid or mutanolysin extracted M protein do not confer a high level of protection against field exposure (*Timoney and Mukhtar, 1993*).

Our data showed that the clinical signs in the diseased horses lasted approximately 45 days, 21 days in other cases; this observation was agreed with (*Dalgleish et al.*, 1993 and *Jonsson et al.*, 1995).

*S. equi* is antigenically homogeneous (*Moore and Bryans 1970*). Virulence factors of *S. equi* include a non-antigenic Hyaluronic acid capsule, hyaluronidase, streptolysin S, streptokinase (*Caballero et al.*, 1999), IgG Fc-receptor proteins, pyrogenic exotoxins, including SePE-1 and H, peptidoglycan, and the anti-phagocytic M- protein (SeM) and a leukocidal toxin, (*Lämmler and Sting, 1989*), 16-18 kd molecular weight ranges protein (*Groschup et al.*, 1990), Hemolysins (*Valentin-Weigand*).
et al., 1988) and proteases (Straus et al., 1980). The virulence of streptococci is considered as a multi-factorial process (Segura and Gottschalk, 2004). This multi-virulence reflect the elevations of serum hyalurnidase as observed in sick horses which seems to be permanent even with treatment and recovered horses.

From the diseased horse’s isolated S. equi, from nasal shedding which begins after a latent period from 4-14 days and ceases between 3-6 weeks after the acute phase, thus explain the sudden and progressive onset of respiratory symptoms in the flock and spreading as fire (Schroeder et al., 1999).

The present data of sick imported horses (table 2) revealed significant increase of ALT, AST, ALP and LDH activity which are consistence with observations of Timoney, (2004). The present data insured the effect of streptokinase (McCay et al., 1991), rather than the effect of streptococci itself (Ofek et al., 1972). In the present paper, the elevations of total protein, albumin, globulin, urea, uric acid and creatinie in diseased horses (group B) revealed the positive effect of streptococci or/and its toxin (M protein or/and streptokinase) on the protein metabolism by it increasing its turn over time. Streptococci are capably of highly specific interactions with proteins (Goran et al., 1982).

The horse albumin showed lower binding activity, the average bacterial cell carries more than 80,000 binding sites (Kronvall et al., 1979, increase a specificity for albumin binding as shown by transmission electron microscopy (Willcox et al., 1993). Streptokinase secreted by Streptococcus equi, it catalyzes the conversion of plasma zymogen, plasminogen to the serine protease plasmin, which subsequently can degrade fibrin, the primary protein component of blood clots (McCay et al., 1991).

There was difference between streptokinase-plasminogen binding (not species-specific) and activation (species-specific) (Nowicki et al., 1994). These complexes may responsible for glomerulonephritis. Bacterin and M protein (SeMe), (M.W. 58.000), (Goran et al., 1982) and in acid extract (41,000-42,000),(Timoney and Eggers, 1985), which was used as epidemiologic determined (Anzai et al, 2005) has antiphagocytic function. The M protein, a super-antigen, elicits very strong B and T cell responses (Timoney and Mukhtar, 1993). Productions of S. equi cytotoxin may be an inducer for more albumin productions; (Longland et al., 1999). In our opinion, This factor can not removed completely, by two reasons, our finding of elevations of lactate level in diseased imported
horses which may due to cecal and colonic bacteria metabolism leading the productions of lactic acid and second Fructans (the oligosaccharides present in grass) (the storage form of carbohydrate), which it is variations level in grasses under climatic conditions, do not appear to be digested by the small intestine.

Binding of host plasma proteins to the surface of the whole organism could be an effective mode of concealment form host cellular recognition mechanisms (Benatey et al., 1986). The high elevations of globulin level may due to M protein (SeM) which has anti-phagocytic property indicated to phagocytes depletions and/or and inefficiently phagocytosed (Raeder et al., 1991). This phenomenon was shown between streptococci and immunoglobulin subclasses IgG1, IgG2 IgG4, IgA2 and IgM2 (Goran et al., 1979).

The present data documented Hyaluronidase activity in this group A, important virulence factors of S. equi (Timoney and Mukhtar, 1993). Certain strains has capsular Hyaluronic acid (Timoney et al., 1982) that resulted in greater phagocytosis of these strains, in addition some strains may be more resistance to intercellular killing than other (Limbert et al., 1991). Increase level hyaluronidase in the present study, indicated the virulence of the infected strain. On the other hand, the data can not insure the source of this enzyme activity either from the body or from the infected srtrain. Virulent isolates of S. equi are almost always highly encapsulated, increase level of Hyaluronic is associated sever to reduce phagocytosis and release of hyaluronidase and fibrinolysin may be of value in tissue penetrations and dissemination (Milinovich et al., 2006).

In diseased horses, statistical significant increase in the lactic acid (lactate) level may due to increase in the concentrations of lactobacilli, streptococci, and lactate-utilizing bacteria in stomach and hind gut accompanied with L-lactate accumulation with increase average concentration of NH3 (Van Eps and Pollitt, 2006 and Varloud et al., 2007). Fermentations of excess carbohydrates were associated with increases in number of streptococci and lactobacilli (2 to 5 log unit) increase (Hussein et al., 2004), with increasing lactic acid, volatile fatty acids, ammonia concentrations, and pH (Medina et al., 2002), which reflect this parameter elevation in serum. Changes in feeding system (Milinovich et al., 2006), also associated with increase serum lactate,NH3 N and urea (Garner et al., 1975), the present data suggested that conceded streptococci cause either direct through the mi-
cograms or/and indirect through it is toxin. All previous led changes in blood flow to the digit and therefore associated with lameness (Deibel and Seely, 1974), with the sustains vasoconstriction (Weaver et al., 2005), concomitant with the severe metabolic crisis (Pollitt and Davies, 1998).

In treated horses (group C), due to statistical problems including; uses different wide variety of antibiotic, low number of individual-drug association’s cases. Wide Variety drugs were observed to be highly effective against beta-haemolytic streptococci like Penicillin, Ampicillin and Cephalothin (Sonea, 1987; Ensink et al., 1996; Moore et al., 1995 and Ensink et al., 2003); erythromycin, chloramphenicol or gentamicin and penicillin, (Higgins et al., 1984) and Cefquinome, (Limbert et al., 1991) rifampin (Kohn et al., 1993), ciprofloxacin, grepafloxacin, moxifloxacin, sparflxacin, (Johnson et al., 1999): benzylpenicillin, ampicillin, oxacillin, (Trolldenier et al., 2000); cefotaxime, trimethoprim /sulfadiazine (TMP/SDZ) (Ensink et al., 2003) and cefpodoxime (Carrillo et al., 2005).

Although the percentages of dead animals reach to (18.18%), which is huge but this observations within the normal percent in imported horses as previously seen by Dalgleish et al., (1993). Two explanations of the appearance of the presence of dead horses which reach to 4 horses (18.18 %) of the imported flock, either failure of immuno system in protection and recovered after acute infections (Varloud et al., 2007), or due to strptococci complications to transformed what called "Bastured strangles" where the lymphatic horse infected with streptococci and further infected other organs (Womble et al., 2007). The biochemical finding was associated with postmortem pathological finding of presence of abscesses pressed on lung tissue on one case. This observable fact was associated with pronounced biochemical profile where ALT, ALP, LDH enzymes, urea, uric acid and creatinine were statistically significant increase in last pre-mortem few days recorded parameter of serum of last week of dead horses (group D). The only recorded point that the decreased level of globulin protein instead of its increase, no exact explanation to this observation except that, the circulated antibodies in horses correlated poorly with protections against S. equi (Timoney and Eggers, 1985), protections against strangles mainly is correlated with the presence of mucosal nasopharyngeal immunoglobulin G and immunoglobulin A antibodies.

The live horse (non-respond
to treatment group (group E) observed during the course of the treatment. The two explanations obtained that wide variety of antimicrobial agents which was active against streptococci equi in vitro, many of these drugs are ineffective in vivo (Hondalus and Mosser, 1994) and/or presence of secondary invasions from other pathogen (Wood et al., 1993); presence post strangles respiratory aspirates yielded a potential microbial pathogen (Crane et al., 1989), Nutritionally variant streptococci (Da Silva et al., 1990), presence of S. zooepidemicus (Hoffman et al., 1993), and/or present of this in dormant stage (Dalgleish et al., 1993).

The present data concerning (group E)(table 2) showed elevation of most of parameters studied with exceptions glucose and albumin level accompanied with two fold increase in lactic acid (lactate) level. This may due to harbor infection on in their guttural pouch (Traore et al., 1991), or binding of host plasma proteins to the surface of the whole organism (Valentin et al., 1990 and Traore et al., 1991) might also block access of C3 or specific antibody to target sites (Nowicki et al., 1994). Furthermore, the shedding of S. equi occurred both from horses with and without clinical signs (Gronbaek et al., 2006).

Binding sites have been described for human serum albumin (Myhre and Kronvall., 1980), fibrinogen (Kronvall et al., 1979a), haptoglobin (Kohler and Prokop, 1978), and aggregated β2-microglobulin and immunoglobulin (Kronvall et al., 1979b,c). Non-immune forms of biological affinities have been described as "short-circuit" reactions (Timohey, 2004). The inability of PMN to phagocytosis and kill the streptococci appears to be due to combinations of the hyaluronic acid capsule, antiphagocytic M protein, and a leukocidal toxin released by organism Benatey et al., (1986); Gursharan et al., (1990); Schroeder et al., (1999); King and Phillips (2001) and Albihn et al., (2003).

Streptococcus equi induces pneumonia in foals (Hoffman et al., 1993) and was responsible for the severe pathological changes developed in lungs of the dead horses which were characterized by pleurities, hyperplasia of the bronchial and alveolar epithelial cells. These results were also concluded by Lakritz et al. (1993).

The present data revealed that the pulmonary tissues were edematous where the alveoli were filled with transudates and hemorrhages. Taha et al., (2007) recorded that pulmonary edema was a common finding within collected pneumatic lung specimens. In these cases oedema was associated with
vascular dilatations and hemorrhages that varied from excessive extra-vascular accumulation of pale eosinophilic, homogenous fluid extending to the pulmonary intera-lobular interstitial within the alveolar and bronchial lumen.

**Hala et al.,** (2001) revealed that, in streptococcus infection, the pulmonary tissue showed inflammatory exudates and hemorrhage, and bronchial epithelium was showed hyperplastic proliferations in some parts. Bacterial causation produce exudates in which both the granulocytes and fibrin predominate. Moreover Focal lymphocytic aggregations and fibrous tissue proliferation were seen. These results were more or less similar to these recorded by **Hala et al.** (2001) and **Taha et al.** (2007).

A strong evidence that *Streptococcus equi* subspecies zoo epidermicus is implicated as a causal factor in ESFP (equine shipping fever pneumonia) (**Oshikawa et al.,** 2003), it grows in the mucous exudates and pulmonary effusions further, the bacteria showed resistance against phagocytosis by pulmonary alveolar macrophages (PAM) and neutrophils. Inhibition of PAM and neutrophils function is considered to be important in the development of pneumonia. With the progression of the diseases, the neutrophils often adhered to the endothelial surface at the alveolar capillary lumen and played a role in generating coagulation necrosis of lung tissues. The bronchial lymph nodes showed edema, lymphocytic depletion, congestion, thickening of the wall of the blood vessels and hemosedrosis. These lesions agreed with **Hala et al.** (2001) and **Taha et al.** (2007).

**Conclusion:**

- Absence of latex test to reach the propionate diagnosis in less time consuming in the horses farm filed. This problem lead to symptomatic treatment which mostly lead to long time, cost money and less efficient and improper treatment.
- Hyaluronidase measurement may be useful and valuable tools in diagnosis, course, direction and sequence of the disease, but need further assessment and evaluation. The biochemical profile associated with different clinical consequences indicated the effectives and efficiency of information’s obtained in follow up and dissection and itemization, we can use during the courses of streptococci infections.
- Our data is not recommended that the imported horse must revaccinate with local vaccine and raise the immunity of diseased horses by regular chick of immunoglobulin level against strangles and other related mi-
croorganism and use bactericidal serum and regular chick up of local vaccine.
• Preventive measures should therefore included segregation and daily observation of new arrivals for at three weeks before they mixed with the resident populations, prompt isolations of affected animals, daily measurements of rectal temperature of intact horses so that they may be segregated immediately if pyrexia is noticed.

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الصور البيوكيمياية والباثولوجيا في حالة الإصابة بالاستريتوكوكى في الخيول

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الختانق في الخيل يشكل خطرا على الخيول مع تطور الحالة إلى الالتهاب. taille الحالات محل الدراسة من نقل خيول مستوردة إلى المزرعة مع ظهور أعراض تنفسية، وحمى وبداء في علاج الآثار وانتهت بوفاة بعض الخيالات. أثناء وعثير من الخيول المستوردة منها خمس عشرة خيل شفيت أسابيع من العلاج ( + المهراس الكبير) وبقيت ثلاثة خيول لم تشفى بالكامل و من الخيول نفت خلال الفترة ذاتها. وتم اخذ العينات من الخيول المعالجة، والمرضية والمنتهية وأجريت التحاليل البيوكيميائية والكيميائية الخيولة والباثولوجيا لتشخيص السبب والمسؤول عن هذه المشكلة.

تم الوصول إلى أن بكتيريا S equi هو السبب الرئيسي لهذه المشكلة في الخيول المصابة. العديد من التغييرات البيوكيميائية المرتبطة بختلف مراحل الأعراض مع ملاحظات سوء الحالات المرضية، والعلاج أو الغير مستجيب للمعالجة. ارتفاع زيادة احصائية كبيرة في LDH، AST، LDH والبوريا وحمض اليوريك والكيراتينات سجل في الخيول المرضية. ورأت كل من البوريا انخفاضا كبيرا.

هناك هناك زيادة كبيرة في نشاط كل من phosphatase واللاكتات ولزلا البيض، والأمونيا، العضيات في الخيول المرضية باستثناء الجلوكوز. وفي الخيول التي تستجيب للعلاج الإشعاعي وجد انخفاض في LDH وانزيم المصب حyaluronidase. وانزيم عدم مستوى الألبومين والأمونيا، وانخفاض مستوى الجلوكوز. اما نشاط انزيمي phosphatase، والجلوكوز Hyaluronidase.

هو لأتغير وتمستاحل حمض اللبنيك زيادة كبيرة في حالات لزلا البيض، انخفاضات كبيرة في انزيم الامام، ومع انخفاض مستوى النسيج في النبضات، ونظام الامام، وانزيم phosphatase، والجلوكوز Hyaluronidase، مع اثنين من ضعف الزيادة في مستوى اللاكتات في حالة عدم الاستجابة للعلاج الخيل.

سجلت نتفاخ في نسبه الزرة فضلا عن نزيف التضخم الكمي بالقصبات
والالتهاب القصبيات، الامتلاك الخلاوي كما تسللت مع خلايا التهابية. الشعب الهوائية وأظهر العقد المفاوية وتسلل خلايا أحادي النواة.

من نتائج البحث نستنتج أن التغيرات الكيميائية الحيوية المرتبطة بظهور ومسار ونتائج الأعراض للالتهابات العقديه في الخيل تعتبر واضحة الدلالات ومرتبطة وأنها كانت تشير إلى تطور كفاءة المعلومات التي تم الحصول عليها من أجل المتابعة المرضية والعلاجية وتمكننا استخدامها أثناء فترة المتابعة السريريه لالتهابات العقديه.

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