Clinicopathological findings of canine distemper virus infection in dogs

M Buragohain, S Goswami and DJ Kalita

Abstract
The present study was undertaken during the period from March, 2016 to February, 2017 in Guwahati, Assam, with the objective of early diagnosis of canine distemper infection on the basis of clinical findings collaborating with haematological and biochemical alterations. The prominent clinical signs were respiratory distress, purulent oculo-nasal discharge, biphasic fever, gastroenteritis, hyperkeratosis of the digital pads and various nervous disorders. Commonly noticed nervous disorders were partial to generalized seizure, chorea, paddling and cycling movement, increase salivation, ataxia, muscle tremor and paralysis. The haematological parameters measured include total erythrocyte count, haemoglobin concentration, packed cell volume, total leucocyte count, platelets count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. The mean total erythrocyte count (4.57 ± 0.23 x10^6/µl), haemoglobin concentration (8.87 ± 0.52 g/dl), packed cell volume (26.72 ± 1.41%); platelets count (114.38 ± 9.26 x 10^3/µl) and mean corpuscular volume (54.17 ± 1.51fl) were found lower than the standard range in all canine distemper infected dogs. These findings, suggested that canine distemper results anaemia with immunosuppression in affected dogs. Biochemical analysis of serum samples revealed increase level of alkaline phosphatase and aspartate aminotransferase, whereas the alanine aminotransferase level remains same.

Keywords: Canine distemper, clinical signs, haematology, biochemical analysis

1. Introduction
Canine distemper (CD) is a highly infectious viral disease of dogs which cause mortality in young population of dogs. CD is a worldwide occurring infectious disease of dogs, belongs to the family paramyxoviridae and genus morbillivirus [1]. Canine distemper virus (CDV) infection is characterised by a systemic and nervous clinical course and viral persistence in selected organs including the central nervous system and lymphoid tissue. Main clinical manifestation comprising of respiratory disorders, gastroenteritis signs and demyelinating leukoencephalomyelitis [2]. Following aerosol infection the virus replicates in the macrophages and lymphoid cells of the upper respiratory tract [3]. Systemic dissemination of the virus is mediated by infected cells, such as lymphocytes, monocytes, platelets and/or occurs through non-cell-associated virus, leading to infection of various organs [4]. Canine distemper virus is a pan tropic virus which led immunosuppression [5]. However, many dogs do not exhibit classical clinical presentation and infected with other pathogens that are responsible for similar signs, rendering CD diagnosis difficult [6]. Some routine laboratorial tests may provide some useful clinical information which can be used in diagnosis of the disease [7]. Lymphopaenia, neutrophilia with elevation of liver and muscle enzymes (aspartate aminotransferase, alanine aminotransferase), blood urea nitrogen and creatinine were consistent clinical findings of canine distemper [8].

As the disease produces wide range of clinical signs, it brings confusion at the time of diagnosis of the disease. Therefore, this study was aimed to diagnose the disease at the early stage on the basis of clinical findings collaborating with haematological and biochemical alterations.

2. Material and Methods
A total of 167 clinically suspected dogs were examined during the study period from March, 2016 to February, 2017. Dogs with the history of fever, seizure, watery or purulent oculo-nasal discharge and gastrointestinal along with nervous disorders were considered as suspected cases of CD for the present study.

References:
[1] Canine distemper virus (CDV) infection is characterised by a systemic and nervous clinical course and viral persistence in selected organs including the central nervous system and lymphoid tissue.
[2] Following aerosol infection the virus replicates in the macrophages and lymphoid cells of the upper respiratory tract.
[3] Systemic dissemination of the virus is mediated by infected cells, such as lymphocytes, monocytes, platelets and/or occurs through non-cell-associated virus, leading to infection of various organs.
[4] Canine distemper virus is a pantropic virus which led immunosuppression.
[5] However, many dogs do not exhibit classical clinical presentation and infected with other pathogens that are responsible for similar signs, rendering CD diagnosis difficult.
[6] Some routine laboratorial tests may provide some useful clinical information which can be used in diagnosis of the disease.
[7] Lymphopaenia, neutrophilia with elevation of liver and muscle enzymes (aspartate aminotransferase, alanine aminotransferase), blood urea nitrogen and creatinine were consistent clinical findings of canine distemper.
[8] As the disease produces wide range of clinical signs, it brings confusion at the time of diagnosis of the disease. Therefore, this study was aimed to diagnose the disease at the early stage on the basis of clinical findings collaborating with haematological and biochemical alterations.

Keywords: Canine distemper, clinical signs, haematology, biochemical analysis
2.1 Haematological examination
About 3 ml of blood was collected from the suspected animals by puncturing cephalic and/or saphenous vein in a vacutainer. Blood samples were immediately brought to the laboratory for routine haematological examination with automated haematological cell counter (Melet Schloesing Lab., MS4e model) [6].

2.2 Biochemical analysis
The serum was collected in a separate eppendorf tube by centrifuged at 3000 rpm speed for 15 mins. The collected serum was stored at -20°C for biochemical analysis by using spectrophotometer as per standard protocol given by manufactured company along with the kits. The kits were provided by Span Diagnostic Ltd. and Coral Diagnostic Limited [9].

3. Statistical analysis
Statistical analysis of haematological and biochemical data was performed using IBM SPSS Statistics 20 software.

4. Results and Discussion
Clinical findings recorded in this study, were respiratory distress characterized by pneumonia, coughing, laboured breathing and severe mucopurulent ocular and nasal discharge. This reports correlate with the findings of previous workers [10, 11, 12, 13]. High rise of body temperature from 102.8-105°F, anorexia, lethargy, dehydration and weight loss were noticed in all the animals at the initial phase of the disease. Hyperkeratosis of the foot pads was observed in animals with the history of chronic CD which was hard to touch and when pressure was applied on it, animal showed the signs of pain. Previous workers also reported similar findings [14, 15]. Various neurological signs included partial to generalized seizure, chorea, ataxia, chewing gum fits, paresis and paralysis. Nervous signs were also described by earlier workers [16, 17, 18, 19] and explained that CDV affect both the grey and white matter of CNS which might be the cause of neurological disorders [20].

4.1 Clinical pathology
4.1.1 Hematological studies
The total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV), platelets count (THR) and mean corpuscular volume (MCV) was found lower than the standard range (Table 1). Earlier workers opined that, persistence of the virus in the bone marrow causes erythroid hypoplasia, results haematological alterations [21]. The consequence of viral persistence in bone marrow has been reported in previous study [22]. CDV infection releases interleukin-6 which causes sequestrating of iron into a less available form, thus iron is not available to the developing reticuloocytes [23]. Other possible causes of decrease TEC in canine distemper infected dogs as recorded in this study might be the production of inflammatory mediators, which could inhibit erythropoesis and also shorten the life span of the RBC [21, 22]. Decrease PCV observed in this study can be correlated with the decreased TEC. The haemoglobin value in the CD affected animals was found to be decreased (8.87 ± 0.52 g/dl) compared to the normal standard range (12-17.8 g/dl) which correlates with previous findings [24]. The reduced platelets count (114.38 ± 9.26 x 10^3/µl) indicating thrombocytopenia occurs as a result of bone marrow depression by the virus [22]. However, few workers observed platelets count within the normal range in CD affected animals [6]. The mean corpuscular volume (54.17 ± 1.51fl) was found to be considerably lower than the normal range (60-77 fl). The mean corpuscular haemoglobin (20.66 ± 1.33pg) and mean corpuscular haemoglobin concentration (31.06 ± 1.06 g/dl) level falls within the normal range. However, they are towards the lower side of normal value. Decreased PCV, MCH and MCHC were reported by earlier workers in CD positive cases [24]. The present study revealed that the TEC, haemoglobin and MCV values were considerably reduced, indicating that the disease produced microcytic hypochromic anaemia. The total leukocyte count was found (16.51 ± 1.34 x 10^9/µl) within the normal range. However, different workers reported leukopaenia in CD affected animals [8, 21, 24]. The absolute lymphocyte count recorded was 1.60 ± 1.26 x10³/µl which are towards the lower value of the normal range. In contrast, many earlier workers have reported lymphocytopenia in CD infected animals [8, 24, 25].

### Table 1: Hematological parameters of canine distemper affected dogs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± S.E.</th>
<th>Normal range [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEC (10^9/µl)</td>
<td>4.57 ± 0.23</td>
<td>6 - 9 (6.8)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>26.72 ± 1.41</td>
<td>37 - 54 (45)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.87 ± 0.52</td>
<td>12 - 18 (15)</td>
</tr>
<tr>
<td>THR (10^9/µl)</td>
<td>114.38 ± 9.26</td>
<td>120 - 600</td>
</tr>
<tr>
<td>TLC (10^9/µl)</td>
<td>16.51 ± 1.34</td>
<td>6 - 20</td>
</tr>
<tr>
<td>Lymphocytes (10^3/µl)</td>
<td>1.60 ± 1.26</td>
<td>0.6 - 5.1</td>
</tr>
<tr>
<td>MCV(µl)</td>
<td>54.17 ± 1.51</td>
<td>60 - 77 (70)</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.66 ± 1.33</td>
<td>20 - 25 (23)</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.06 ± 1.06</td>
<td>31 - 34 (33)</td>
</tr>
</tbody>
</table>

4.1.2 Biochemical studies

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Mean ± S.E.</th>
<th>Normal range [27]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>20.79 ± 2.51U/L</td>
<td>10-109 U/L</td>
</tr>
<tr>
<td>AST</td>
<td>29.78 ± 2.84U/L</td>
<td>13-15 U/L</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>363.81 ± 2.74 U/L</td>
<td>1-114 U/L</td>
</tr>
</tbody>
</table>

The alanine aminotransferase (ALT) level in serum (20.79 ± 2.51U/L) was found within the normal range (10-109 U/L) whereas, increase level of serum aspartate aminotransferase (AST) (29.78 ± 2.84 U/L) and alkaline phosphatase (363.81 ± 2.74 U/L) were recorded in all infected animals (Table 2). These findings were agreed with earlier finding [28]. However, increased level of ALT was reported by other workers and opined it is non-specific, they also reported that rise of AST level in the blood were consistent findings [6]. The alkaline phosphates value in the CD affected animals was found to be much higher than the normal range (1-114 U/L). ALP is located in the tip of the villi of enterocytes and in gastrointestinal disorders increases its concentration in blood [29]. Previous workers also viewed those serum ALP level increases during different intestinal mucosal diseases [30,31]. CD causes gastrointestinal disorder [2,12] as also recorded in our present study that might be explained for higher level of ALP found in this study.

5. Conclusions
Clinical signs such as respiratory distress, mucopurulent ocular and nasal discharge, fever, gastroenteritis, hyperkeratosis and nervous disorders are common clinical manifestations of the disease. The mean TEC, PCV, Hb, THR and MCV were found reduced with normal MCH and MCHC value. These indicate microcytic hypochromic anaemia.
Estimation of serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of the CDV infected animals revealed increased level while alanine aminotransferase (ALT) remained unaltered in all the infected animals.

6. Acknowledgement

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7. References