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# URINALYSIS AND GROSS PATHOLOGY OF NIGERIAN DOGS INFECTED WITH LOCAL ISOLATES OF THE *CANINE DISTEMPER VIRUS*

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***ABSTRACT***

***Clinical differentiation of canine distemper (CD) from other tropical diseases of dogs is difficult because of the many forms it presents. In search of additional features of CD, to aid accurate tentative diagnosis, urinalysis and gross pathology of Nigerian dogs infected with local isolates of the canine distemper virus (CDV) were studied. A group of five dogs was infected with CDV isolated from dogs that died of outbreaks of CD in Nigeria. A second group of same breeds and of same age was used as controls. From the first day of second phase of fever, urinalysis was done daily for each dog for five days. The gross pathology of 19 dogs that died following experimental infections with the local isolates of CDV was also studied. Protein, red blood cells, ascorbic acid and sediments (tube casts, RBCs and WBCs) were detected in urine of the dogs infected with the local isolates of CDV. The most consistent gross pathology/lessions of CD in Nigerian dogs infected with local CDV isolates were enteritis (78.9 %), gastritis (73.7 %), pneumonia (68.4 %) and nephritis (57.9 %). A lesion not yet reported for CD, zebra marks-like haemorrhages in the large intestines, was observed in two of the 19 dogs (10. 5 %).***

**Key words:** Canine distemper virus, Nigerian isolates, dogs, urinalysis, gross pathology

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## INTRODUCTION

Canine disemper (CD) is a viral disease of dogs and of wild canids caused by a *Morbillivirus.* It affects many body systems of infected animals including the nervous, respiratory, gastrointestinal and integumentary systems [1]. Clinical signs of the disease include nervous signs, undulating fever, anorexia, conjuctivitis, photophobia, cloudiness of the eyes, congestion of oral and ocular mucous membranes and ocular discharges [ 2, 3, 4] . Hagan [1] and Heller *et al* [5] reported that *Canine distemper virus* parasitizes lymphnodes and bone marrows of infected dogs. Consequently, lymphoadenopathy and anaemia are also features of canine distemper [6].

Most of these clinical signs of CD are also features of other tropical diseases of dogs such as trypanosomosis, parvovirus enteritis, infectious canine hepatitis, leptospirosis, lead poisoning and rabies

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[7]. Canine trypanosomosis is prevalent in Nigeria. The clinical signs common to CD and to canine trypanosomosis include undulating fever, anorexia, photophobia, conjuctivitis, enlargement of peripheral lymph nodes, anaemia, occular discharges, corneal opacity and nervous signs [8,9]. Similarity of these clinical signs make it difficult to clinically differentiate the diseases.

To overcome this difficulty in differentiating CD from other diseases, it has been suggested that full blood count, serum biochemistry and urinalysis be carried out on suspected cases. Already, determination of blood coagulation time and assessment of heamatology of cases of CD have been reported to aid tentative diagnosis of the disease in Nigerian dogs [10].

This paper reports results of urinalysis and of gross pathology of Nigerian dogs infected with Nigerian isolates of the *canine distemper virus*.

**MATERIALS AND METHODS**

Ten dogs of Nigerian local mongrel breed were used for the experiment on urinalysis. They were acclimatized for one week during which they were treated with penicilline, streptomycine and ivermectin to elliminate bacterial and parasitic infections. To ensure they were free of canine distemper infection, their sera were tested by haemagglutination – inhibition (HI) test with PPR vaccine as antigen [11] as already reported [12]. Only dogs that tested HI negative for *CDV* antibodies were used for experimental infection in the study.

Canine distemper virus was isolated in chorioallantoic membrane of chick embryos by inoculating embryonated eggs with cerebrospinal fluid of dogs that died from confirmed outbreaks of CD in Nsukka, Nigeria. Five of the experimental dogs were infected with the CDV while another five dogs of the same age and breed were used as control. Infection of the dogs was achieved by instilling 0.5 ml of the local isolate of CDV with $EID_{50}$ of $10^5$ into their nostrils. Following the observation of clinical signs, including fever, in the experimental dogs, their serum were collected for confirmation of canine distemper by HI test [12].

Urine was collected from all dogs in the infected and uninfected group by catheterization and massaging the urinary bladder early in the morning. The urine collection was done daily for five days, starting from the day second phase of fever was observed in the infected dogs, to mimick history often presented to clinicians. Each urine sample was tested within 30 minutes of collection by the qualitative dipstick method. Parameters assessed in the urinalysis included, colour, turbidity, biochemical constituents (billirubin, protein, ascorbic acid, glucose, nitrites and urobilinogen) as well as presence of blood. To assess urine for presence of sediments, urine samples were centrifuged at 3000 revolutions per minute for five minutes and the sediments obtained were observed under the low power objective of the microscope ( × 10) for presence of tube casts, crystals, red blood cells (RBC), white blood cells (WBC) and bacteria.

Information on the gross pathology of CD in Nigerian dogs were obtained from observations on 19 dogs that died following infection with local isolates of CDV in earlier studies [10, 13, 14]. The dead dogs were subjected to standard necropsy and observations were recorded. Frequency of occurrence of each lession was calculated and presented as percentages.

**RESULTS**

The urine of the CDV – infected dogs changed from amber colour to yellow while that of the uninfected controls remained amber throughout the study (Table 1). Red blood cells (blood) was observed in the urine of the CDV – infected dogs on varied days from onset of fever but no red blood cells were detected in the urine of the uninfected controls (Table 2). Protein and ascorbic acid were detected in varying levels in the urine of the infected dogs but these were not detected in the urine of the uninfected group (Tables 3

and 4). Renal casts, triphosphate crystals and other pathologically important sediments were observed in the urine of the infected dogs but not in the controls (Table 5).

Table 1: Colour and turbidity of urine of dogs infected with a Nigerian isolate of the c*anine distemper virus*.

| Dogs/Status | | Days from onset of second phase of fever | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| A | Infected | Lgt Amb/Cdy | Dead | | | |
| B | Infected | Lgt Amb/Cdy | Lgt Amb/Cdy | Dead | | |
| C | Infected | Amb/Tsp | Amb/Tsp | Lgt Yel/Cdy | Lgt Yel/Cdy | Dead |
| D | Infected | Amb/Tsp | Amb/Tsp | Amb/Tsp | Yel/Cdy | Yel/Cdy |
| E | Infected | Amb/Tsp | Amb/Tsp | Amb/Tsp | Lgt Yel/Cdy | Yel/Cdy |
| F | Uninfected | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp |
| G | Uninfected | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp |
| H | Uninfected | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp |
| I | Uninfected | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp |
| J | Uninfected | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp | Amb/Tsp |

Key: Amb = Amber; Lgt Amb = Light amber; Yel = Yellow; Lgt Yel = Light yellow; Cdy = Cloudy; Tsp = Transparent.

Table 2: Presence of red blood cells (erythrocytes/µl) in urine of dogs infected with a Nigerian isolate of the *canine distemper virus*.

| Dogs/Status | | Days from onset of second phase of fever | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| A | Infected | 100 | Dead | | | |
| B | Infected | 100 | 0 | Dead | | |
| C | Infected | 0 | 0 | 0 | 5 – 10 | Dead |
| D | Infected | 0 | 0 | 0 | 0 | 250 |
| E | Infected | 0 | 0 | 0 | 0 | 250 |
| F | Uninfected | 0 | 0 | 0 | 0 | 0 |
| G | Uninfected | 0 | 0 | 0 | 0 | 0 |
| H | Uninfected | 0 | 0 | 0 | 0 | 0 |
| I | Uninfected | 0 | 0 | 0 | 0 | 0 |
| J | Uninfected | 0 | 0 | 0 | 0 | 0 |

Observations at necropsy of the CDV – infected dogs showed the following general patterns of distribution of lessions: Pustules on the lower abdomen and on the inner thigh (36.8%), congestion or haemorrhages on the liver (31.7%), pneumonia (68.4%), enteritis (78.9%), tracheitis (26.3%), gastritis (73.7%) and nephritis (57.9%). Also, streaks of haemorrhages ("zebra marks") were seen in the large intestines of two of the dogs (10.5%) (Table 6).

Table 3: Protien (mg/dl) in urine of dogs infected with a Nigerian isolate of the *canine distemper virus*.

| Dogs/Status | | Days from onset of second phase of fever | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| A | Infected | 100 | Dead | | | |
| B | Infected | 100 | 100 | Dead | | |
| C | Infected | 30 | 100 | 100 | 100 | Dead |
| D | Infected | 30 | 30 | 30 | 30 | 100 |
| E | Infected | 0 | 30 | 100 | 100 | 100 |
| F | Uninfected | 0 | 0 | 0 | 0 | 0 |
| G | Uninfected | 0 | 0 | 0 | 0 | 0 |
| H | Uninfected | 0 | 0 | 0 | 0 | 0 |
| I | Uninfected | 0 | 0 | 0 | 0 | 0 |
| J | Uninfected | 0 | 0 | 0 | 0 | 0 |

**DISCUSSION**

It was observed that once the urine changed colour to yellow and became cloudy with proteinuria, haematuria and ascorbic acid in urine of dogs infected with *CDV*, they died within 24 to 48 hours. This finding could be useful in prognosis. Cole [15] reported that proteinuria occurs in cases of glomerular nephriris. This is due to increased permeability of the renal basement membrane [16]. So, proteinuria may not be a specific lesion of canine distemper but is indicative of nephritis.

Table 4: Ascorbic acid (mg/dl) in urine of dogs infected with a Nigerian isolate of the *Canine distemper virus*

| Dogs | | Days from onset of second phase of fever | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| A | Infected | 20 | Dead | | | |
| B | Infected | 20 | 20 | Dead | | |
| C | Infected | 10 | 20 | 20 | 20 | Dead |
| D | Infected | 10 | 10 | 20 | 10 | 20 |
| E | Infected | 0 | 10 | 20 | 20 | 20 |
| F | Uninfected | 0 | 0 | 0 | 0 | 0 |
| G | Uninfected | 0 | 0 | 0 | 0 | 0 |
| H | Uninfected | 0 | 0 | 0 | 0 | 0 |
| I | Uninfected | 0 | 0 | 0 | 0 | 0 |
| J | Uninfected | 0 | 0 | 0 | 0 | 0 |

The proteinuria recorded for the infected dogs correlated with the nephritis recorded for 57.9 % of the dogs that died at post mortem. Both the observation of proteinuria in urinalysis and the nephritis seen at post mortem of dogs infected with local isolates of *canine distemper virus* agree with report of Cantile *et al* [17] that CD causes renal pathology. Willard [18] also reported that presence of renal casts, leucocytes and red blood cells in urine is evidence of congestion, inflamation or degeneration of the renal tubules. Detection of these sediments in urinalysis can also be used to diagnose nephritis in dogs. Haematuria,

albuminuria and presence of hyaline casts in urine have also been reported as signs of pathology of the kidney [15, 19]. Detection of these clinical pathologies in the dogs is a further confirmation of damaged kidneys in the infected dogs.

The observed loss of vitamine C and protein in urine in the infected dogs in this study is worthy of note. The losses may account for the reported success and relief achieved in CDV – infected dogs treated with high doses of vitamine C and immunoglobulin [20]. The treatment thus served as replacement for the vitamine and globuluin lost through urine.

The pustules observed on the under belly and on the inner thigh, hepatitis, pneumonia, enteritis, tracheitis, gastritis, and nephritis seen in the infected dogs are in agreement with reports of other authors as lesions of CD in other breeds of dogs [21, 22, 23 ]. However, the "zebra marks" pattern of haemorrhage observed in the large intestines of 10.5% of Nigerian dogs infected with *Canine distemper virus* appears to be an uncommon lesion of the disease. It is however worthy of note that "zebra mark" lesion in the intestine has been reported in Rinderpest of cattle and Peste des Petits Ruminants (PPR) of sheep and goats [24, 25]. The observation that this lession also occurs in canine distemper buttresses the fact that *Rinderpest virus*, *PPR virus* and *Canine distemper virus* are closely related viruses [26].

Table 5: Sediments in urine of dogs infected with a Nigerian isolate of the *Canine distemper virus.*

| Dogs/Status | | Sediments |
|---|---|---|
| A | Infected | Triphosphate crystals, RBCs (+), WBCs (+++), epithelial cells and tube cast |
| B | Infected | Triphosphate crystals, RBCs (+++), WBCs (++), epithelial cells and tube cast |
| C | Infected | Triphosphate crystals, RBCs (+), WBCs (+++), epithelial cells and tube cast |
| D | Infected | Triphosphate crystals, RBCs (+), WBCs (+++), epithelial cells and tube cast |
| E | Infected | Triphosphate crystals, RBCs (+), amorphous crystals, calcium oxalate crystals and tube casts |
| F | Uninfected | WBC (+) |
| G | Uninfected | WBC (+) |
| H | Uninfected | WBC (+) |
| I | Uninfected | None |
| J | Uninfected | WBC (+) |

Key: + = slight; ++ = moderate and +++ = severe

Table 6: The frequency of distribution of lesions in Nigerian local mongrel dogs infected with local isolate of the *Canine distemper virus*.

| Lesions | Frequency (%) |
|---|---|
| Enteritis | 78.9 |
| Gastritis | 73.7 |
| Pneumonia | 68.4 |
| Nephritis | 57.9 |
| Pustules on abdomen/inner thigh | 36.8 |
| Hepatitis | 31.6 |
| Tracheitis | 26.3 |
| Zebra marks – like haemorrhages in large intestine | 10.5 |

Nephritis as suggested by urinalysis and confirmed at post mortem and the observation of pustules on the skin, pneumonia, gastritis and enteritis are indications that the dogs suffered a disease that affected the urinary, integumentary, respiratory and digestive systems. Fracer [7], reported that a disease of dogs that affects many body systems at the same time is likely to be canine distemper. So, analysis of urine of sick dogs and necropsy of dead ones could be added to observation of clinical signs and haematology, to assist clinicians differentiate tentative diagnosis of canine distemper from other tropical diseases that have similar clinical signs.

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