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BLOOD COAGULATION TIME AS A POSSIBLE TOOL FOR THE CLINICAL DIFFERENTIATION OF CANINE DISTEMPER AND TRYPANOSOMOSIS

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ABSTRACT

Blood coagulation time was used for the clinical diagnosis and differentiation of canine distemper virus and Trypanosoma brucei brucei infections in dogs. The coagulation time of blood of the infected dogs was determined by the glass slide method. Following experimental infection, the mean blood coagulation time of the canine distemper infected group (1.4 ± 0.7 min) varied significantly (P < 0.05) from those of the Trypanosoma brucei brucei infected (6.16 ± 0.96 min) as well as from those of the uninfected control (4.8±0.7 min) dogs. In naturally infected dogs, the mean coagulation time of the canine distemper virus infected dogs (1.7±0.95 min) was also significantly (P < 0.05) shorter than those of trypanosome infected (6.83 ± 1.69) and apparently healthy (4.96± 1.31 min) dogs. The results therefore suggest that blood coagulation time on glass slides may be a useful tool for the tentative diagnosis and differentiation of canine distemper from canine trypanosomosis in places where the two diseases are endemic. However, there is need for more elaborate studies to establish the cut off point of blood coagulation time for the two diseases.

Key words: Blood, coagulation time, canine distemper, trypanosomosis.

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INTRODUCTION

Classical cases of canine distemper are characterized by diphasic fever, leukopaenia, gastroenteritis, respiratory disorder and at times, nervous signs [1]. Such cases are easy to recognize clinically. However, early cases of canine distemper manifest clinical signs which are not characteristic. These include, fever, anorexia, conjuctivitis, ocular discharges, photophobia and cloudiness of the eyes [2].

Canine distemper virus multiplies in the lymph nodes of infected dogs before getting into the blood stream [3]. Consequently, lymphoadenopathy, including enlargement of peripheral lymphnodes is often a feature of the disease [4]. Canine trypanosomosis is also characterized by biphasic fever, corneal opacity, enlargment of the peripheral lymphnodes, ocular discharges and at times facial oedema [5, 6]. Therefore, early cases of canine distemper present clinical features that are similar to those of canine trypanosomosis which may confuse the clinical differentiation of the two disease in endemic areas like Nigeria.

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Furthermore, in the tropics where the two diseases are endemic, there is need for a simple and rapid technique for the clinical differentiation of the two diseases. This will assist clinicians collect appropriate specimens and make correct requests for laboratory tests for the diagnosis of the two diseases in sick dogs.

MATERIALS AND METHODS

Experimental infections

Fifteen puppies of both sexes, aged 4 – 5 months, were used for the experimental infections. The puppies were housed in fly-proof cages in the Experimental Animals Unit of the Department of Veterinary Medicine, fed on house hold diet with water provided *ad libitum*. The animals were observed for one week before the experiments during which period they were treated with levamisole (10 mg/kg) and Oxytetracycline LA (7 mg/kg) to eliminate any existing, inapparent helminth and bacterial infections respectively. They were randomly assigned to three groups of five puppies each. Puppies in group I were infected with *Canine distemper virus* by intranasal innoculation of each with 0.5ml of a local isolate of *CDV* [Effective Infective $Dose_{50}$ (EID_{50}) = 10^5]. Group II puppies were infected by intraperitoneal innoculation of 1 ml of blood containing $1x10^6$ *T. brucei* [7]. Puppies in group III served as uninfected control. The establishment of CDV and *T. brucei* infections were respectively confirmed using heamagglutination-inhibiton test [8] and microscopic examination of buffy coat [9, 10]. The dogs in all groups were bled through the cephalic vein for glass slide whole blood coagulation time on day 21, post infection, when clinical manifestations of their respective infections have fully established.

Natural infections

Seventy-five dogs of different ages and sexes that visited the University of Nigeria Veterinary Teaching Hospital (VTH), Nsukka for various reasons between February and July 2000 were used for the second study. Dogs showing clinical signs such as fever, corneal opacity, anorexia, lymphadenopathy and photophobia that are common to canine distemper and canine trypanosmosis were randomly selected for inclusion in the study. Twenty-five dogs confirmed by the haemagglutination – inhibition test (HIT) to be infected with CDV [8] were used as group I while another set of 25 dogs confirmed by the microscopic examination of buffy coats to be infected with *T. brucei* [9, 10] were used as group II. A third set of 25 apparently healthy dogs not infected with either CDV or *T. brucei* served as control (Group III).

In all cases, the dogs were bled through the cephalic vein. Blood for the HIT was collected without anti-coagulant and the serum separated routinely [11]. Blood for buffy coat method was placed directly into microhaematocrit centrifuge tubes and processed routinely [11].

Determination of blood coagulation time

To determine the coagulation time, a drop of blood collected from the cephalic vein of each dog in the six groups (natural and experimental) was placed on a clean, grease-free, glass slide immediatly on collection. The time it took each drop of blood to coagulate was recorded as coagulation time for the dog. Mean coagulation time was calculated for each of the three groups of dogs.

Statistical analysis

The mean blood coagulation time of the CDV and *T. brucei* experimentally infected dogs were compared with that of the control, using one way analysis of variance (ANOVA). Variant means were seperated by the least significant difference (LSD) at $p<0.05$. Also, the coagulation time of CDV and trypanosome naturally infected dogs were compared as done for the experimentally infected dogs.

RESULTS

The results showed that compared to the uninfected control (Group II), infection with CDV lowered the coagulation time whereas *T. brucei* infection increased the same irrespective of whether the dogs were

infected experimentally or naturally (Table 1). The difference in blood coagulation time between the three groups (I – III) was significant ($P < 0.05$); the CDV group being the lowest, followed respectively by the controls and the trypanosome infected groups. A similar pattern was also noted in the mean coagulation time of the naturally infected groups.

Table 1: Blood coagulation time of dogs with natural and experimental *Canine distemper virus* and *Trypanosoma brucei brucei* infections.

Dog groups	No. of animals	Coagulation time in minutes Mean ± S. D.	Range
Natural infections			
CDV infected	5	1.4 ± 0.7^{a}	0.5 – 2.0
T. brucei infected	5	6.2 ± 1.0^{b}	5.0 – 7.3
Uninfected control	5	4.8 ± 0.7^{c}	4.0 – 8.0
Experimental infections			
CDV infected	5	1.7 ± 1.0^{a}	0.7 – 3.8
T. brucei infected	5	6.8 ± 1.7^{b}	5.0 – 11.0
Uninfected control	5	$5.0 – 1.3^{c}$	3.9 – 8.0

[abc]Mean values with different superscripts in the same column for natural or experimental groups are significantly different ($P < 0.05$).

DISCUSSION

The results of this study showed that canine distemper infection significantly shortened the blood coagulation time of dogs while *T. brucei* infection significantly prolonged the blood coagulation time. No report exists on effects of *Canine distemper virus* and Trypanosomes on coagulation time of blood of infected dogs. However, in other disease conditions, several factors including anaemia and haemoconcentration have been known to affect blood coagulation time [12, 13, 14]. It is also known that while anaemia prolongs coagulation time [12], haemoconcentration shortens it [13, 14].

Canine distemper is a viral disease of dogs which affects the nervous, respiratory, gastrointestinal and integumentary systems [15]. The various clinical manifestations of CD, including nervous signs, undulating fever, anorexia, conjuctivitis, photophobia, cloudiness of the eyes, congestion of oral and ocular mucous membranes, ocular discharges, vomition, salivation and diarrhoea usually culminated in haemoconcentration which is thought to be responsible for the shortening of the blood coagulation time of such animal [1, 13, 15, 16, 17] . Furthermore, CD and other chronic diseases result in increased activities of macrophages, an inflamatory mediator that may cause increased fibrinogen synthesis [18, 19]. Therefore, a combination of haemoconcentration, activation of the procoagulant activity of the macrophages and increase in synthesis of fibrinogen may be responsible for the shortening of coagulation time observed in *CDV* infected dogs.

Canine trypanosomosis, another tropical disease, is known to present anaemia and other similar clinical signs as canine distemper [5, 6]. The anaemia in canine trypanosomosis is known to result from to the lysis of red blood cells which leads to haemodilution; a condition thought to prolong blood coagulation time as recorded in the trypanosome infected group of dogs in this study [12, 20, 21].

In conclusion, therefore, the results of this study showed that canine distemper infection significantly shortened the blood coagulation time of dogs while *T. brucei* infection significantly prolonged it;

suggesting that the estimation of blood coagulation time may form the basis for the clinical differentiation of the two diseases in endemic regions**.**

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