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Necropsy Findings and Histopathological Changes in Dogs with Conjunct Experimental *Trypanosoma congolense* and *Ancylostoma caninum* Infections

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Authors' contributions

This work was carried out in collaboration between both authors. Author RION designed the study and wrote the protocol including the first draft of the manuscript, managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process. Author IRO prepared the photomicrographs of the histopathological sections. Both authors read and approved the final manuscript.

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ABSTRACT

Trypanosoma congolense and *Ancylostoma caninum* are parasitic diseases of dogs which commonly occur in the south-eastern part of Nigeria. There appear to be dearth of information on the histopathological changes in dogs with mixed infections of both parasites which necessitated the study. There were marked necropsy and histopathological changes in the vital organs of 4 dogs experimentally infected with both *Ancylostoma caninum (A. caninum)* and *Trypanosoma congolense (T. congolense)*. *Trypanosoma congolense* was inoculated post establishment of *A. caninum* infection in the dogs. The prepatent period of *T. congolense* was 10±2.0 days and 14±1.2 days in *A. caninum*. The disease ran a course of 45 days before death. Necropsy findings include, haemorrhages in the stomach mucosa, splenomegally, enlargement of the heart, congested and emphysematous lungs, congested and necrotic areas on the liver and normal kidneys. The histopathological changes were classified as degenerative, emphysematous, infiltrations of immune

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complexes and anaemia. The gross morphology of organs at necropsy may not reveal the degree of histopathological changes. The study however reveals extensive histopathological changes in vital organs of dogs with mixed infections of *T. congolense* and *A. caninum*.

Keywords: Trypanosoma congolense; Ancylostoma caninum; necropsy findings; histopathological changes.

1. INTRODUCTION

Canine trypanosomosis is a disease of public health importance, causing as much disease in humans as in dogs [1,2,3]. In Nigeria, African canine trypanosomosis is caused mainly by *T. brucei* and *T. congolense* [4]. Other species of canine trypanosomes are of various virulence and produces disease of varying severity [5]. Infections with *T. brucei* and *T.congolense* usually produce severe disease conditions in dogs [6,7]. [8] confirmed that under natural conditions, trypanosomosis and helminthosis often occur in mixed infections. Hence both infections in dogs in endemic areas may produce more severe disease conditions. Previously several reports have been made on the pathological effects of canine trypanosomosis on vital organs and systems of the body [9,10,11]. However there is paucity of information on necropsy and histopathological changes in vital organs in conjunct infections of *Trypanosoma congolense* and *Ancylostoma caninum* in dogs hence the essence of study of on necropsy findings and histopathological changes in dogs with conjunct experimental *Trypanosoma congolense* and *Ancylostoma caninum* infections.

2. MATERIALS AND METHODS

Twelve mongrel breed of dogs of both sexes weighing between 4.0 and 8.0 kg were used in the experiment. They were acclimatized for 3 weeks during which they were screened for blood and gastrointestinal parasites and confirmed negative before use in the experiment.

2.1 *Ancylostoma caninum* Infection

A dose of 200 infective L_3 suspended in 1 ml of distilled water was used to infect the dogs *per os* and infection confirmed by faecal flotation as described by [12].

2.2 *Trypanosoma congolense* Isolate

The Kilifi strain of *T. congolense* was obtained for use from the National Institute of Trypanosomosis and Oncocerciasis Research (NITOR) Nigeria. The strain was first isolated from a cow in Kaduna and was maintained in rats and subsequently passage in a donor dog from where parasites were collected for infection of the experimental dogs.

2.3 Experimental Design

Twelve mongrels were randomly grouped into 2 of 6 dogs each. Group (GPA) was the uninfected control and GP B was infected with 2.5×10^6 of *T. congolense* via the intraperitoneal route (i.p.). The quantity of parasites inoculated was estimated using the rapid matching method of [13]. Trypanosome infection was done post establishment of *A. caninum.* Parasitaemia was determined using wet mount and buffy coat techniques [14].

With progress in experiment, causalities were sent for necropsy examination. Samples for histopathology were collected and preserved in 10% formalin solution, and processed through fixation, dehydration, clearing and embedding in paraffin wax for normal haematoxylin and Eosin procedures as described by [15]. Slides were prepared and viewed using Olympus Vanox microscope and representative photomicrographs were made using the motic camera (Moticam 1000, 1.3M Pixel USB 2.0).

2.4 Ethical Approval

The care of the animals was in conformity with the guideline for animals' experimentation of Council for International Organization of Medical Sciences (CIOMS) for biomedical research involving animals. The dogs were humanely cared for and treated throughout the study. They were comfortably housed in properly ventilated pens in good hygienic condition and provided good and adequate feeding with clean portable drinking water. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

3. RESULTS

The trypanosome infection was established by day 10±2.0 post infection and 14±1.2 days in *Ancylostoma caninum* infection. The infection ran a course of 34 days before mortality.

Table 1 shows various necropsy changes in organs of infected dogs. The changes were observed on the heart, lungs, liver, stomach and the spleen. Table 2 shows histopathological changes in the heart, lungs, liver, stomach and the spleen.

4. DISCUSSION

The gross morphology of most of the processed organs especially the heart, lungs, spleen and liver were abnormal. This contradicts earlier observation of normal organs from trypanosome infected animal [16]. The disparity in our findings is dependent on differences in species and virulence of the infecting trypanosome parasite. It may seem that conjoined infection with *A. caninum* downgrades immunity and enhanced development of abnormalities in the vital organs [17]. The necropsy changes though severe in most organs were near consistent with previous findings in trypanosomosis in animals [18,4]. The presence of trypanosomes in cardiac tissues elicits immune release of inflammatory cells which over time induces heart disease and heart failure. Cardiomegally results from compensatory effect in a diseased heart in attempt to sustain normal cardiac function [19].

Anaemia in trypanosomosis manifests through several mechanisms in infected animals [20,21,22]. It was enhanced in complications of hookworm infection through excessive blood loss from their blood sucking and blood letting activities as observed by the presence of haemorrhages in the stomach mucosa.

Table 1. Necropsy changes in organs of dogs with conjunct infection of *T. congolense* and *A. caninum*

Organs AB	Normal	Normal	Observed disorder
Heart	Fig. 1	Fig. 1a	Enlargement of the heart with rounded apex
Lungs	Fig. 2	Fig. 2a	Areas of Emphysema with congested areas in the lungs
Liver	Fig. 3	Fig. 3a	Areas of congestion with tiny areas of necrosis in the liver
Spleen	Fig. 4	Fig. 4a	Splenomegally
Stomach		Fig. 5	Haemorrhages in the stomach

Table 2. Histopathological changes in organs of dogs with conjunct infection of *T. congolense* and *A. caninum*

Organs	Abnormal	Observed disorder
Spleen	Fig. 6	Showing a reduction in red pulp population (almost absence of red blood cells) and presence of mainly plasma cells (black arrow) and macrophages (white arrow).
Liver	Fig. 7	Showing dilated and congested sinusoids (arrow)
Kidney	Fig. 8	Showing mononuclear cell infiltration of the glomerulus (G) and mild tubular degeneration (arrows).
Lungs	Fig. 9	Showing emphysema (black arrows) and mononuclear cell infiltration (white arrows).
Heart	Fig. 10	No remarkable histologic changes except few mononuclear cell Infiltrations.

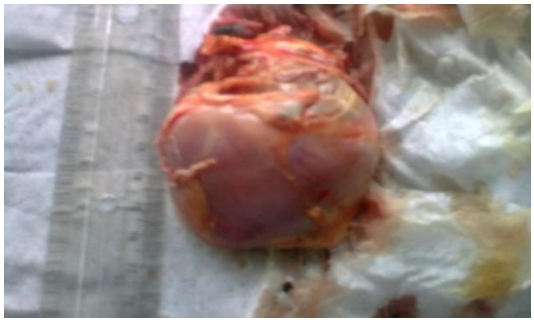

Fig. 1. Enlarged heart with rounded apex from a dog with conjunct *T. congolense/ A. caninum* infections

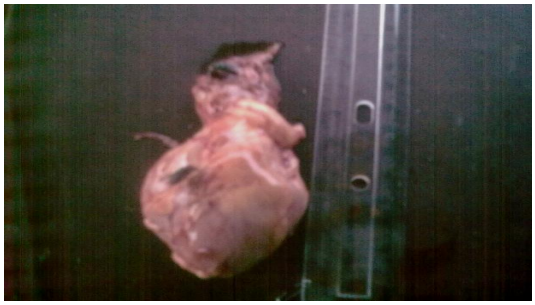

Fig. 1a. Normal heart of a dog

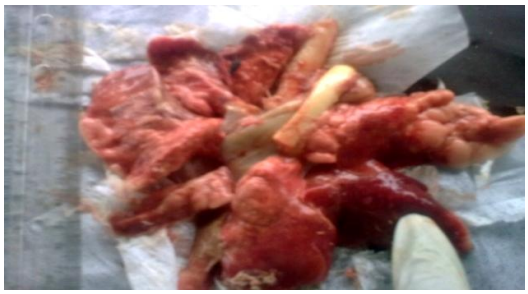

Fig. 2. Emphysematous lungs with congested areas in a dog with conjunct *T. congolense/A. caninum*

Fig. 2a. Normal lungs of a dog

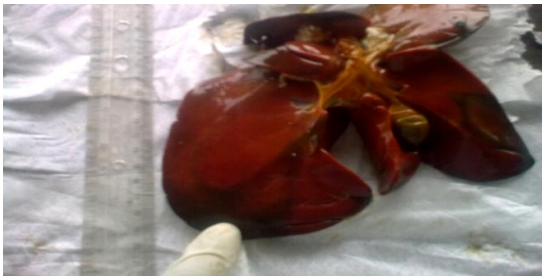

Fig. 3. Tiny areas of necrosis with congested areas on the liver of a dog with conjunct infections of *T. congolense* / *A. caninum* infections

Fig. 3a. Normal liver of a dog

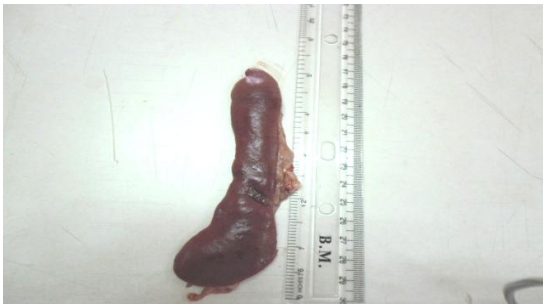

Fig. 4. Enlarged spleen with rounded sides in a dog with conjunct *T. congolense/ A. caninum* infections

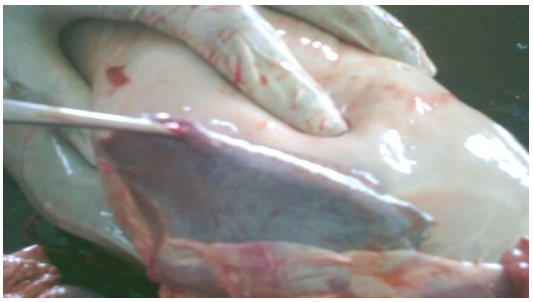

Fig. 4a. Normal spleen of a dog

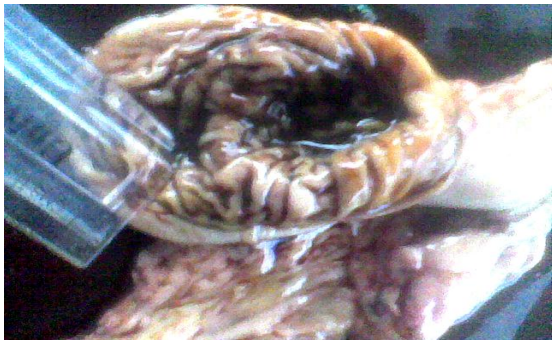

Fig. 5. Haemorrhages in the stomach of a dog with *T. congolense/A. caninum* infections

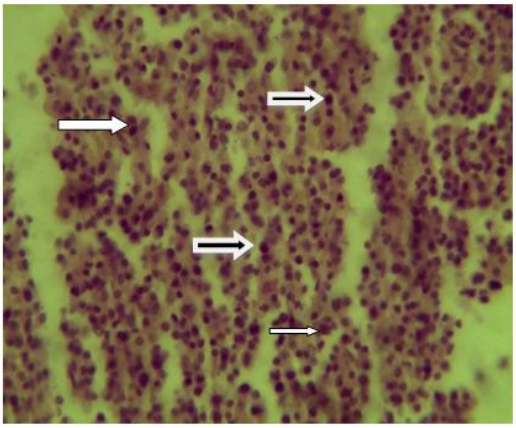

Fig. 6. Photomicrograph of spleen from *T. congolense/A. caninum* infected dog showing a reduction in red pulp population (almost absence of red blood cells) and presence of mainly plasma cells (black arrow) and macrophages (white arrow). H and E x40

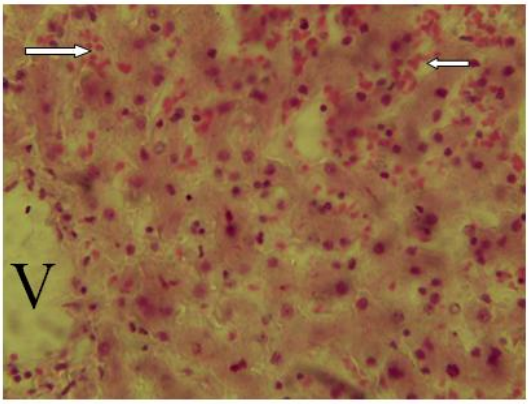

Fig. 7. Photomicrograph of liver from *T. congolense/A. caninum* infected dog showing dilated and congested sinusoids (arrow). See the central vein (V). H and E x40

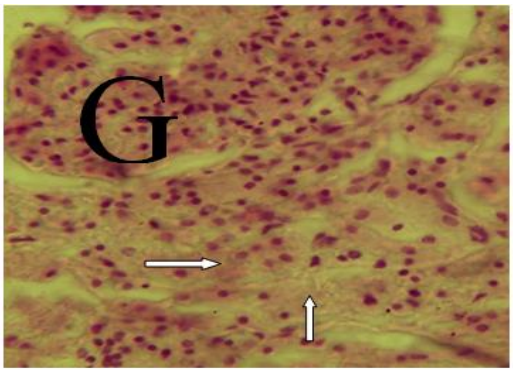

Fig. 8. Photomicrograph of kidney section from *T. congolense/A. caninum* infected dog showing mononuclear cell infiltration of the glomerulus (G)) and mild tubular degeneration (arrows). H and E x40

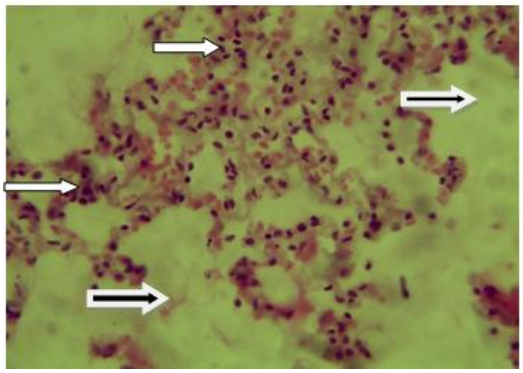

Fig. 9. Photomicrograph of the lungs section from *T. congolense/A. caninum* infected dog showing emphysema (black arrows) and mononuclear cell infiltration (white arrows). H and E x40

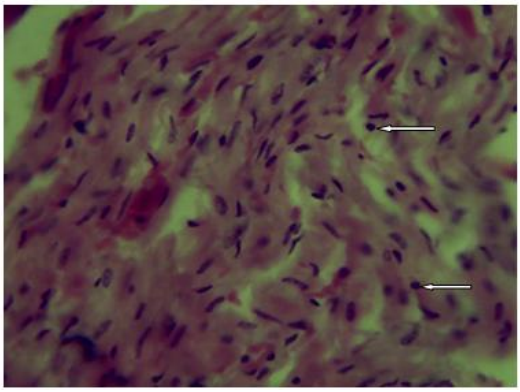

Fig. 10. Photomicrograph of a section of the heart from *T. congolense/A. caninum* infected dog. No remarkable histologic change except few mononuclear cell infilterations were seen. H and E x40

Splenomegally results from massive sequestration of red cells and lymphocytes and expanded macrophage populations in response to trypanosome infection [23,11]. Splenomegally is a feature of acute or parasitemic phase of infection signifying acuteness of both disease conditions in the dogs. However with progress in the disease, the cellular components of the spleen gradually depletes as observed histologically as reduction in red pulp population and near absence of red blood cells. Similar observation was made in *T. evansi* infection in donkey [16]. The effect of both parasites caused disease of the lungs which appeared in form of emphysema and gas accumulation in the tissue. This condition causes respiratory compromise and eventual mortality. This was as observed by [24] in *T. evansi* infection in bandicoot rats. Comparatively, there is paucity of information on the histopathological changes in conjunct *T. congolense / A. caninum* infection in dogs. The classical histological changes observed in the vital organs of the dogs were mostly anaemia, degeneration, congestion, emphysema, enlargement and inflammatory cell infiltrations. This somehow corroborates the findings of [24] in *T. evansi* infection in bandicoot rats. The presence of trypanosomes in tissues elicits systemic formation of antigen-antibody complexes in various tissues [25]. These Inflammatory cells are directly and or indirectly involved in tissue damage and sometimes mere presence of trypanosomes in tissue could be a direct cause of tissue damage [26,27]. The degenerative and necrotic changes in the hepatic sinusoids maybe due to right sided heart failure and congestion from damming back of blood into the inferior vena cava and hepatic veins [28]. This condition alters the physiologic functions of the liver as the “General of the Army” of the body. The reddish brown congested areas on the liver contrasts with the normal tissue. This in a way corroborates the report of [10] in *T. brucei* infection in dogs. Similarly [18] observed enlarged hepatocytes with numerous vacuoles and dissociated hepatic cords in *T. congolense* infection in rats. [29] served disorganized hepatic cords in *T. congolense* infection in cattle.

5. CONCLUSION

Ironically there was absence of apparent change in kidney morphology whereas there was histopathological evidence of presence of massive tissue destructive immune complexes in the glomerulus. This somewhat corroborates the works of Ohaeri [18], who observed thickening of basement membrane and intense cell infiltration of macrophages and lymphocytes around glomeruli and blood vessels in rats with *T. congolense* infection. Saror and Coles [29] observed accumulation of mononuclear cells around the glomeruli and blood cells in *T. congolense* infection in cattle. On the contrary, gross distortion of the cardiac morphology presents histological evidence of only few inflammatory cells infiltrations in the tissue. This may imply a clear distinction between morphology and microscopic histological changes in various tissues and organs. In conclusion, conjunct *T. congolense* and ancylostomosis produced relatively severe necropsy and histopathological changes in organs of infected dogs which could account for increase in mortality.

DISCLAIMER

This manuscript was presented in the 50th Anniversary- Annual congress”. Nigeria Veterinary Medical Association” available link is “http://xa.yimg.com/kq/groups/17723053/1429206117/name/LIST+OF+ABSTRACT+FINAL+ABUJA+2013+(2).doc?download=1”
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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