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Pathogenicity Of Some Trypanosoma Species On The Endocrine Glands Of Experimentally Infected Yankasa Rams

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Abstract: ***The study was designed to determine the pathological changes in endocrine glands of Yankasa rams experimentally infected with Trypanosoma brucei, Trypanosoma evansi and mixed infection. Sixteen Yankasa rams were divided into four groups comprising four rams in each group. Group A was inoculated with T.brucei, group B with T.evansi, group C with mixed infection (both T.bruei and T.evansi) while group D served as control. Each were infected with approximately*** 1.0×10^6 ***of Trypanosomes through the jugular vein. They were closely monitored for trypanosomosis from the time of infection up till the end of the experiment. Two rams from group A and C while group B and D was one each. They were humanely sacrificed 91 days postpartum and examined for pathological changes. The infected rams had histopathological changes in the hypothalamus characterized by neuronal degeneration and microglial infiltration, adenohypophysitis while in the pituitary gland, there was mononuclear cell infiltration, necrosis and disruption of the architecture of the adenohypophysis. The thyroid gland was infiltrated by inflammatory macrophages and lost its glandular secretions, and presence of trypanomastigotes nest within the muscle fibres. All these histopathological changes were absent in the uninfected rams, suggesting that T.brucei and T. evansi is capable of disrupting the endocrine function of reproduction in Yankasa rams.***

Keywords: Pathology, Endocrine glands, Trypanosomes, Yankasa rams

I. INTRODUCTION

Livestock production plays important roles in the provision of high-quality protein to consumers and regular income to producers. To fulfill their potential sustainability, they must be managed with care. From time immemorial livestock rearing is given much importance not only in developing countries but also in developed countries (Babatunde and Qaim, 2010). Trypanosomosis is an immuno-suppressive disease (Sulaiman and Adeyemi, 2010). Livestocks diseases remain a veritable threat to the animal production industry. Animal products are constantly under threat by diseases that affect livestock and hence reduce productivity (Bamaiyi, 2013). Trypanosomosis in sheep is caused by *Trypanosoma brucei*; *T.congolense*(Okubanjo *et al*., 2014b);*T. vivax* (Rodrigues *et al*., 2013) and *T. evansi* . The post-mortem lesions in animals infected with trypanosomes have been documented by several authors. However, the carcasses are generally emaciated and pale (Okubanjo *et al*., 2014b). There is peri-orbital and scrotal oedema (Kilekoung *et al*., 2014; Okubanjo *et al*., 2014b). Others are scrotal dermatitis; sterility; epididymitis, penile protrusion with haemorrhages and posthitis (Victor *et al*., 2012). Petechial and ecchymotic haemorrhages are common with congestion in most tissues and organs. There is enlargement of the lymph nodes (Odeyemi *et al.*, 2015); congestion and enlargement of the liver, lungs and kidney with petechial haemorrhages (OIE, 2013). Oedema, hydroperitoneum and severe myocarditis are also common findings in *Trypanosoma* infected animals (OIE, 2013; Okubanjo *et al*., 2014b). In aborted animals, the cotyledons are enlarged, haemorrhagic and friable with focal areas of necrosis on the placenta (Leigh and Fayemi, 2013; Silva *et al*., 2013). The roles played by endocrine glands especially the hypothalamus and anterior pituitary in reproductive process are well documented (O'Donnell *et al*., 2017). The

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gonadotrophin releasing hormone (GnRH) produced from the hypothalamus induces release of gonadotropins, follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary. FSH and LH are required for spermatogenesis and sperm maturation, while development of male secondary characteristics and libido depends on the testosterone. Luteinising Hormone (LH) stimulates Leydig cells to produce testosterone and small quantities of other androgens. Secretion of testosterone by the Leydig cells provides a high concentration of testosterone around the seminiferous tubules which is essential for spermatogenesis. In other words, GnRH, LH and FSH directly or indirectly affect the level of testosterone produced by the interstitial cells, which reportedly has been shown to be a good marker for semen quality and production. Several studies have reported involvement of central nervous system under trypanosomosis infection and impairment of the hypothalamic-pituitary-gonadal axis (Batista *et al.,* 2012). Testosterone, the male reproductive hormone belongs to a class of steroids called androgens. It is the most important of all the steroids produced. Testosterone is produced mainly by the Leydig cells of the testis with a limited amount by the adrenal cortext. The Leydig cells are the main sites of testosterone synthesis and are stimulated by pulse of pituitary gonadotropins, lutenizing hormone (LH) or interstitial cell stimulating hormone (ICSH) to secret this androgens (Verhoeven *et al., 2010*).

II. MATERIALS AND METHODS

The study was carried out at the animal fly-proof pen located at the Department of Animal Production Teaching and Research Farm, Faculty of Agriculture Adamawa State University, Mubi. Sixteen apparently healthy and intact Yankasa rams aged between 24 and 30 months and weighed between 22 to 25 kg was purchased from local market around Mubi. Their age was estimated using the pattern of eruption of their dentition, while breeding history will be obtained from the sellers where possible. Ethical clearance was sought for and approval was obtained from the Adamawa State University Committee on Animal use and care. The animals on arrival were housed in an insect-proof animal pen at the Department of Animal Production Teaching and Research Farm Adamawa State University, Mubi, where they were screened for the presence of haemoparasites, as well as endo and ectoparasites. The rams were thereafter treated with Oxytetracycline (Tridax®) intramuscularly, at a dose of 20 mg/kg body weight and Albendazole orally, at a dose of 7.5 mg/kg body weight. The rams were sprayed against ectoparasites with Diazinon (Diaznol®, Animal Care, Nig. Ltd.), at concentration of 2 ml/litre of water. They were allowed to acclimatized for eight weeks and neck-tagged for the purpose of identification They were intensively managed by housing in fly-proof pens throughout the period of the experiment. Before commencement of the experiment, the rams were ensured to be clinically free of trypanosomes and other haemoparasites in their blood using buffy coat centrifugation technique (Biryomumaisho *et al*., 2013). *Trypanosoma evansi* was obtained from the stabilates maintained at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State while that of *Trypasoma brucei* (Emodike strain) was obtained from National Animal Production Research Institute (NAPRI) Zaria, Kaduna State Nigeria. Rats were inoculated with the contents and immediately transported to the Department of Zoology Adamawa State University Mubi where they were passage into new rats for maintenance until the commencement of research proper in the Department of Animal Production Teaching and Research Farm Adamawa State University, Mubi. By the end of the eight weeks acclimatization period all sixteen rams were clinically fit (due to good semen characteristics) and were randomly grouped into four experimental groups (GI, GII, GIII and GIV) of four rams each, based on their weights. The rams in groups I, II and III were experimentally infected with *T.brucei brucei*, *T. evansi* and mixed inoculum of both parasites, respectively while those in group IV served as the uninfected control. At the end of the experiment, six (6) rams were selected and sacrificed and subjected to post mortem examinations and biopsies was taken for histopathology. Sections from the hypothalamus, thyroid gland, pituitary gland, adrenal gland, testes was taken and fixed in Bouin`s solution.

III. RESULTS

The testes of the control Yankasa rams (group IV) showed normal tissue architecture with normal active semiferous tubules containing proliferating spermatogenic cell layers and supportive sertoli cells. There are matured spermatid within the lumen of the seminiferous tubule (Plate VII-a), while those of the infected Yankasa rams (groups I, II and III) showed moderate degeneration (T. evansi-infected group and mixed infection) (Plate VII-b,c) to severe (*T. brucei*-infected groups) (Plate VII-d), atrophic and distorted seminiferous tubule containing degenerating spermatogenic cells and sertoli cells. There are few presence of inmatured cells arising from the germinal epithelium with reduced tubular wall thicken. In-addition, interstitial cells are inadequate and some of the tubules show foci of calcification and proliferating myofril within the semiferous tubules and hyper chromatic nucleus were observed.

Hypothalamus gland in the infected *T.brucei* (group I) rams shows neurohypophysis tissue displaying bundles of supporting neuroglial cells,pituicytes and unmyelinated cells that are deeply eosinophilic and degenerative. Blood channels are mainly capillaries and sinusoid and plexus (Plate XIII-b), while *T.evansi* (group II) (Plate XIII-c) shows slight atrophy. Mixed infection rams (group III) showed severe perivascular infiltration of inflammatory cells (Plate XIII'-d). Uninfected group (IV) shows normal tissue architecture (Plate XIII-a).

The pars distalis of the pituitary gland in the uninfected controls (group IV) was unremarkable and the section shows normal numerous cells of adenohypophysis and hypophysis while the pars intermediate contained numerous well-formed secretary colloid and blood vessels (Plate XIV-a). However, in the infected T.brucei (group I) rams, there was slight adenohypophysitis and deeply stained par distalis with course chromatin and prominent nucleoli (Plate XIV-b). There was

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slight atrophy of the adenohypophysis cells and chromophobes in the par distalis appear eosinophilic, the nuclei are degenerative with pale chromatin in *T.evansi* rams (Plate XIV-c). There was slight atrophy in the adenohypophysis cells and chromophobes of mixed infection rams (group III), the par intermediate of the hypophysis show vesicles containing secretary colloid and active blood vessels (Plate XIV-d).

Section of thyroid gland in the control group (plate XV-a) showed numerous follicles surrounded by a fibrous capsule, which forms septae that divide the parenchyma into multiple lobules. The septae also contain the nerves and blood vessels supplying each lobule,the nuclei of the epithelial cell are spherical and vesicular with cytoplasm and *T.brucei* infected rams (Plate XV-b) shows thyroid parenchyma infiltrated by lymphocytes and reactive follicles. In mixed infection thyroid gland (Plate XV-c) it shows moderate infiltration of inflammatory cells by lymphocytes and presence of trypanomastigotes nest within the muscle while that of T.evansi groups shows mild inflammation.

No overt gross changes were observed in the adrenal glands of infected rams. Apart from the hypertrophy of the zona fasciculata- reticularis, only mild histopathological changes were observed in the adrenals of infected rams. In contrast, adrenals from infected *T.brucei*) rams showed marked changes including localised and diffuse subcapsular and cortical mononuclear cell infiltration (Plate XVI-b); hypertrophy of the zona fasciculata-reticularis and accumulation of adipocytes in the zona glomerulosa of *T.evansi* (Plate XVI -c) and focal coagulative necrosis in the zona fasciculata of mixed infection (Plate XVI- d). Plate (XVI –a) shows a normal adrenal gland from uninfected control sheep.

IV. DISCUSSION

The roles played by endocrine glands especially the hypothalamus and anterior pituitary in reproductive process are well documented. The gonadotrophin releasing hormone (GnRH) produced from the hypothalamus induces release of gonadotropins, follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary. FSH and LH are required for spermatogenesis and sperm maturation, while development of male secondary characteristics and libido depends on the testosterone. GnRH, LH and FSH directly or indirectly affect the level of testosterone produced by the interstitial cells, which reportedly has been shown to be a good marker for semen quality and production (Raheem, 2014). The lesions associated with experimental *T. brucei* infection in this study included hypothalamitis, adenohypophysitis, in the rams as well as hepatocellular degeneration, atrophy. Although *T. brucei* is both haematic and tissue-invading trypanosome, parasites were observed in the blood stream at day 28th post infection but later disappear in the blood stream at day 56th post infection till the end of the experiment but were later found in the tissues examined. This result is in agreement with the report of Leigh *et al*., (2015) on the histopathological changes in some reproductive and endocrine organs of *Trypanosoma brucei* infected WAD goat Does.

The pituitary gland, which is connected to the central nervous system (CNS) through the hypothalamus, is one of the endocrine organs affected by trypanosomiasis. Histopathological examination of *Trypanosoma brucei* infected West African dwarf does by Leigh *et al*. (2015) revealed subacute necrotising adenohypophysitis characterized by widespread necrosis and disruption of the architecture of the adenohypophysis, multiple foci of inflammatory cells which were mostly lymphocytes and a few neutrophils were also observed in the pituitary gland. This report is in cognizance with our report on the pathogenicity of some trypanosome species on the endocrine gland of yankassa rams. Our report is also in agreement with the report of Adeyeye *et al*., (2016) who reported infiltration of mononuclear cell in the pituitary gland of postpartum Yankasa ewes experimentally infected with *Trypanosoma evansi* during pregnancy as well neuronal degeneration and microglia infiltration of the hypothalamus.

The thyroid gland is known to play a role in the regulation of the neuroendocrine system at the hypothalamic-pituitary axis (Fonseca *et al*. 2013). Histopathological examination of the thyroid glands of *T.brucei* infected rams shows thyroid parenchyma infiltrated by lymphocytes and reactive follicles and presence of trypanomastigotes nest within the muscle fibres. This result is in agreement with the report of Adeyeye *et al*., (2016) and that of Beynon and Pinneri (2016) who reviewed Thyroid Gland and Thyroid-Related Deaths for the Forensic Pathologist.

Chronic infection could impair the function of thyroid gland and recorded hypothyroidism associated with decrease in triiodothyronine (T3) and thyroxin (T4) levels in T. evansi infected camels (Sivajothi *et al*.,2015).

Adrenal gland is one of the most common endocrine organs affected by chemically induced lesions. The adrenal gland consist of three layers; Capsule, Cortex and Medulla. The adrenal cortex is required for life, particularly the secretion of aldosterone, but the functions of the medulla are not essential for life. The adrenal cortex consist of three distinct zones: zona glomerulosa, zona fasciculate and zona reticularis (Bornstein *et al*.,2020). The adrenal glands are the major source in the body of the steroid hormones (NIH, 2021). No overt gross changes were observed in the adrenal glands of infected rams. Infected rams showed marked localised and diffuse subcapsular and cortical mononuclear cell infiltration, hypertrophy of the zona fasciculata-reticularis and accumulation of adipocytes in the zona glomerulosa.

The histopathology of the testis from infected yankassa rams in the current study showed degenerative changes of the seminiferous tubules characterized by vacuolation with dead spermatocytes in *T.evansi.* The same result was recorded in dromedary camels (Amin *et al*., 2020). Also same results were detected in the experiment of rams infected with *T. brucei* and *T. evansi* (Wada *et al.*, 2016). Furthermore; necrosis and inflammation, calcified cells and monocytes infiltrations, interstitial cells with white blood cells, myofibrin were recorded in this our study which is also in agreement with the report of Amin *et al*., (2020), Ogundele *et al*., (2016) and Kothari *et al*., (2017) who reported on the effect of *T.vivax* on reproductive organs of sheep and goats. Another factor implicated in tissue and organ degenerative changes is

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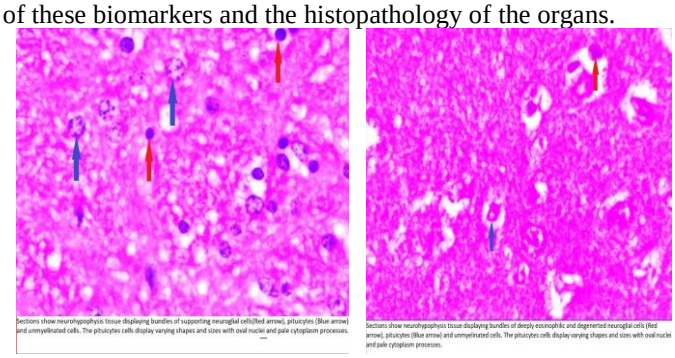
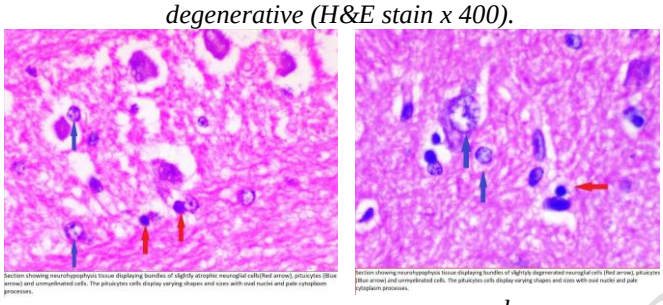
oxidative stress imposed by trypanosomes and macrophage activities. Therefore cell injury was confirmed by the increase of these biomarkers and the histopathology of the organs.



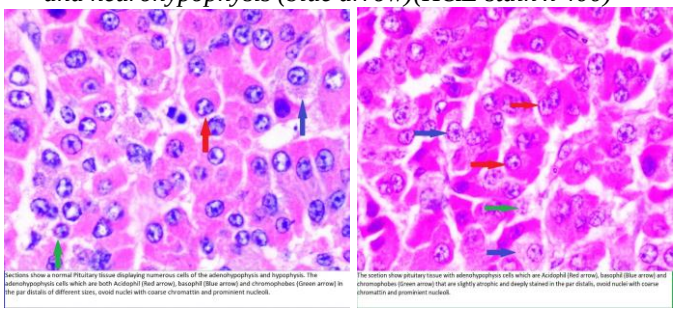
a *b*

Plate XIII-a,b: Photomicrograph of the Hypothalamus of the control animal(a) showing normal architecture and T.brucei infected group (b) showing neurohypophysis tissue displaying bundles of supporting neuroglial cells,pituicytes and unmyelinated cells that are deeply eosinophilic and degenerative (H&E stain x 400).



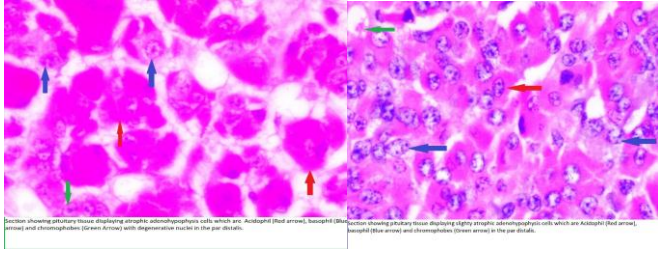
c *d*

Plate XIII-c,d: Photomicrograph of the Hypothalamus of T.evansi (c) animal showing atrophic neuroglial cells(red arrow) and pituicytes(blue arrow) and mixed infection group (d) showing perivascular inflammation (red arrow) and neurohypophysis (blue arrow)(H&E stain x 400)



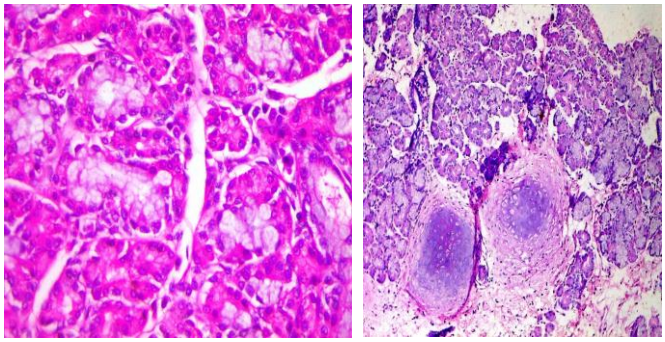
a *b*

Plate XIV-a,b: Photomicrograph of the Pituitary gland of the control(a) animal showing normal adenohyphosis and hyphosis architecture(red arrow), basophils(blue arrow) and T.brucei infected group(b) showing slight adenohyphositis (H&E stain x 400)



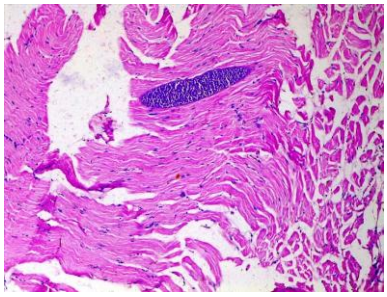
c *d*

Plate XIV-c,d: Photomicrograph of the Pituitary gland of T.evansi(c) animal showing atrophic adenohyphosis(red arrow) and chromophobes with degenerative nucei (green arrow) and mixed infection group(d) showing slight adenohyphositis (red arrow) and chromophobes (green arrow) (H&E stain x 400).



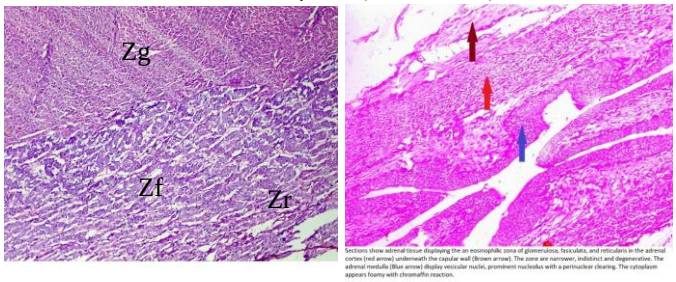
a *b*

Plate XV-a,b Photomicrograph of control rams(a) showing normal thyroid follicular cells and T.brucei infected animals(b) showing thyroid parenchyma infiltrated by lymphocytes and reactive follicles (H&E x400).



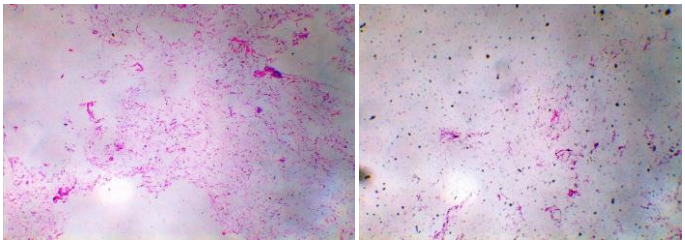
c

Plate XV-c: Photomicrograph of mixed infection thyroid gland(c) showing moderate infiltration of inflammatory cells by lymphocytes and presence of trypanomastigotes nest within the muscle fibres(H&E x 400)



a *b*

Plate XVI –a,b showing a normal adrenal gland from uninfected control sheep(a) and T.brucei (b) showing eosinophilic zona glomerulosa (red arrow), capular wall (brown arrow) and indistinct and degenerative adrenal medulla(blue arrow).(H&E x400)



c *d*

Plate XVI –c,d showing hypertrophy of the zona fasciculata-reticularis and accumulation of adipocytes in the zona glomerulosa of T.evansi(c) and focal coagulative necrosis in the zona fasciculata of mixed infection(d)).(H&E x400)

V. CONCLUSION AND RECOMMENDATION

The study shows that the *T.brucei* isolate used in this study produced gross histopathological changes in the hypothalamus, pituitary, thyroid, adrenal glands and testis in yankasa rams than *T.evansi*. We therefore recommended that T. *brucei* isolate be used to determine its effects on the estrus cycle of Yankasa ewes considering the pathology seen on endocrine organs. Yankasa ewes may also be experimentally infected to determine the effect of the parasite on the endocrine (production of progesterone) of the ovaries and other aspects of female reproductive system.

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