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Age, and non-age-related cytoarchitectural changes in the brain of some Nigerian cattle

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Abstract

Brain is the most vulnerable organ in the body to the ageing processes which operates at variable levels between organs and species. In this study we examine brains histopathologically for architectural changes in four Nigerian cattle breeds. Brains from 246 cattle (Bunaji=80, Muturu=78, Rahaji=62 and Sokoto Gudali=26), aged 1–≥12years were examined at 8 different neuroanatomical locations. All the cattle used were obtained from Abattoir in Ibadan, Nigeria. They were examined at ante-mortem and those without clinical signs suggestive of neurological disease were sampled. Major changes observed were intracellular accumulation of substances (61.4%), neuronal and or axonal degenerations and loss (51.6%), perivascular cuffing (50.0%), extracellular accumulation of substances (29.7%), hypercellularity (19.1%) and spongy state (0.4%), malacia (0.0%), demyelination (0.0%) in 233 cattle. Intraneuronal vacuolations (35%), lipofuscin accumulation (42%), axonal spheroids (50%), inflammatory changes (50%), and brain sand (22%). Although perivascular cuffing was high, there was low incidence of lymphocytic infiltration in the pineal gland in both sexes. Intracellular accumulation of substances, neuronal and or axonal degenerations and loss, and extracellular accumulation of substances display levels of significant changes with age ($p<0.0001$). Changes starts at the age of 2 years in life in these breeds of cattle due probably to the adverse stress exerted by the tropical climate. The description of histological findings in the brain of symptomless cattle in the present study provides a useful background for diagnostic bovine neuropathology in the tropics.

Key words: age-related, brain, cattle, changes, Nigeria.

Introduction

The brain of the vertebrate animal receives the most structural protections from the external environment. This is evident from the levels of protection it receives from the cranium, cushioned by cerebrospinal fluid, and well protected from noxious agents by the lepto-meninges, the blood brain barrier, and perivascular spaces (blood-CSF barriers) (40). It is probably because it's a highly specialized and structurally complex organ within the body. Its functional complexities of coordination and integration of complex signals often underscore its importance to the individual. However, spontaneous changes, a phenomenon that affects all organs and parts of the body (11) has proved devastating on the brain making it the most vulnerable to ageing processes. This is due probably to its high O_2 requirement that is hardly met, low regenerative capability and low antioxidant synthetic capability (5, 12). This could probably explain how cellular and molecular changes that occur during normal ageing in the brain, render neurons vulnerable to degeneration, environmental influence and how they determine which neuron succumb (29).

Neuronal cells of the various neuroanatomical locations of the nervous system have variable degrees of vulnerabilities and affected by degeneration and ageing

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(48). The compact anatomy of the brain means that even mild background changes could produce severe functional disturbances such as observed in the frailty of the elderly and loss of cognition seen in animals with higher cognitive abilities. Changes in the brain could be the result of any of complex biological processes of ageing, as postulated (44). In cattle, neurological disturbances are easily masked leading to wrong diagnosis since neurological signs in these animals are easily manifested in responses to strange beings, environments or presence of diseases of other systems. Spontaneous changes in the brain have been shown to operate physiologically, genetically or at molecular organizational level in most cases with no gross lesions (11) and could be due to cellular mutations (33, 36) or metabolic imbalances (10, 17). These may result into cellular or extracellular accumulations of substances and in severe cases with reciprocal behavioral changes in the affected individual (29, 37). Traces of abnormal tissue deposits to specific sites were common features reported in affected brain samples (13, 47).

The factors determining the sites of depositions of the abnormal substances are still unknown (37). This is commonly seen in the decline of sensory, motor, and cognitive functions in humans and primates with increase in time (21). However, there lies considerable variability in ageing process among individuals, species and organs (13). The different environments, lifestyle and genetics could be responsible for the variations. That has led to efforts in searching for suitable animal model for the evaluation of ageing diseases in man; probably because research in primates and other small animals has some limitations (13, 26). The many reports as seen in rodents (41), in dog (7, 13), in the horse (22) and in primates (16) attest to this fact. Little is known about cattle and age-related brain changes in the tropics. Although, cattle do not share physiological similarities with man, they share similar tendencies to increase in weight as they age which may result in hormonal imbalances (49). This work demonstrates the age-related structural changes, cellular and extracellular substance accumulations, vascular and histo-pathological architecture in the brain of some Nigerian cattle breeds. It is believed that it would provide information for research and neuropathological diagnosis of disease of this species in the tropics.

Material and methods

Two hundred and forty-six (246) brain samples from the four major cattle breeds of Bunaji (n=80), Muturu (n=78), Rahaji (n=62) and Sokoto Gudali (n=26) were obtained from the main abattoir in Ibadan, Nigeria (Table 1). Cattle were physically examined and screened for apparent disease and aged by dentition, a method described by Johnson (23) for cattle. Brains were collected and preserved in 10% formalin solution and processed as described by Bolon et al. (6) and (45). Eight (8) coronal and hemicoronal neuroanatomical areas of the brain including the proximal medulla oblongata, cerebellum, midbrain, the cerebrum, thalamo-hypothalamic area, the basal ganglion, hippocampus and the pineal gland were studied histopathologically at the Friedrich Loeffler Institute Germany. A total of one thousand nine hundred and sixty-eight (1,968) sections were studied. Further necessary evaluation was done using Luxol fast blue, PAS, cresyl violet stains. Histopathological observations were presented as photomicrographs. All data generated from the variables in this study were calculated in percentages using Microsoft excel version 2010 and presented as pie chart, histograms and tables. Data obtained from breeds, sexes and different age groups were also subjected to correlation analysis by Fisher's Exact Test using the statistical package R version 3.3.1 (39) and presented. All values $p \leq 0.05$ were considered significant.

Table 1. Cattle breeds used in the study.

Breed	Sex		Total
	Female	Male	
Bunaji	51	29	80
Muturu	17	62	78
Rahaji	34	25	60
Sokoto Gudali	12	14	26
Total	116	130	246

Tissues were removed from the 10% formalin solution and processed based on the method outlined by Garman (14). They were blocked using paraffin wax, trimmed to size and sectioned with Zeiss M55® microtome at 4µm thick. Sections were picked on Superfrost® (positively charged) slides, air dried and incubated at 63°C for a minimum of 2 hours. They were deparaffinized through 2 changes of xylol at 5 minutes each, 2 changes of Isopropanol at 3 minutes each and hydrated through descending orders of 96%, 70%, 50% ethanol and distilled water at 3 minutes each.

Hematoxylin and eosin (H&E) staining was carried out based on Romeis Mikroscopiche technik (32). Sections were immersed in Hematoxylin solution for a minimum of 5 minutes and a maximum of 10 minutes depending on the strength of the stain, washed in running tap water for 10 minutes. They are removed and dipped 50% ethanol for 2 minutes and quickly counter stained with eosin for 3 minutes. Sections were dehydrated in 2 changes of 96% ethanol and absolute Isopropanol for 2 minutes each and finally in 2 changes of xylol for 3 minutes each. Tissues were mounted with an adhesive and cover slip. Slides were studied using the Zeiss® Axioskop 2 plus SIP 429-76 light microscope. Micrographs were obtained by using Zeiss Axiocam HRC BD 04065 microscopic camera version Axiovision Release 4.8.2 SP3 (08-2013).

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Results

Age distributions of animals used in the study are shown at Fig. 1. All microscopic findings are presented at Table 2.

Light-microscopic background changes were predominantly intracellular accummulations (lipofuscin, vacuoles, neuromelanin), inflammations, hypercellularity and extracellular accumulations (*Corpora arenacea*, Buscaino bodies, Psammoma bodies, vascular mineralization) (Figs. 2, 3, 4, 5). One hundred and fifty one (61.4%) of the 246 cattle had intracellular accumulation of substances in 212 neuroanatomical locations of medulla oblongata (n=81), cerebellum (n=24), midbrain (n=81), thalamus (n=9), hippocampus (n=7), basal ganglion (n=5), cerebrum (n=3), pineal gland meningis (n=2) in various degrees. One hundred and two (42%) of 246 cattle were observed to express intraneuronal lipofuscin accummulation and most (n=71) had lipofuscin in the midbrain, 37 cattle had lipofuscin in the medulla oblongata, 16 cattle were observed to express lipofuscin in the nuclei of the cerebellum, 6 cattle expressed lipofuscin in the thalamic nuclei, 2 cattle each showed lipofuscin in the hippocampus and basal ganglion and none had lipofuscin observed in the pineal gland. Lipofuscin accumulation was bilaterally observed in the olivary nucleus in all animals with lipofuscin accumulation.

Intraneuronal vacuolations were observed in medulla oblongata (n=49), Purkinje cells (n=8), midbrain (n=17), thalamus (n=2), hippocampus (n=3), basal ganglia (n=3). Vacuolations are variable in sizes, shapes and contents, in some cases large ovoid and empty whereas others were single to multiple with or without vacuolar contents. Seventy five (75) cattle (31%) were observed to have intracellular vacuolations in 82 neuroanatomical locations. One hundred and twenty three (50%) of the 246 cattle were observed to have neuronal/axonal degenerations in 141 neuroanatomical areas of medulla oblongata (n=112), cerebellum (n=2), midbrain (n=13), thalamus (n=5), hippocampus (n=4), basal ganglia (n=3), cerebrum (n=2) and pineal gland (n=0). Overall, age has a significant influence on selective loss of myelin axons, but not in any specific brain area. There is a statistically significant (P=0.029) effect for the cerebrum, although only 2 animals expressed selective loss of myelin/axons in cerebrum.

Forty seven (19.1%) of the 246 cattle had hypercellularity involving microgliosis in 61 neuroanatomical areas of medulla oblongata (n=8), cerebellum (n=8), midbrain (n=6), thalamus (n=15), hippocampus (n=6), basal ganglia (n=8), cerebrum (n=8) and pineal gland (n=2). The thalamus had the highest (6.10%) and the lowest in basal ganglion (0.81%). One (0.4%) of the 246 cattle had spongy state in 3 Neuroanatomical areas of medulla oblongata (n=0), cerebellum (n=0), midbrain (n=1), thalamus (n=1), hippocampus (n=0), basal ganglion (n=0), cerebrum (n=1) and pineal gland (n=0). Seventy three (29.7%) of the 246 cattle had extracellular accumulation of substances ranging from vascular mineralization, buscaino bodies and Psammoma bodies (p-value=0.004263). In total, age have significant influence on extracellular accumulation of substances. Examining each brain area separately extracellular accumulation of substances appears insignificant except in the medulla oblongata (Table 2). Notifiable pathohistologic lesions consisted mainly of inflammation (lymphohistioplasmacytic), and neuronal and/or axonal degeneration and loss (spheroids, chromatolysis). Age-related brain changes starts as early as two years and most changes were apparent by the age of 4 years in these breeds.

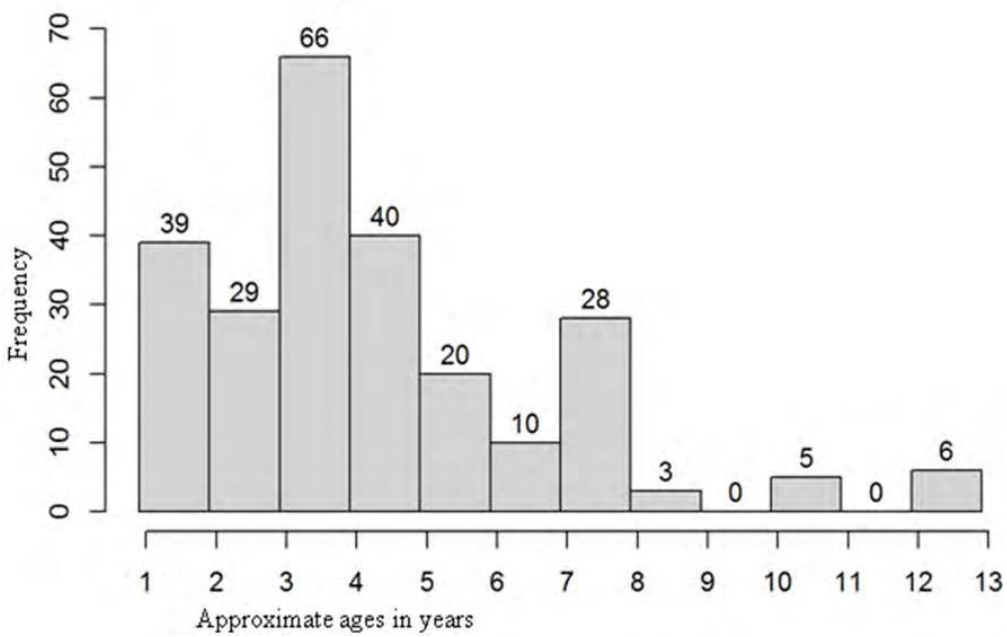

Figure 1. Age distributions of animals used in the study.

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Table 2. Histopathological observations in the brains of 233 cattle obtained from slaughter house in Nigeria.

Neuropathological changes present (n=233)	**Main areas affected**	**Evidence that affected change is age related (p-value)**
Intracellular accumulation of substances (n=151)		0.0005168 *
Lipofuscin	Red nucleus Olivary nuclei(bilaterally) Hypoglossal nucleus Dorsal vagal nucleus Solitary tract nucleus Caudate nucleus Paraventricular nucleus Dentate nucleus of the cerebellum Entorhinal cortex Dentate gyrus Caudate nucleus Putamen Dentate gyrus Lateral geniculate nucleus Purkinje's cells	
Neuronal vacuoles	Red nucleus Solitary tract nucleus Olivary nuclei Nucleus gracillis Nucleus cuneatus Nucleus funiculus Medulla oblongata Cerebellum Midbrain	
Neuromelanin	Thalamus Hippocampus Leptomeninges Pineal gland	
Neuronal and/or axonal degeneration and loss (n=127)		0.03321 *
Spheroids	Medulla oblongata Cerebellum Midbrain Thalamus Hippocampus Cerebrum	
Chromatolysis	Medulla oblongata Hippocampus Basal ganglion	

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Hypercellularity (n=47)		0.7062
	Medulla oblongata	
	Cerebellum	
	Midbrain	
	Thalamus	
	Hippocampus	
	Basal ganglion	
	Pineal gland	
Extracellular accumulation of substances (n=73)		0.004263 *
Buscaino bodies	Medulla oblongata	
	Hippocampus	
Psammoma bodies	Medulla oblongata (1)	
	Leptomeninges (3)	
Corpora arenacea (brain sand)	Pineal gland	
Vascular mineralization (calcium)	Medulla oblongata	
	Cerebellum	
	Midbrain	
	Thalamus	
	Pineal gland	
Inflammatory changes (n=123)		0.1924
	Medulla oblongata	
	Cerebellum	
	Midbrain	
	Thalamus	
	Hippocampus	
	Cerebrum	
	Basal ganglion	
	Pineal gland	
Spongy state (n=1)		1
	Cerebellum	
	Midbrain	
	Thalamus	
	Cerebrum	

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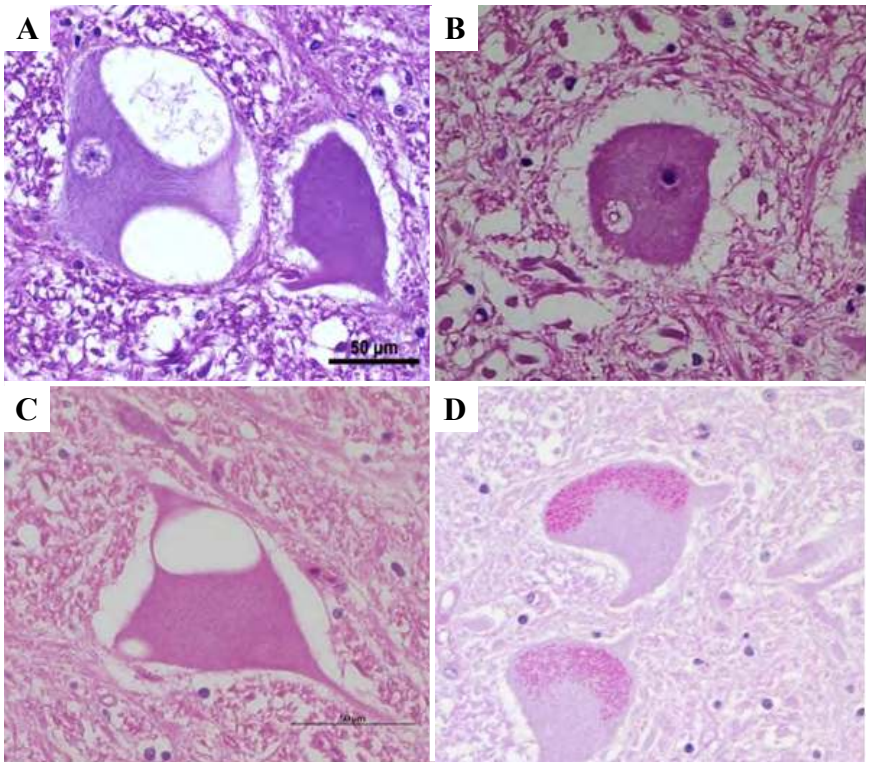

Figure 2. A. Intraneuronal vacuolation in the red nucleus of Male Sokoto Gudali 3½ years- Midbrain (HE x400). **B.** Early stage of intraneuronal vacuolation in the midbrain of a 2 years male Sokoto Gudali (HE x 400). **C.** Intraneuronal vacuoles in the solitary tract nucleus of a 1½ year old female Bunaji (HE x 400). **D.** Lipofuscin granules accumulation in hypoglossal nucleus of a 4½ years old female Sokoto Gudali (PAS x 400).

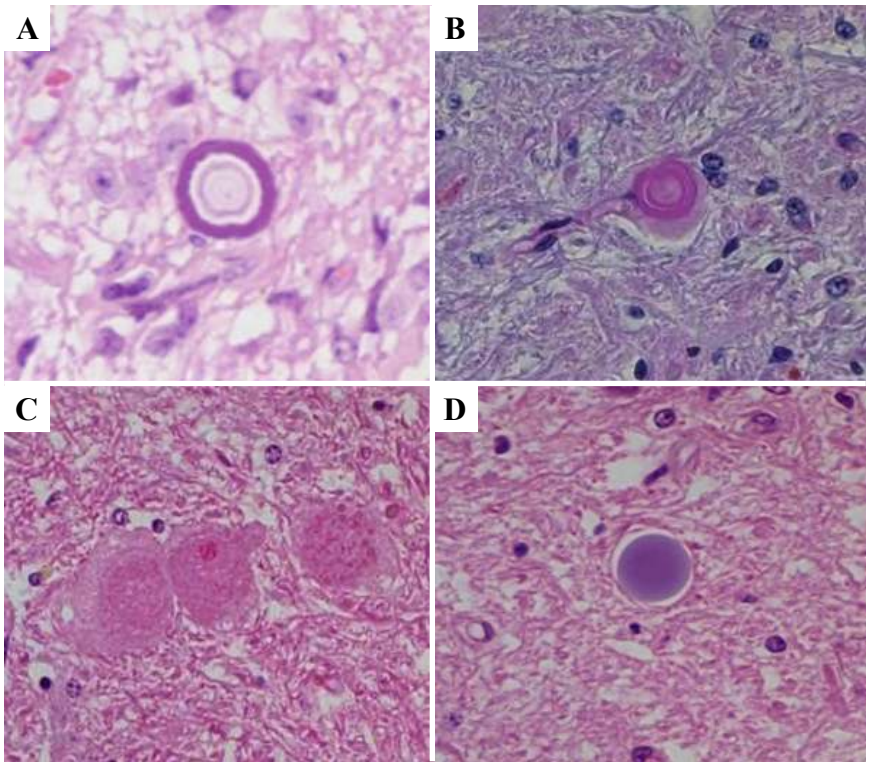

Figure 3. A. Psammoma body seating next to pinealocytes in the pineal gland of 2 years old female Rahaji (HEx400). **B.** Concentric body (Psammoma body) over laying a neuron in the medulla oblongata of 2 years old male Sokoto Gudali (PAS x 400). **C.** Axonal spheroids in the medulla oblongata of a 10 year old female Bunaji (HE x 400). **D.** Bluish smooth round body (Buscaino/Lafora body or mucocyte) in the medulla oblongata of 10 years old female Bunaji (HE x 400).

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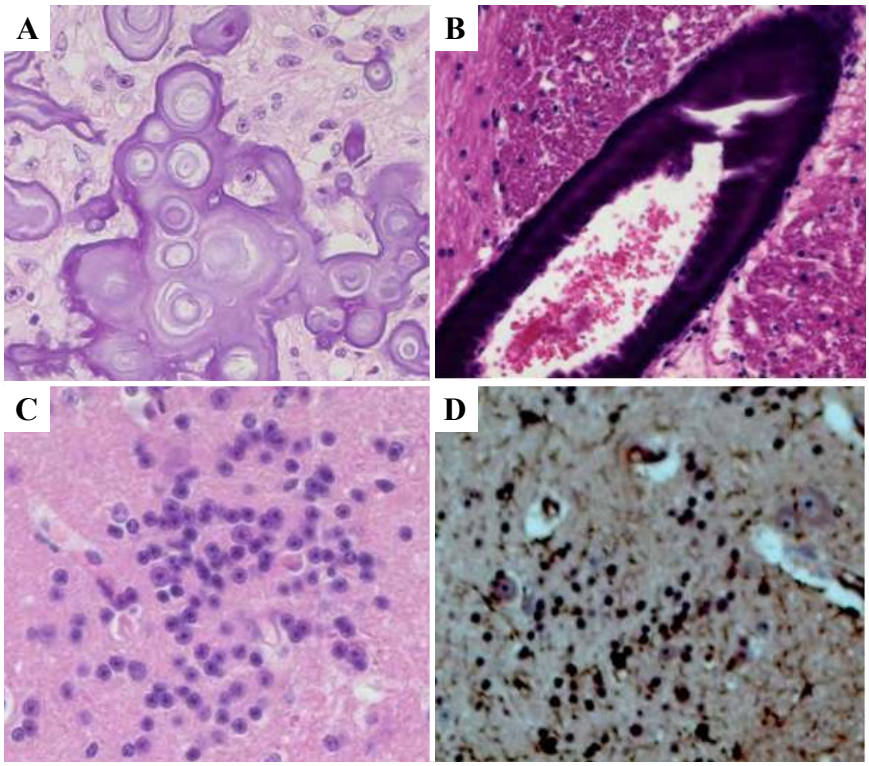

Figure 4. **A.** Corpora arenacea in the pineal gland of a 4 years old male Bunaji (HE x 400). **B.** Mineralized vascular wall and thickening in a 6 years old male Sokoto Gudali (HE x 400). **C.** Focal aggregation of heterogeneous progenitor/germinal cells in the basal ganglia of a 6 year old male Sokoto Gudali (HE x 400). **D.** Confirmation of heterogeneous progenitor/germinal cell aggregation. Granule cell-like and immature cells are shown, by Iba1 staining in the basal ganglia and cattle (IHC-Iba1 x 200).

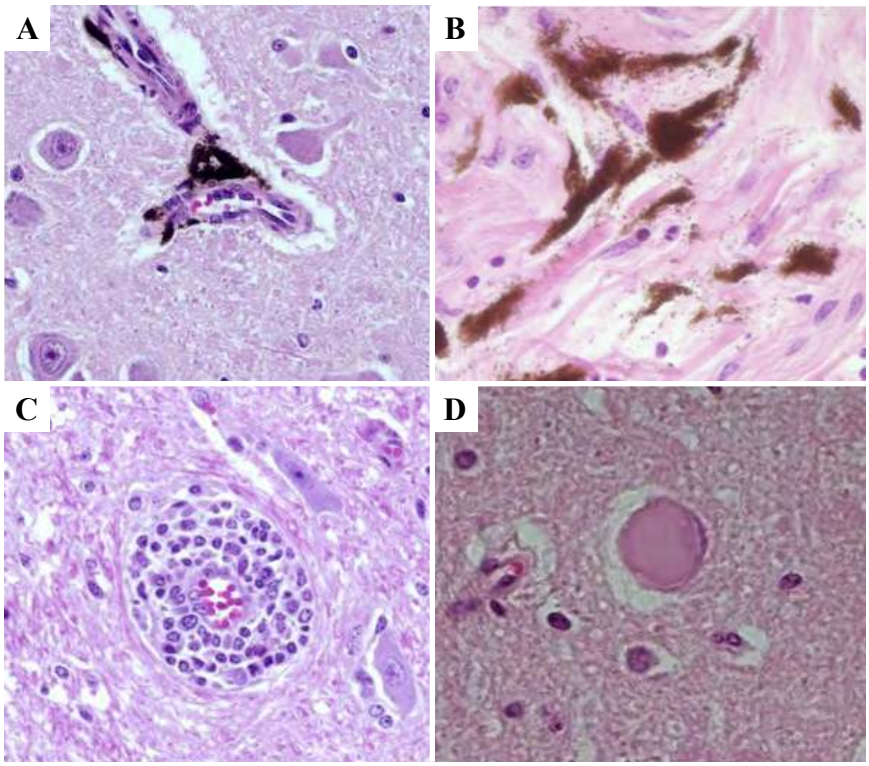

Figure 5. **A.** Melanosis arround the vessel in the hippocampus of a male Sokoto gudali ≥12 years (HE x40). **B.** Lepto-meningeal melanosis in a 3 years old male Muturu (HE x40). **C.** Lymphohistioplasmacytic perivascular infiltration with plasma cells in the thalamus of female Bunaji 3½ years (HE x 40). **D.** Chromatolytic neuron in the hippocampus of a ≥12 year old male Sokoto Gudali (HE x 40).

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Discussion

Eight coronal and hemi-coronal areas of medulla oblongata (at the level of the obex), midbrain, thalamo-hypothalamic area, hippocampus, basal ganglia, cerebrum, cerebellum and pineal gland were studied. These neuroanatomical areas represent important sites which comprise the population of neurons and glia cells vulnerable for neurodegerative conditions and or ageing processes in vertebrate animals. These also correspond to the neuroanatomical parts of the brain outline for neurologic studies in large animals (6, 14, 38, 45). Changes observed in this study were consistent with changes reported by Gavier-Waden et al.(15) who reported them as incidental findings in older cattle. It also corresponds to the report of Jahns et al. in horses (22). These changes were observed to start early in life (between the ages of 1½ to 4 years) in these breeds. This is probably due to prolonged exposure to the adverse tropical conditions and stress leading to a premature or early ageing in these breeds and corresponds to the report by Alegre (3).

Exposure to the high levels of ultraviolet radiation damages the DNA and fasten the ageing processes (2). The age-related cytoarchitectural changes observed consist of intracellular accumulation of substances involving intraneuronal lipofuscin deposition, leptomeningeal melanosis and intraneuronal vacuolations, neurodegeneration and axonal degeneration and loss, extracellular accumulation of substances involving corpora arenacea, Buscaino body, polyglycosan bodies, psammoma bodies and vascular wall mineralization. These changes started early in life in these animals which is at variance with the report of Jahns et al. (22) who reported these changes in older horses. It is probable that exercise can strengthen muscles, improve mobility, and reduce frailty even among older individuals. The same may be true for the brain harbouring significant potential for plastic change well into old age (45).

Intracellular vacuolations were all observed in neuronal cells and this depicts the vulnerability of neurons to injury or trauma in the nervous system; this was reported by Miller and Zachary (30) who stated that neurons are the most vulnerable cells in the body due to large requirement for energy. Lipofuscin storage is a well-known finding in aged nervous tissue and muscles of many species (13). Lipofuscin is a lipid pigment composed of peroxidized lipids and proteins with poorly understood metabolic effects (8, 18, 42). It is present in children and in young animals. In most domestic animals and in humans, some neuronal populations are prone to pigment storage (34). In dogs, early storage can be found in hypoglossal and oculomotor nuclei (7). Aged dogs have showed different degrees of pigment accumulation, a finding that was more pronounced in the older animals (33, 36). Lipofuscin accumulation is a product of oxidation of unsaturated fatty acids and may be symptomatic to membrane, mitochondria or lysosomal damage. Its accumulation is a result of imbalance between formation and disposal. Although lipofuscin storage does not induce cerebral dysfunction in young individuals, some deleterious effects cannot be ruled out in aged individuals in which lipofuscin are present in large amount. This possibility is reinforced when we consider ceroid lipofuscinosis, an inherited lysosomal disease described in many canine breeds. In this disease, lipofuscin storage is the pathogenic basis underlying functional disturbances, including visual and behavioral alterations and this also occurs in man (31).

Polyglycosan bodies (inclusion of PAS positive nature including buscaino bodies) were a constant finding in all aged cattle in this study. Of great concern was the severity of axonal spheroids and vacuolations which was common to all age groups of all the breeds. In most cases they were characterized by large foamy axonal swellings affecting dorsal rootlets and axons in the cuneate fascicle and gracile fascicle of the medulla oblongata and in agreement to the report by Borras et al. (7). Spheroids are swollen axons and can result from diverse insults, including trauma, hypoxia, intoxications, nutritional deficiencies, and storage disorders. No changes were observed that could give any indication of the origin of the swollen axons. Similar lesions occurred throughout the brain, being particularly severe in the trigeminal nerve radix, inferior cerebellar peduncle and pons as reported in Merinesco sheep (25). They have been reported as frequent incidental findings in sheep, cattle, dogs, pigs, man, and horses with no history of clinical neurologic disease (13).

Histopathological changes were observed at both intracellular (intracellular accumulation of lipofuscins, vacuolations and leptomelanosis) and extracellular levels (mineralization, fibrosis and *corpora arenacea* in pineal gland, vascular walls, psammoma and buscaino bodies) in the neuropil. The PAS positive intravacuolar granulated materials observed in one cattle corresponds to the description of "hybrid organelle" with accumulated polyglycosilated complex (44) observed in Alzheimer's disease and causes damages in the nervous system and gangliosides (20) leading to mental retardation, and death in early childhood. The neuronal vacuolations observed in younger cattle corresponds to the findings reported by Johnstone and Thomson (24) in 2 of 3 years old cattle.

There was increased lipofuscin accumulation within large neurons with age in all breeds. Neuronal lipofuscin accumulations were observed early in life of all breeds. Bilateral lipofuscin accumulation was uniquely observed in the olivary nuclei in all the cattle that express lipofuscin accumulations. Neuronal loss and chromatolysis were less observed in this study. Neuronal cell loss occurs due to necrotic or apoptotic processes, which are induced by mitochondrial or endoplasmic reticulum stress or the disturbance of autophagy or ubiquitin/proteasome systems. They however did not show age related significance and this is in agreement with the report by Wang and Michaelis (48)

that most brain regions do not suffer an age related neuronal loss. Psammoma bodies were recorded and correspond to the report by Hamir et al. (19) who reported the prevalence of 46% in lepto-meninges in racoons.

The non-age-related lesions observed included inflammatory changes involving lymphohistiocytic mononuclear cells, hypercellularity involving astrogliosis, microgliosis and spongy state changes. The infiltrates of lymphoid cells in perivascular spaces and nonsuppurative meningoencephalitis, were observed in 50% of the animals studied and these corresponds to the report of Butt et al. (9) who observed that the predominant findings in cynomolgus monkeys initially kept for toxicological studies were spontaneous changes. The multifocal areas of germinal and specialized ependymal cells observed in the basal ganglion correspond to the report by Wreiole (50), who observed nests of Island of Calleja, adjacent to lateral ventricles in Feline brain. However, the circumscribed choroid plexus within the parenchyma of the medulla oblongata was not reported. Multifocal, lymphohistioplasmacytic, perivascular infiltrations was very high and observed in 50% of the animals studied. Most findings were of slight or minimal severity, lacked an apparent cause, and were considered incidental and of negligible biologic significance.

Histopathological changes, some of which were age related, were identified in the brains of 233 cattle involving all the four breeds. All cattle studied were slaughtered for consumption and were without obvious clinical neurologic disease. There were no consistent differences in the distribution and frequency of non-age–related findings in cattle of all breeds. The fact that the non-age–related changes were very common throughout all breeds and sexes presents compelling evidence that such changes are present in Nigeria cattle brains, regardless of clinical history. This could also be as a result of the environmental factors such as toxicities from substances in the grazing field. This study compare well with previous studies on incidental and age-related neuropathological changes in cattle (15), horses (22) and other domestic animals (51). Focal to multifocal hypercellularity was also common in all breeds studied. These were observed as grave yards of dead neurons or around degenerating neuronal cells. Intracellular changes including neuronal vacuolations within the medulla oblongata, midbrain and in most cases with multiple vacuoles or with degenerative debries were observed.

Further evaluation of inflamatory changes for possible agents common within the study area such as rabies, pseudorabies and rift valley fever viral antigens using specific antibodies to identify currently common or unknown dangerous and/or zoonotic agents and to help increase awareness in human and animal health were negative for viral agents. We therefore conclude that most neuronal and cellular accumulations of substances although started early in life, were age related changes in these breeds of cattle.

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Conflict of Interest

The authors declare no competing interests.

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