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Sero-diagnostic studies on the occurrence and prevalence of bovine spongiform encephalopathies in Nasarawa state, Nigeria

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Abstract

The study was conducted to determine the occurrence and the prevalence of bovine spongiform encephalopathy among cattle herds in Nasarawa State, Nigeria using targeted sampling approach. The procedure of sampling adapted was a "double" targeted method for survey. First, a BSE risk group was targeted 'emergency or casualty slaughter' and within this Group, animals with signs indicative for BSE were sub-targeted. Two sampling locations were chosen; Akwanga central abattoir and Lafia central abattoir, both located in Nasarawa North and Nasarawa south senatorial district. Immediately the suspected animals were slaughtered, the caudal brain stem was harvested and examined for the disease specific form of the prion protein, PrPSc, using Western Blot technique after proteinase K digestion. A total of 2000 cattle of local breeds, aged ≥ 24 months and above were clinically examined. A total of 147 (7.4 %) of the cattle sampled were clinically suspicious for BSE. No sample was positive for BSE. Fluorescent antibody test for rabies and H&E staining on samples were carried out to observe for differential diagnosis. These showed no pathological lesions indicative for neurological disease. While our findings do not exclude the presence of BSE in Nasarawa State, we demonstrate that targeted sampling of ruminants for neuroinfectious disease is possible in developing countries, pointing to the possibility of implementing such a monitoring scheme in Nigeria to prevent economic losses in ruminant livestock as BSE caveats from endemic countries have shown.

Keywords: Bovine Spongiform Encephalopathy; Cattle; Occurrence; Dementia; Nigeria

1. Introduction

Bovine spongiform encephalopathy (BSE), popularly known as 'mad cow disease', is so named for the reason been that it affects the animal's nervous system, causing the animal with the disease to behave abnormally and lose control of its capacity to do normal activities, such as eating, walking and social interaction [1].

Bovine spongiform encephalopathy was discovered first in 1986 in United Kingdom [2]. Epidemiological studies recognized the carrier of this infection to be meat and bone meal (MBM) incorporated as a protein source in concentrated feedstuffs [3]. Also, specific risk materials (SRM) like animal brain, cerebrospinal fluid, spleen, tonsil etc have been incriminated. Although the number of cattle confirmed with BSE in the United Kingdom as of 2003 is greater than 180,000, the total estimated number of U.K. cattle potentially infected with BSE is in excess of 2 million [4].

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Not long after BSE was diagnosed in cattle, sporadic cases of BSE in exotic ruminants such as Kudus, Elands, Arabian Oryx, Ankole cows, Nyala, Gemsbock and Bison were also diagnosed in British zoos. One Zebu in a Swiss zoo was also BSE positive [5]. In the majority of these cases, exposure to animal feed produced with animal protein (and therefore potentially containing BSE infectivity) was either documented or could not be excluded. Moreover, there has been a long concern that sheep and goats could have been exposed to BSE via the oral route having been fed with meat and bone meal preparations from BSE infected cows. Besides, it has been experimentally demonstrated that BSE can be orally transmitted to small ruminants [6].

However, unusual or "atypical" cases of BSE are being reported by investigators from several countries. Atypical cases are distinct from previously found BSE and classified in two different forms based on biochemical characteristics, very low prevalence, aged affected animals with some of them displaying clinical signs These forms are: (i) a H-type that was first described in France [7] characterized by a higher apparent molecular mass of unglycosylated PrPres (ii) a bovine amyloid spongiform encephalopathy (BASE) that was first described in Italy on two animals [8]. However, the origin and possibility of natural transmission is unknown [9].

Unusual cases of BSE were considered an unexpected finding since it was previously believed that BSE disease in cattle was caused by a single strain of infectious agent, which has been shown to be very consistent and uniform in appearance, even after transmission to other species. The reports of unusual phenotypes of BSE in cattle suggest that different PrPSC phenotypes exist cattle with BSE [9]. Cases with atypical BSE have only been found in countries having implemented large active surveillance programmes. It is noteworthy that atypical cases have been found in countries that did not experience classical BSE so far, or in which only few cases of classical BSE have been found.

2. Material and methods

2.1. The Study Area

This study was carried out in Nasarawa State, Nigeria, a States located at north central part of Nigeria which is a center of livestock that passed through the middle belt and northern parts of the country. It is a part of the country noted for large expanses of montane grasslands. It is considered a hub for livestock traversing the middle belt and northern Nigeria. It also serves as a dispatch point for livestock to the eastern and Western parts of the country.

2.2. Samples Collection

The primary sample for BSE diagnosis was the obex region of the caudal brain stem. For confirmatory purposes the whole brain, spinal cord, lymph nodes, tonsils and spleen were also collected immediately the suspected animals were slaughtered according to the recommendation of [10]. Harvested samples were placed in separate specimen bottles, properly labeled before being placed in an ice pack and transported to National Veterinary Research Institute Vom Plateau State for processing and storage.

2.3. Sample Processing

From each caudal brain stem sample, two-gram (2g) of the obex region were cut out. The first portion was immersed in a bottle of formalin and kept for disease confirmatory purposes. The second portion was packed in specimen transport box (STB) containing dry-ice, likewise the remaining portion of the brain stem. The two-gram frozen portions were later shipped to University of Science and Technology Namibia for detection of disease specific form of prion protein, PrPSc.

2.4. Sample Analysis

Samples were analyzed using OIE approved testing procedures for BSE's, employing approved OIE rapid tests for BSE [10]. Samples were analyzed using Prionics western blot kit, a rapid western blot test developed for BSE. The test was carried out according to the manufacturers guide after sample preparation and proteinase K digestion. Positive samples were further confirmed by carrying out gel electrophoresis in line with the method of [11]; [10] and immunohistochemistry (IHC) staining [12] of tissue sections prepared from formalin fixed tissues.

2.5. Controls

Positive and negative controls using confirmed BSE positive and negative samples were set up alongside kit controls during testing.

3. Results and discussion

A total of 2000 cattle, 1000 each from Akwanga and Lafia central abattoir brought in for emergency/casualty slaughter were clinically examined. A total of 147 (7.4 %) of all cattle examined were identified as BSE suspicious (Tables 1 and 2). Five breeds of cattle were identified and examined amongst all cattle brought in for emergency or casualty slaughter. They were all aged ≥ 24 months which qualified them to be in this sample group.

Using ataxia as focal points, these criteria identified the following as clinical suspicious cattle: Akwanga central abattoir: Bunaji (15 males and 5 females), N'dama (23 males and 3 females), Muturu (11 males and 4 females), Keteku (4 males and 2 females) and Kuri (8 males and 2 females) giving total of 77 (7.7 %) clinically suspicious cattle for BSE (Table 1). Lafia central abattoir, we identified: Bunaji (16 males and 3 females), N'dama (15 males and 3 females), Muturu (14 males and 2 females), Keteku (7 males and 1 females) and Kuri (7 males and 2 females) giving a total of 70 (7.0 %) clinically suspicious cattle for BSE (Table 2).

Laboratory testing using Prionics®-Check Western to screen for BSE in cattle revealed that none of these suspected cattle were positive for BSE (Table 3). Samples collected from clinically suspicious livestock were subjected to Fluorescent Antibody test for the possible identification of an underlying rabies disease that could be differential to prion disease. All samples gave a negative result as compared to control samples. None of them showed green oval or ellipsoidal fluorescing virus particles on examination which is diagnostic for rabies using the fluorescent microscope (Table 5). Bearing in mind that typical signs are not consistently expressed in animals reaching the clinical phase of BSE and can be confused with those of other diseases a search for such other neurologic diseases apart from that of rabies was undertaken. This search via H&E staining on brain stem of sampled livestock gave negative results. (Table 5).

Table 1 Demographics and Clinical Examination of Cattle for BSE in Akwanga central Abattoir.

Demographics/ Clinical examination parameters		**Breeds (Number examined)**										**Total**
		Bunaji (507)		N'dama (275)		Muturu (115)		Ketetu (70)		Kuri (33)		1000
		M	F	M	F	M	F	M	F	M	F	
Demographics												
	Age (≥23 months)											1000
	Cohort data/herd size											Nil
	Anecdotal report											Nil
	Emergency/casualty slaughter	471	36	249	26	96	19	58	12	29	4	1000
Clinical Examination												
1) Changes in mental status	Self-isolation	40	2	11	2	20	4	20	7	12	7	125
	Disobeys herdsman's call	29	1	15	3	25	2	5		11		91
	Flighty on approach	29	4	15	4	25	2	5		11		95
	Hyper-alert	41	3	18	5	24	10	52	7	13	5	178
	More excitable											Nil
	Exaggerated responses to external stimuli	41	1	18	6	24	10	4	7	13	5	129

	Somnolent /dull/vacant/gazed/lowered head	21	2	5	7	15	3	4	7	11	3	78
	Often lies down	15	3	5	8	25	1	7	4	11	5	84
	Reduced social interaction	29	4	15	9	25	2	5	5	11	5	110
	Frequent teeth grinding	40	5	12	2	11	3	5	2	11	4	95
	Loss of appetite	30	15	105	3	112	10	15	5	16	8	324
2) Puritic Activities	Increased grooming behavior											Nil
	Rubs on objects frequently											Nil
	Licking and smacking of mouth	19	6	13	2	9	3	5	2	7	4	70
	Nibble reflex response											Nil
	Rapid tongue extrusion	29	7	11	4	17	4	26	7	11	7	122
	Poor response to scratch test											Nil
	Body discoloration											Nil
3) Postural changes	Abnormal hind limb stance	40	8	13	2	9	4	4	2	8	2	92
	Lowered hind with flexed limbs	40	9	13	1	9	4	4	2	8	2	92
4) Gait changes	Ataxia	15	5	23	3	11	4	4	2	8	2	77
5) Involuntary movements	Head tremors	29	2	20	5	5	1	2	5	1	4	74
	Seizures											Nil
6) Weight loss	Weight loss	300	15	151	6	112	10	16	7	30	5	652
7) Others	Increased salivation	19	3	13	1	9	3	5	2	7	4	66
	Ruminal fluid dribbling	19	4	13	1	9	3	5	2	7	4	65
	Cardiac/respiratory abnormalities											Nil
	Ruminal impaction											Nil
Clinically positive (%)		15 (1.5	5 (0.5)	23 (2.3)	3 (0.3)	11 (1.1)	4 (0.4)	4 (0.4)	2 (0.2)	8 (0.8)	2 (0.2)	77 (7.7)

Key: M=male, F=female

Table 2 Demographics and Clinical Examination of Cattle for BSE in Lafia central Abattoir.

Demographics/ Clinical examination parameters		**Breeds (Number examined)**										**Total**
		Bunaji (392)		N'dama (305)		Muturu (203)		Keteku (96)		Kuri (54)		1000
		M	F	M	F	M	F	M	F	M	F	
Demographics												
	Age (≥24 months)											1000
	Cohort data/herd size											Nil
	Anecdotal report											Nil
	Emergency/casualty slaughter	347	45	278	27	185	18	83	13	46	8	1000
Clinical Examination												
1) Changes in mental status	Self-isolation	20	9	10	17	19	9	17	5	14	4	134
	Disobeys herdsman's call	10	6	13	1	11	9	21	2	10	2	84
	Flighty on approach	21	12	13		12	4	6		9		77
	Hyper-alert	11	15	14	16	13	19	10	10	19	10	137
	More excitable											Nil
	Exaggerated responses to external stimuli	17	9	5	16	16	6	16	10	13	3	111
	Somnolent /dull/vacant/gazed/lowered head	67	58	10	19	6	17	16	5	9	5	212
	Often lies down	21	10	21	11	17		10	15	14	5	124
	Reduced social interaction	12	10	13	14	16	14	19	10		3	111
	Frequent teeth grinding	15	13	14	4	13	16	12	6	5	2	100
	Loss of appetite	200	12	13	31	23	13	9	6	4	2	313
2) Puritic Activities	Increased grooming behavior											Nil
	Rubs on objects frequently											Nil
	Licking and smacking of mouth	1	12	17	14	16		11	9	10	5	95
	Nibble reflex response											Nil
	Rapid tongue extrusion	19	9	23	9					6	3	69
	Poor response to scratch test											Nil
	Body discoloration											Nil
3) Postural changes	Abnormal hind limb stance	16	15	10	7	3	2	2	3	2	1	61
	Lowered hind with flexed limbs	16	15	10	7	3	2	2	1		1	57

4) Gait changes	Ataxia	16	3	15	3	14	2	7	1	7	2	70
5)Involuntary movements	Head tremors	10	5	8	5	5	4	9	7	14	1	68
	Seizures											Nil
6) Weight loss	Weight loss	21	12	23	14	22	11	4	3	10	5	125
7) Others	Increased salivation	19	10	21	10	16	7	15	5	7	2	112
	Ruminal fluid dribbling	10	5	7	3	7	7	10	5	12	6	72
	Cardiac/respiratory abnormalities											Nil
	Ruminal impaction											Nil
Clinically positive (%)		10 (1.1)	9 (0.9)	10 (1.0)	5 (0.5)	7 (0.7)	6 (0.6)	4 (0.4)	4 (0.4)	10 (1.0)	5 (0.5)	70 (7.0)

Key: M=male, F=female

Table 3 Prionics Western Blot (PWB) Test on Cattle Brain Stem for BSE from Akwanga central Abattoir.

Diagnosis	**Breeds (Number examined)**										
	Bunaji		N'dama		Muturu		Keteku		Kuri		Total
	507		275		115		70		33		1000
	M	F	M	F	M	F	M	F	M	F	
Clinically Positive Cattle (%)	15 (1.5)	5 (0.5)	23 (2.3)	3 (0.3)	11 (1.1)	4 (0.4)	4 (0.4)	2 (0.2)	8 (0.8)	2 (0.2)	77 (7.7)
PWB test result (%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Key: M=male, F=female

Table 4 Prionics Western Blot (PWB) Test on Cattle Brain Stem for BSE from Lafia central Abattoir.

Diagnosis	**Breeds (Number examined)**										**Total**
	Bunaji		N'dama		Muturu		Keteku		Kuri		
	155		123		103		71		48		1000
	M	F	M	F	M	F	M	F	M	F	
Clinically Positive Cattle (%)	16 (1.6)	3 (0.3)	15 (1.5)	3 (0.3)	14 (1.4)	2 (0.2)	7 (0.7)	1 (0.1)	7 (0.7)	2 (0.2)	70 (7.0)
PWB test result (%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Key: M=male, F=female

Table 5 FAT and H&E test on Cattle Brain Stem for Differential Diagnosis from Akwanga central abattoir.

Diagnosis	**Bunaji (507)**		**N'dama (275)**		**Muturu (115)**		**Ketetu (70)**		**Kuri (33)**		**Total (1000)**
	M	F	M	F	M	F	M	F	M	F	
FAT	0		0		0		0		0	0	
H&E test	0		0		0		0		0	0	

Key: M=male, F=female

Table 6 FAT and H&E test on Cattle Brain Stem for Differential Diagnosis from Lafia central abattoir.

Diagnosis	**Bunaji (155)**		**N'dama (123)**		**Muturu (103)**		**Ketetu (71)**		**Kuri (48)**		**Total (1000)**
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	
FAT	0		0		0		0		0	0	
H&E test	0		0		0		0		0	0	

Key: M=male, F=female

4. Discussion

A "double" targeted method of sampling was used in this research. First the risk groups emergency/casualty slaughter animals were targeted and within this group those animals with signs indicative for BSE were sub-targeted. Cattle with two permanent central incisors were considered to be over 24 months. This was in line with guideline provided by [10].

Clinical examinations led to the identification of 96 (9.6 %) BSE suspicious cattle in Akwanga central Abattoir (Table 1) and a total of 91 (9.1 %) BSE suspicious cattle in Lafia central abattoir (Table 2).

Laboratory testing of all suspected cattle revealed that none (0 %) of the clinically suspicious animals were positive for BSE. This finding is in line with the results of [14] and [4] which showed that none of the samples taken from clinically suspicious animals were positive for BSE.

The public health important of this study's findings may finger a disease free status for vCJD with minimal risks of contraction, owing to the absence of confirmed cases of BSE's in the study area but it should always be borne in mind that BSE risk can still exist in a country even if no cases are found with surveillance as reported by [15].

The only differential disease with a similar sample type to BSE is rabies hence fluorescent antibody testing for rabies was carried out on all the samples collected. Examining for neuropathology to rule out the other aforementioned neurologic diseases H&E staining on brain stem sections was carried out. These analyses gave a negative result for rabies (Table 5) and no signs of neuropathology from H&E staining. This also agrees with the procedures provided by provided by [13].

This study lays the groundwork for an efficient and cost-effective surveillance of scrapie and other neuroinfectious diseases of livestock in resource-limited countries, such as Nigeria. This we have demonstrated by systematic ante mortem inspection of emergency and casualty slaughter animals that would have identified neurologically diseased small ruminant livestock in a relatively high proportion. We advocate the use of our findings as a platform for the setup of an active surveillance for scrapie and other animal BSEs in Nigeria so as to determine the scrapie status for economic reasons.

5. Conclusion

This study did not identify any BSE disease in the study area implying that the public health implication of this zoonosis may be temporarily overlooked, though subject to a wider survey. Methods employed 'clearly indicated that neurologically diseased livestock can be identified by systematic ante mortem inspection of emergency and casualty slaughter livestock in a relative high proportion. This lays the groundwork for an efficient and cost-effective surveillance of BSE and also other neuroinfectious livestock diseases in resource limited countries, such as Nigeria.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors whose names are mentioned hereby declare that they have no conflict of interest in this research article and that in case any of such comes up, it will be resolved hitch-free. The authors also declare that this research is solely sponsored by them without any external intervention.

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