British Journal of Medicine & Medical Research
13(8): 1-6, 2016, Article no.BJMMR.21721
ISSN: 2231-0614, NLM ID: 101570965

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Transmissible Spongiform Encephalopathy and Cattle in Enugu State of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author FOU designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors CNS and RCD managed the literature searches and analyses of the study. Author FOU managed the experimental process. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/21721
Editor(s):
(1) Mari Nevas, Helsinki University, Finland.
Reviewers:
(1) Anonymous, National Veterinary Research Institute, Nigeria.
(2) Anonymous, Hospital "F. Perinei", Altamura, Italy.
(3) Panchanan Maiti, University of Tennessee Health Science Center, Memphis, USA.
(4) Dejun Yang, University of Maryland, Baltimore, USA.
Complete Peer review History: http://sciencedomain.org/review-history/13181

Original Research Article

Received 31st August 2015
Accepted 16th January 2016
Published 6th February 2016

ABSTRACT

Bovine spongiform encephalopathy (BSE) is the Transmissible spongiform encephalopathy (TSE) found in cattle. The disease can be transmitted by ingestion of prion infected beef. The aim of this study is to determine whether the beef consumed in Enugu area of Nigeria can transmit TSE to humans and animals. Apparently healthy cattle from abattoir of Enugu State Main Market, numbering eighty, were used in this research work. The cattle were slaughtered and their brain tissues collected and stored in 10% phosphate buffered saline respectively for tissue preservation, shortly before assay. Part of each cattle's brain tissue was homogenized and subjected to prion protein (PrP) extraction procedure. Aliquots of the PrP extract and commercial positive PrP^c control

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were treated with proteinase K solution to eliminate proteinase K labile proteins, especially cellular prion protein (PrP^c). An aliquot of the proteinase K treated extract was further treated with 26.8% Sodium sulfate solution and ethyl ether to remove any immunoglobulin protein which may be present thereby leaving the remnant solution with abnormal prion protein (PrP^{res}) if present. Aliquots of the protein extract and control without proteinase K treatment, the ones that were treated with proteinase K, the other that was treated with 26.8% Sodium sulfate solution and ethyl ether were assayed respectively for proteins using biuret method. The results showed the presence of prion protein in the aliquots without proteinase K treatment, trace of protein in the aliquot of protein extract treated with proteinase K and no prion protein in the PrP^c control treated with proteinase K. The aliquot that was treated with both proteinase K and 26.8% Sodium sulfate solution / ethyl ether showed zero protein. The results suggest that there was no abnormal PrP in the eighty samples of brain tissues of cattle used for this work and they did not have BSE.

Keywords: Spongiform encephalopathy; transmissible; prion; prion protein; cattle.

1. INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) are a group of progressive disease conditions that affect the brain and nervous system of many animals, including humans [1,2]. They are also known as prion diseases [2]. They are transmitted by prions. Prions are the abnormal forms of prion protein. Prions are also the only infectious pathogens containing protein but apparently lacking nucleic acid. Prions are also the only protein with the ability to transmit biological information through the propagation of alternative protein folding without changes in the genome [3,4]. Unlike the normal prion protein (PrP^c), which does not cause any harm, the misfolded prion protein called prion, designated as PrP^{res}, causes a number of neurological diseases. In some prion infected brain, large deposits of prions are found in form of plaques which are believed to be the result of the attempts by the brain to detoxify the infectivity of prions [5]. The formation of prion (PrP^{res}), the infectious prion protein could occur when PrP^{res}, also known as the misfolded cellular prion protein, attach to normal cellular prion protein (PrP^c). This prion could accumulate in the brain in form of plaques [6].

The accumulation of prion in form of plaques result to the TSE diseases. The type of TSE may depend on the subject infected [2,3,7]. TSE can be transmitted either by inoculation or ingestion [1]. It can also occur by genetic mutation and be inherited [1].

TSE can be identified in various forms: as scrapie in sheep, Bovine Spongiform encephalopathy (BSE) in cattle, Creutzfeldt Jacobs Disease (CJD), Kuru, Gerstman Straussler Schemker Syndrome (GSSS), Variant Creutzfeldt Jacobs disease (VCJD) and Iatrogenic TSE in humans [8]. Also identified among these TSE include chronic wasting disease (CWD) in deers and elks, fatal familial insomnia (FFI) in humans and Transmissible Mink encephalopathy (TME) in ranch raised minks. In addition to their presence in sheep, cattle, elks and deer and humans, TSEs occur in many other different animal species including non-human primates, mice, hamsters, rats, guinea pigs, goats, pigs, cats and a variety of exotic felines and bovid in zoos [8].

Bovine spongiform encephalopathy (BSE) is the TSE disease found in cattle. Since 1986, the BSE epidemic in United Kingdom has focused international attention on TSE family of diseases. Although the origin of BSE was said to be unknown, it was first recognized among cattle in United Kingdom in 1986. An epidemic of BSE in United Kingdom was noted to be caused by the recycling of processed waste parts of cattle, some of which were infected with BSE agent [9]. BSE was said to be derived from an unusual strain of scrapie or represented cattle TSE disease present in a low level as to have escaped detection previously [10]. However, several laboratory tests have identified similarities in BSE from sources tested, in contrast to most commonly known isolates of feeding of protein supplements contaminated with rendered tissues of BSE positive cattle. Changes in rendering processes such as exchanging of fat extraction by organic solvents and switching from batch heating to continuous flow heating apparently led to the survival of sufficiently infectivity in the final meat and bone meal products, to allow transmission by feeding these materials to other cattle [11]. Predominant clinical signs of TSE in cattle include gait ataxia and changes in behaviour or personality such as aggressiveness or wariness.

Infectivity and PrP^{res} are found almost exclusively in nervous system; especially the brain, spinal cord and the lymphoid tissues [12,13].

The disease predominantly affects dairy cattle, presumably because these cattle are fed more with the contaminated high protein supplement than other classes of cattle. In cattle, the age of onset of BSE is 2-5 years whereas the human TSE age onset of disease is variable, depending on the type of TSE, ranging from 30-60 years, with an average of 50 years. The incubation period may present a minimum of six months. Death usually occurs between 7 and 36 months from onset [11,14].

Since TSE is also a burning issue among some incurable diseases menacing animals and humans today, it became necessary to investigate cattle in Enugu area of Nigeria for prion and BSE. Majority of the cattle in this area pass through Enugu Main Market Slaughter house for the meat of the people. This work therefore aims at screening cattle in Enugu metropolis to determine whether there are abnormal prion proteins for possibility of TSE disease.

Pathological prion protein implicated in TSE is detected by antibody based tests or bioassay and histological demonstration of prion plaques in tissues. These techniques include the Western blot [15], Enzyme linked immunosorbent assay (ELISA) [16], Immunopolymerase chain reaction (IPCR) [17] and Immunohistochemistry [18]. Although not yet prevalently used, the Enhanced quaking-induced protein conversion (Equic) method seem to be the latest. This is a prion disease blood test using immunoprecipitation and quaking- induced technique [19].

However, in this work, after the extraction of PrP using the technique of Grassi et al. [20], we combined the Proteinase K digestion technique with the biuret method [21,22]. Prion protein has to be extracted from brain tissues before applying the methods because it is a cell membrane protein.

2. MATERIALS AND METHODS

Among the cattle in the Enugu State main Market abattoir, eighty of them within the average age of one and half years were used for this study. The cattle used were certified apparently healthy by the veterinary doctors in that abattoir. With a view to ruling out or finding abnormal prion protein, the cattle were screened for prion proteins using a combination of two techniques, Proteinase K resistant method according to Reza et al. 2008 and Biuret method. The standard biuret colour developing reagents were used along with 26.8% Sodium sulphate and ethyl ether to be able to remove immunoglobulin if present and estimate the PrP in the extract [21,23].

After the usual slaughter of the cattle for public meat, the brain tissue samples were collected (Usually from the obex region of the hind brain). Four grams of each cattle brain tissue was collected into a 10% phosphate buffered saline shortly before the prion protein extraction. PrP was extracted using a mixture of detergents prepared as extraction buffer. The buffer was made up of; 10 mM TrisHCl (P^H7.4), 0.1M NaCl, 0.01M EDTA, 0.5% IGEPAL CA-630 (SIGMA ref 1-3021), 1% Deoxicholic acid and 0.01% Sodium azide, as stated in 'SPIbio kit' [20]. Extraction was done by homogenization of the tissues in the buffer by violent agitation using, homogenizers (Ribolyser and Moter) [20]. Subsequently,the prion protein extract was separated from the tissues with centrifugation at 5000 rpm for 10 minutes.

An aliquot of the PrP extract and PrP commercial control were treated with 60 ug/ml of proteinase k solution and incubated at 37^{oc} for 40 minute. An aliquot of the test sample treated with proteinase k was further treated with 26.8% Sodium sulfate and ethyl ether to precipitate immunoglobulin if present and reduce its density respectively. Centrifugation was applied to separate the immunoglobulin precipitate from the supernatant expected to contain the PrP^{res} if present. Aliquots of the test and control samples not treated with proteinase k were reacted with biuret colour reagent respectively. Another aliquot of test and control samples respectively, treated with proteinase k were also reacted with biuret colour reagent. Finally, the third aliquot treated with proteinase k, 26.8% Sodium sulfate and ethyl ether was again reacted with biuret colour reagent. Their respective optical densities were determined spectrophotometrically at 520 nm wavelength and their protein concentrations estimated.

3. RESULTS

The data obtained from the assay on PrP extract and the PrP control without proteinase k treatment, the ones treated with proteinase k and the sodium sulfate/ether solutions as stated in the materials and methods are as shown in the Tables 1, 2 and 3.

Table 1. Mean PrP concentration of brain tissue extract and control

Parameter	PrP after extraction n = 80	PrP^C control n = 2
Prion protein (ug/l)	1.2±0.04	2.3±0.01

Table 2. Mean PrP concentration of brain tissue extract and control before and after treatment with proteinase k (pk)

Parameter	PrP after extraction n = 80	PrP of extract after pk treatment n=80	PrP^c control n = 2	PrP^c of control after pk treatment n=2
Prion protein (ug/l)	1.2±0.04	0.4±0.01	2.3±0.01	0±0

Table 3. Mean PrP concentration of brain tissue extract, the exract after pk treatment and the extract after treatment with pk, 26.8% sodium sulfate and ethyl ether

Parameter	PrP after extraction n = 80	PrP after treatment with pk n=80	PrP after treatment with pk, 26.8% sodium sulfate & ether n=80
Prion protein (ug/l)	1.2±0.04	0.4±0.04	0.0±0

4. DISCUSSION

Although the combination of techniques used to determine prion protein in this research work did not entirely follow any of the conventional methods for determination of prion protein such as Western blot, Enzyme Immunosorbent assay or Immunohistochemistry, they still followed established scientific principles for determination of proteins of which prion protein is one. The prion protein extraction protocol used, agreed with the principle that because prion protein is a cell membrane protein, it has to be extracted from brain tissues using a mixture of buffered detergent [24]. The use of proteinase k, 26.8% Sodium sulfate and ethyl ether is in agreement with the principle of proteolytic enzymes for digestion of cellular prion protein (PrP^c) and the removal of high molecular weight immunoglobulins from mixed solution which may contain non proteinase k digestible abnormal prion protein, PrP^{res} [22,23]. The application made with biuret method is in consonance with the principle that peptide bondages react with biuret reagent and are spectrometrically or colorimetrically detectable for determination of concentration [21,23]. Due to the fact that prion protein has peptide bondages and other properties referred to above, attempt on the combination of the techniques used in this research work, resulted to this scientifically discussable results.

The value of prion protein of the extract and that of PrP^c commercial control as shown in Table 1, presented evidence of PrP in the brain tissues of the eighty cattle used for this work [8,20]. Prion ptotein is expressed predominantly in the brain, spinal cord and lymphoid tissues [13] and the result in Table 1 also agrees that the extraction buffer used was functional. The result of this work as shown in Table 2 indicated that there was proteolysis or digestion of PrP^c by the proeinase k used. PrP^c is readily digested by proteinase k like other common proteins [8]. The same table also showed that although there was digestion of PrP after treatment with proteinase k, there was a remnant of undigested protein in the brain tissue extract. The protein remnant may have been the proteinase k resistant PrP^{res}, high molecular weight immunoglobulin or both as reported by Reza et al. [22]. The result of the brain tissue protein extract treated with proteinase k, reacted with 26.8% Sodium sulfate and ethyl ether as seen in Table 3, showed that there was no form of protein left over. This suggests that the remnant of protein after treatment with proteinase k as indicated in Table 2 may have been the high molecular weight globulin and not PrP^{res} [22]. If prion, the abnormal PrP was present in the brain tissue protein extract, there wouldn't be a zero protein result after treatment with proteinase k, and the removal of globulin with 26.8% Sodium sulfate, ether and centrifugation [22].

It is pertinent to note that any finding in this work is important because the presence of cellular prion protein ensures the integrity and hemodynamic activity of brain cells and vessels, respectively. Also the presence of prion, presents a possibility of not only destabilizing the integrity

of the brain cells but the formation of prion plaques which may cause cerebral vein stenosis, blockage of flow of internal jugular vein and reversal of cerebral venous outflow [25,26,27].

Although the techniques used in this research work may need confirmation by a universally accepted method, the data in this work above show that prion, the only proteinaceous infectious particle that can cause TSE in vertebrates and BSE in cattle was not found in the extract. This work therefore suggests that the cattle used did not have PrP^{res}. This implies that beef meat consumed in Enugu area of Nigeria have no BSE and cannot transmit TSE to human and animal subjects in this area.

5. CONCLUSION

We therefore conclude that:

1. Since prion protein extraction procedure used in this study was a research acceptable procedure, the content of the extract was prion protein and color detectable reaction with biuret reagents was observed.
2. Following the data obtained from the technique used, BSE marker in cattle, the PrP^{res}, was not detected in the cattle used. It may be said that the beef meat consumed in Enugu area of Nigeria does not have BSE, therefore, cannot transmit TSE to human and animal subjects in this part of the country. However, a confirmation may be needed by a universally accepted method.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13181