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Active and passive surveillance of bovine spongiform encephalopathy in Bangladesh

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Abstract

The aim of the present study was to investigate whether Bovine Spongiform Encephalopathy (BSE) is present in this country and to analyze the Global BSE Risk (GBR) status in Bangladesh. A total of 2,000 brain samples were collected from cattle older than 30 months of age, slaughtered for human consumption in the district slaughter houses from 2005 to 2006. The brainstem (obex), Pyriform lobe, cerebrum and cerebellum were subjected to histopathological study. Samples that showed some nonspecific lesions were subjected to immunohistochemistry and only brain stem to ELISA for the detection of abnormal prion protein PrP^{sc}. In passive surveillance, annual overall diseases of cattle, buffalo, sheep and goats in Bangladesh were collected from Department of Livestock Services (DLS), Dhaka to investigate the occurrences of neurological diseases. Import related data were collected from "National Export Promotion Bureau" Kawran Bazar, Bangladesh Bank and DLS to analyze the importing products of animal origin (cattle, buffalo, sheep and goats) from different countries to find whether or not the imported products posed any risk for the BSE. In an actire surveillance conducted in slaughter house, histopathologically BSE specific lesions were not detected in any of the brain samples, but other nonspecific lesions were observed. No PrP^{sc} was detected from the samples by immunohistochemistry and ELISA. DLS report also supported the absence of BSE in cattle and buffalo and scrapie in sheep and goats in Bangladesh. It was also clearly recorded that Bangladesh imported livestock products from countries in GBR level I and II but not from countries in GBR level III and IV. From this study it apparently seems that BSE is not currently present in the indigenous animals in Bangladesh and poses no or negligible risk to human and animal health.

Key words : Bovine spongiform encephalopathy (BSE), Histopathology and immunohistochemistry, Brain tissues, Active and passive surveillance

INTRODUCTION

Bovine Spongiform Encephalopathy (BSE), commonly known as "Mad cow Disease" is a transmissible neurodegenerative diseases of cattle over 30 months of age (Wells et al, 1987). BSE was first observed in Great Britain in April 1985 and was officiallydiagnosed in November 1986 (Wells et al,1987; Kimberlin, 1992). BSE belongs to the group of Transmissible Spongiform Encephalopathies (TSEs), together with scrapie of sheep, a chronic wasting disease of free-ranging and captive deer and elk, feline spongiform encephalopathy and

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Creutzfeldt-Jakob disease (CJD) and Kuru of humans (Gabizon and Prusiner, 1990; Prusiner 1991; Weissmann, 1991a, 1991b). The disease has a causal link with an old disease "Scrapie" which has been prevalent in sheep for over 200 years (Almond and Pattison, 1997; Chowdhury, 2005). Most scientific evidence suggests that prions are the causative agent of BSE (Prusiner, 1982). Transmission of BSE is thought to primarily occur through ingestion of feedstuff, especially ruminant-derived meat-and-bone meal (MBM), contaminated with the BSE agent (Wiles-mith et al, 1992; Wilesmith et al, 1988). BSE was linked with a human disease "Varient Creutzfeldt-Jakob Disease" (vCJD) linked to humans eating of contaminated beef products infected with BSE. Most of the developed coun-tries of the world impose restriction on importation of livestock products. The developed countries want to know the status of BSE surveillance in the exporting country prior to export to exclude the risk of BSE (Prusi-ner, 2000; Vanopdenbosch and Roels, 2004; Chowd-hury, 2005).

Bangladesh is a Muslim populated country. So beef-meat is very popular in this country. Various commodities produced from livestock by-products (e.g. bone, hoofs, horn, gelatin) was also exported to different countries of the world.

Although Bangladesh is free from BSE, it is not recognized by other countries due to the lack of official BSE surveillance. For this reason nationwide official surveillance program is required. This study reports a pilot surveillance of BSE in Bangladesh.

MATERIALS AND METHODS

Study area

Study was conducted in three districts slaughter houses of Bangladesh namely Dhaka (Centre of the country), Mymensingh (North east) and Barishal (South east).

Collection of samples

A total of 2,000 brain samples were collected from cattle older than 30 months of age, slaughtered for human consumption. Out of 2,000 samples 1,000 samples from Dhaka, 500 samples from Mymensingh and 500 samples from Barishal. The brainstem (obex), Pyriform lobe, pieces of cerebellum and cerebrum were collected for histopathology and immunohistochemistry and the brainstem (obex) alone for ELISA.

Transportation and storage of samples

The samples after collection was kept in ice box containing sufficient amount of dry ice packs. The samples for histopathology and immunohistochemistry werekept in 10% neutral buffered formalin and the samples for ELISA were kept in −20°C until use.

Histopathology

The formalin fixed sections were trimmed, processed, paraffin-embedded, sectioned and were routinely stained with Hematoxylin and Eosin (H and E) as per standard procedure (Luna, 1968).

Immunohistochemistry

Immunohistochemistry was done by Labelled Streptavidin-Biotin (LSAB) Method using: DAKO Chem-Mate™ detection kit. Anti PrP^{sc} monoclonal antibody (mAb 6H4) a mouse IgG1 subtype antibody: Light chain: K subtype with a dilution of 1 : 800 (Prionic AG, Switzerland) was used. The reaction was visualized using the AEC. Parallel negative and positive controls were run in each time. The formalin fixedpositive control was collected from Institute of Veterinary Pathology, University of Zurich, Switzerland.

ELISA

Samples that showed some non-specific lesions were subjected to ELISA and were done by the BetaPrion® BSE EIA test kit (Roboscreen, Germany) which consists of two modules, the BetaPrion® BSE Purification Kit, which includes the purification tools and the Beta Prion® BSE Detection Kit, which is based on a sensitive ELISA.

Quality of data

In suspected cases ELISA and immunochemistry was done. When more confusion arises then the samples were compared with the positive BSE control slide.

Clinical data

Data collection

Clinical data on various diseases of cattle, buffalo, sheep and goatswere provided by the Department of Livestock Services (DLS), Dhaka, Bangladesh, in order to get precise and comparable data on neurological disease incidence.

Data analysis

An analysis of the reasons of sickness/death was conducted. Under reporting often results from difficulties in identifying in clinical BSE suspects, as the clinical sings of BSE are variable, often subtle and not pathognomic and therefore, the cattle that had suffered or died with neurological signs were given special consideration.

Import related data

Data collection

Import related data from sixty four different countries on animal (cattle, buffalo, sheep and goats) and animal related commodities were provided by the "National Export Promotion Bureau", Kawranbazar, Bangladesh Bank and DLS, Bangladesh, to analyze the importing products of animal origin (cattle, buffalo, sheep and goats) from different countries to find whether or not the imported products posed any risk for the BSE. The special attention was given to livestock commodities, cattle, meat-and-bone meal (MBM) and bone importation. Data were also checked for the presence or absence of national import ban on MBM, ruminant by-products. BSE diagnostic services, reporting systems, public awareness programs and contingency plan.

RESULTS

Histopathology

The specific lesions of BSE were not found in the histopathological examination but other nonspecific lesions were recorded in 27.4% brain samples (Fig. 1). These lesions include microgliosis (Fig. 2, 4), oligodendrogliosis (Fig. 3), satellitosis (Fig. 3), perivascular edema (Fig. 4), congestion and hemorrhages.

Immunohistochemistry

Two BSE positive controls and one negative control were run in each time. All of the collected field samples and the negative control were found negative for PrP^{sc} (Fig. 5). In each time, positive control revealed clear, red colored intranuclear granular staining in specific gray matter nuclei (Fig. 6) indicating that immunohistochemistry worked properly.

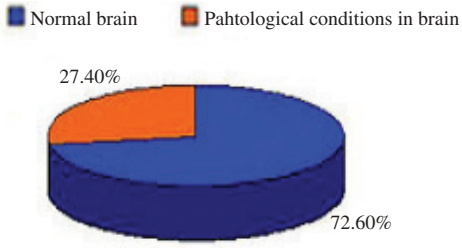

Fig. 1. Pathological conditions in brain.

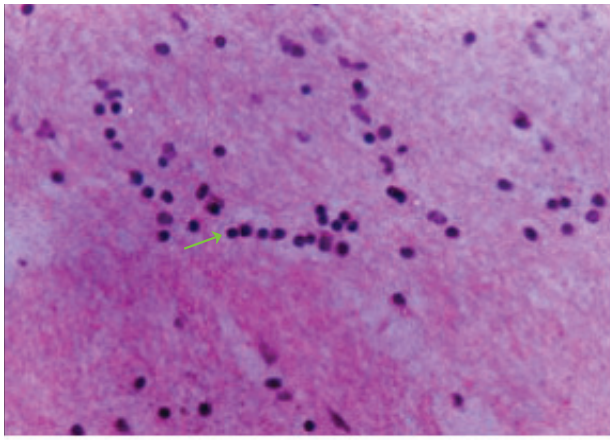

Fig. 2. Figure shows the gliois characterized by proliferation of glial cells (→) (H & E, X 330).

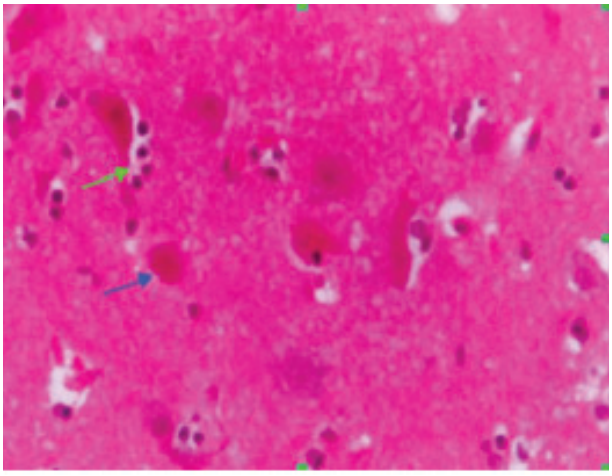

Fig. 3. Figure showing the satellitosis characterized by proliferation and accumulation of oligodendrocytes near the neurons (→) and degeneration of some neurons (→) (H & E, X 330).

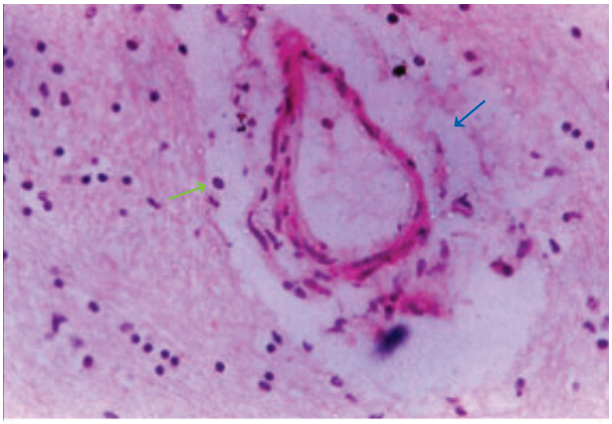

Fig. 4. Figure showing the microgliosis characterized by proliferation of microglial cells (→) and perivascular edema (→) (H & E, X 330).

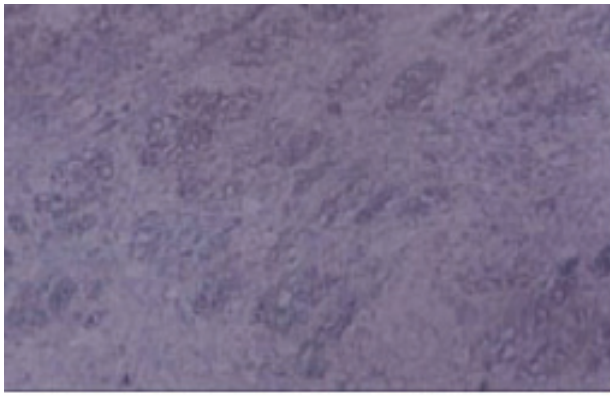

Fig. 5. PrP^{SC} negative control where no positive reactions were labeled.

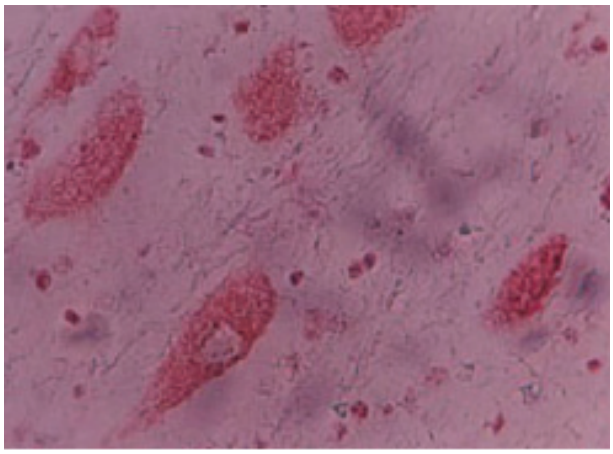

Fig. 6. PrP^{sc} positive control showing clear positive reaction (red colored distinct granular precipitates in the neurons) and almost free from nonspecific reactions (LSAB method of immunohistochemical staining, AEC substrate, Mayer's hematoxylin counterstain, X 330).

ELISA

Ninety two out of 2,000 samples (obex) which showed nonspecific lesions on the histopathological examination were analysed using BetaPrion® BSE EIA test kit for the detection of PrP^{sc} protein. The resulted optical density (OD) values lower than 0.2 were considered as BSE negative and higher or equal to 0.2 were considered as BSE positive. Results showed all 92 samples revealed OD values below 0.2 indicating that all samples were negative for BSE.

Clinical data

The diagnoses of neurological diseases in cattle and buf-falo during the period of 2002～2006 were rabies, tetanus, listeriosis, bovine tuberculosis, bovine babesiosis and actinomycosis. Details were shown in Table 1. However, there was no record of BSE in cattle and buffalo.

The diaghoses of neurological diseases diagnosed in sheep and goats during the period of 2002～2006 was rabies, lister-iosis and gid diseases. Details were shown in Table 2. However, there was no record of scrapie in sheep and goats.

Table 1. Incidence of neurological diseases in cattle and buffalo

Animal	Diseases	Year (%)					
		2002	2003	2004	2005	2006	Cumulative total
Cattle and buffalo	Rabies	167 (10.44)	–	268 (0.14)	87 (0.14)	150 (0.18)	10.90
	Tetanus	–	1200 (2.59)	–	–	–	2.59
	Listeriosis	68 (4.25)	–	–	160 (0.26)	–	4.51
	Bovine tuberculosis	41 (2.56)	–	–	–	–	2.56
	Bovine babesiosis	7 (0.43)	4 (0.008)	2034 (1.13)	459 (0.76)	360 (0.44)	2.76
	Actinomycosis	–	103 (0.22)	579 (0.32)	132 (0.22)	–	0.76
	Total	283 (17.69)	1307 (2.82)	2881 (1.61)	838 (1.40)	510 (0.62)	24.08

Table 2. Incidence of neurological diseases in sheep and goats

Animal	Diseases	Year (%)					
		2002	2003	2004	2005	2006	Cumulative total
Sheep and goats	Rabies	92 (9.90)	–	122 (1.71)	–	–	11.61
	Listeriosis	–	179 (4.19)	–	–	110 (1.83)	6.02
	Gid diseases	–	–	–	125 (3.37)	–	3.37
	Total	92 (9.90)	179 (4.19)	122 (1.71)	125 (3.37)	110 (1.83)	21.0

Source: Department of Livestock Services (DLS) 2007

Importation of commodities

So far Bangladesh has imported commodities from countries in the classification of GBR level I and GBR II, but not from countries in GBR level III and GBR IV. As shown in Table 3.

Importation of animals from countries with bovine spongiform encephalopathy

Bangladesh imported live animals from different countries having GBR level I and GBR II. However, a few dairy cattle have been imported from Spain (GBR level III) in early eighties. Most of them died due to acute respiratory illness. BSE related signs are not observed in the survived cows. Details may be seen in Table 3.

Import bans and importation of by-products of ruminant origin

Although import ban have been imposed in bangladesh from 2007. Import data before 2007 (Table 3) showed that MBM was imported only from countries in GBR level I and II not from countries in GBR level III and IV.

Public awareness program and contingency plans

In Bangladesh there were no public awareness program and contingency plans for BSE till 2006. However, recently in 2007, DLS in collaboration with Department of Pathology, Bangladesh Agricultural University (BAU), has finisheda training course to train 700 veterinarian and policymaker on different aspects of BSE.

Ban on feeding ruminants with feedstuffs derived from ruminants

In the Bangladesh livestock rearing system, it is very common that dairy animals (cattle and buffaloes) are fed green fodder, dry fodder and feed concentrates from vegetable origin (oil-cake). Industries which use animal by-products in Bangladesh produce only bone grist, gelatin and gum glue, but not MBM. So, feeding of MBM for cattle is not popular in Bangladesh.

Rendering of ruminant by products

In Bangladeshthere is no rendering plants for ruminant by-products. Rendering of sheep and bovine bone and offal is one of the important sources of transmission

Table 3. Imported products of animal origin (cattle, buffalo, sheep and goats) from different countries during 1999 ~ 2006

Commodity	Countries
1. Live animals (cattle, buffalo, sheep and goats)	Australia (I), India (II), New Zealand (I), Singapore (I), Spain (III), Norway (II), Pakistan (II), Argentina (I)
2. Meat and edible meat offal of bovine, sheep and goats	Australia (I), India (II), Brazil (II), New Zealand (I), Singapore (I), Pakistan (II)
3. Dairy products (milk and cream, butter, whey butter, cheese and curd)	Australia (I), India (II), Brazil (II), New Zealand (I), Singapore (I), Iceland (I), Pakistan (II), Sweden (I), Argentina (I)
4. Products of animal origin (guts, bladders and stomach, blood, hides or skins, furskins, tails, bones and horn-cores, hooves, glands, semen)	Australia (I), India (II), New Zealand (I), Singapore (I), Pakistan (II), Argentina (I)
5. Animal fats and oils and their cleavage products, Prepared edible fats, animal waxes	Australia (I), India (II), Brazil (II), New Zealand (I), Singapore (I), Iceland (I), Norway (II), Pakistan (II), Sweden (II), Uruguay (I), Argentina (I)
6. Raw hides and skins and leather	Australia (I), Germany (III), Hungary (III), India (II), U.K (IV), USA (III), Brazil (II), New Zealand (I), Singapore (I), Austria (III), Belgium (III), Denmark (III), Italy (III), Japan (III), Spain (III), Kenya (II), Mexico (III), Portugal (IV), Pakistan (II), Sweden (II), Switzerland (III), Argentina (I)
7. Articles of leather, handbags and similar containers, articles of animal gut (Catgut)	Australia (I), India (II), Netherlands (III), UK (IV), USA (III), New Zealand (I), Poland (III), Singapore (I), South Africa (III), Belgium (III), Denmark (III), Italy (III), Japan (III), Bulgaria (III), Greece (III), Pakistan (II), Sweden (II), Switzerland (III)
8. Furskins and artificial fur	Germany (III), Hungary (III), India (II), UK (IV), USA (III), Singapore (I), Iceland (III), Italy (III), Japan (III), Bulgaria (III), Greece (III), Mexico (III)
9. Woll, fine or coarse animal hair	Germany (III), India (II), Netherlands (III), UK (IV), USA (III), Brazil (II), Poland (III), Singapore (I), Canada (III), Iceland (III), Italy (III), Japan (III), France (III), Finland (III), Greece (III), Pakistan (II), Turkey (III)

(I)=GBR level I, (II) GBR level II, (III)=GBR level III and (IV) =GBR level IV
Source: National Export Promotion Bureau, Kawran Bazar, Dhaka and Bangladesh Bank, Dhaka, Bangladesh

of BSE to cattle deu to the recycle of infected materials. So, there is no possibility of recycling of bone and offal from ruminants affected by BSE in Bangladesh.

Diagnostic services

In Bangladesh has so far no national OIE reference laboratory for BSE. Although technological know how and methods have been developed in the Department of Pathology, Bangladesh Agricultural University, Mymensingh, by funding from a private company "Global Capsules Ltd." (GCL), Dhaka, Bangladesh, but it is not OIE validated.

DISCUSSION AND CONCLUSION

Histopathological examination of the brain is still considered a gold standard for the diagnosis of BSE (Chowdhury, 2005; Kubler et al, 2003). The characteristic histopathological lesions in the central nervous system are vacuolation of neuropil (spongiform change) or vacuolation of neuron, astrocytosis and neuronal degeneration (Prusiner, 2001). In the present study, the specific lesions of BSE were not found, but other nonspecific inflammatory lesions were found in 27.4% cases. However, the greatest proportion 72.6% of animals had no neurohistiological lesions. A number of non-specific inflammatory lesions such as gliosis, encephalitis, perivascular cuffing have been observed which arenot related to BSE. The non-specific lesions like, congestion, cuffing, satellitosis was probably due to stress related condition thatusually occurs during transportation from rural area to district slaughter houses.

In the present study, another part of experiment was immunohistochemistry and ELISA for the detection of PrP^{sc} protein in some selected brain samples. The result of the ELISA test was prepared and PrP^{sc} prion protein was not detected. PrP^{sc} was not detected by using immunohistochemistry. So, it may confirm that PrP^{sc} was not present in the tested samples.

In the present study, clinical data from DLS during 2002 to 2006 showed that the most frequent diagnosis for neuroogical symptom in cattle was rabies (10.9%) followed by listeriosis (4.51%), bovine babesiosis (2.76%), tetanus (2.59%), bovine tuberculosis (2.56%) and actino-

mycosis (0.76%). But there was no out break or report of BSE in Bangladesh.

Almost similar findings have been found from clinical data investigation of BSE in Korea from 1996 to 1999, the most frequently neurological symptoms found were 81.5% with parturient paresis, followed by metabolic disease 4.3%, traumatic injuries 4.3% and unknown causes 3.7% (Braun et al, 1998; Ikegami et al, 1991).

Bangladesh imported commodities, live animal from countries having GBR level I and II not from countries having GBR level III and IV (Annual report of Bangladesh Bank, 1999-2006; Report of Bangladesh Customs Tariff, Economic law, 2006). But some products like, raw hides and skin, leather, wool and fine or coarse animal hair were imported from some countries having GBR level III and IV. But according to OIE these products are not risk factor for BSE. Bangladesh has imported some cattle only from Spain (GBR III) for breeding purpose only. During that time some animal died due to acute respiratory problem and the rest were retrain-ed till their old age and did not enter the food chain at all.

In Bangladeshstill there was no import restriction till 2006. However, import ban have been imposed from 2007. In Bangladesh there was no public awar-eness and contingency plans for BSE. However, recen-tly DLS in collaboration with Department of pathology, BAU, has completeda training course on different aspects of BSE for their filed and executive veterinarian. In Bangladesh animal by product producing industries produce only bone grist, gelatin, gum-glue but not MBM. So there is no chance of use of MBM in cattle feed. And in Bangladesh livestock rearing system, dairy animals (cattle and buffalo) are fed green fooder and rice straw, and very seldom urea mollases block (UMB), mustard cake but not fed MBM. In Bangladesh there are no rendering plants for ruminant by-products. Therefore, it apparently seems that BSE may not prevalent in Bangladesh.

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