DOI: 10.1002/vms3.627

ORIGINAL ARTICLE

WILEY

# Usage of meat and bone meal in animal, poultry and fish feeds: A survey and risk analysis for the occurrence of bovine spongiform encephalopathy in Bangladesh

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**Funding information**
Bangladesh Agricultural Research Council (BARC); Ministry of Agriculture, Government of the Peoples Republic of Bangladesh, Grant/Award Numbers: 29, NATP Phase-1, BARC

## Abstract

**Background:** Bovine spongiform encephalopathy (BSE) is an emerging zoonotic disease of cattle associated with pathological prion protein ($PrP^{sc}$) transmitted via meat and bone meal (MBM). Although Bangladesh did not experience a BSE outbreak but the country could not export animal products to developed countries as has not yet been declared BSE free country by OIE due to lack of scientific risk evaluation for BSE. The objectives were identification of hazard, release and exposure pathways of pathological prion protein through MBM and analysis of risk for the occurrence of BSE in Bangladesh.

**Methods:** The scientific data were reviewed, hazards were scheduled and surveys were conducted on livestock production system, import of MBM and its use to identify the hazards present in Bangladesh context. The analysis was done by the 'OIE Risk Analysis Framework 2006 and European Union (EU) Scientific Steering Committee (SSC) 2003'. From the historical reviews, import of MBM and its use was identified, as external hazards.

**Results:** The analysis revealed that these hazards had negligible or moderate risk for the introduction of infectious $PrP^{sc}$ as Bangladeshi cattle are vegetarian cattle. No milk replacer was used and use of slaughtered waste in the animal feed industry is absent. Unconsumable bones are processed to produce bone chips, fertilizers and bone meal for poultry feeds. Scrapie was never prevalent in Bangladesh. Therefore, risk from the internal challenge was negligible in Bangladesh for the occurrence of classical BSE. These prevented the propagation of BSE infectivity and eliminated BSE infectivity from the system very fast, if that was present.

**Conclusions:** It was concluded that introduction of $PrP^{sc}$ into cattle population of Bangladesh through MBM was very negligible. Therefore, Bangladesh can be considered as BSE negligible risk country.

KEYWORDS
Bangladesh, BSE, cattle, MBM, risk analysis, risk assessment

## 1 | INTRODUCTION

Livestock populations in different countries are facing troubles with emergence of Bovine Spongiform Encephalopathy (BSE), one of the most important emerging zoonotic diseases of cattle usually over 30 months of age. BSE is not a contagious disease of cattle in the usual sense. There is no evidence for horizontal or vertical spread of classical BSE between animals. The origin of BSE remains unknown, but meat and bone meal (MBM) derived from infected cattle is considered to be a major vehicle of BSE infectivity (Wilesmith et al., 1988). BSE agent may transmit through mechanically meat processing (Anil et al., 1999; Garland et al., 1996; Grandin, 1997). Since this agent is highly resistant to heat, rendering processes for the production of MBM from carcasses might not be adequate for complete inactivation of the infectivity. MBM has frequently been used as the protein source in concentrate of cattle. Therefore, the agents that were derived from infected animals were recycled through the use of these feeds. The contamination of cattle feed with MBM from other feeds at feed manufacturing plants (cross contamination) exposed the cattle to MBM. Oral ingestion of feed contaminated with the abnormal BSE prion protein is the only documented route of field transmission of BSE (Prince et al., 2003; Wilesmith et al., 1988, 1991, 1992).

The Scientific Steering Committee (SSC) of the European Union in a geographic BSE risk assessment exercise (GBR) listed several risk factors for BSE propagation (spread within a cattle population) including the structure and intensity of the cattle population and other livestock populations, production and use of ruminant-derived meat-and-bone meal (including feed bans), the use of specified risk material (SRM) and carcasses (including SRM bans) and the rendering industry (structure, technology, rendering parameters) (Alban et al., 2000; SSC, 2000a). The most important measures to prevent exposure of cattle to BSE are the ban of feeding ruminant protein back to cattle ('MBM ban'), the exclusion of all high risk material such as brain and spinal cord of cattle and cattle carcasses from MBM production ('SRM ban'), the treatment of produced MBM at 133°C and 3 bars for 20 min (EU standard), and the prevention of cross contamination during feed production and use. Blocking of the known and suspected feed-related routes of BSE transmission has resulted in a documented decline in the number of new infections in subsequent birth cohorts in the United Kingdom, in Switzerland and in other countries.

The dairy industries of Bangladesh are based on low-cost production systems using straw, green grass, Kitchen waste, oil cake, molasses and agroindustrial by-products. Concentrated ready feeds are not used for sheep, rarely for beef cattle and to a comparatively modest extent for dairy cows. Milk replacer also was not used in Bangladesh. Particularly important facts that scrapie and BSE never been reported in Bangladesh. The use of slaughtered waste in the animal feed industry is absent. Most of the offals are consumed by the human. Unconsumable bones are crushed to produce bone chips, fertilizers and bone meal for poultry feed. Imported MBM is used for poultry and fish feed. MBM is not used in concentrated feed for cattle at any level in Bangladesh. There is a risk of BSE having been introduced via cross-contamination of monogastric feed and cattle feed during processing, preservation, transportation and even on farm. The imported MBM from BSE risk countries are the sole potential risk for introduction of BSE in Bangladesh. Bangladesh has no active surveillance system to assess the risk of BSE in regard to MBM. Therefore, a qualitative risk analysis was made for the occurrence of bovine spongiform encephalopathy (BSE) in Bangladesh according to OIE risk analysis model in regard to MBM.

## 2 | MATERIALS AND METHODS

### 2.1 | Data collection and survey

#### 2.1.1 | Collection of MBM import related data

MBM import related data were collected from Department of Livestock Services (DLS), Krishi Khamar Sarak, Farmgate, Dhaka (DLS, 2016) and personal communication with the owner of the Gochihata Dairy & Fish Farm.

#### 2.1.2 | Cattle feeding practice survey

A cross-sectional study was conducted on 476 farms of 34 upazilas (third tier of regional administrative unit). As several upazilas are governed under a district (second tier of regional administrative unit), first, we have randomly selected 17 districts out of 64 and then 34 upazilas having two from each selected district. The list of the cattle/dairy farms was collected from Upazila Livestock Offices and then 476 farms having 14 from each upazila were randomly selected with a condition that the farmer rears at least three lactating cows or three bulls/bullocks for fattening or meat purpose. We calculated minimum number of farms to be 384 based on the formula, $n = Z^2PQ/L^2$, where $n$ = sample size, $P$ = expected proportion of farms being exposed, $Q = 1-P$, $L$ = required precision (Thrusfield, 2005). We used $P = 0.50$, a precision of 5 % ($L = 0.05$), and confidence level 95 % (i.e. $Z = 1.96$).

#### 2.1.3 | Rendering mill and slaughterhouse survey

*Selection of rendering mill and butchers area for slaughterhouse survey*
A list of rendering mills was collected from the bone mills exporters' association office. Among the rendering mills, 10 rendering mills and respondents were randomly selected for the study. For the collection of information about the fate of the specific risk materials (SRMs) in Bangladesh, a total of 146 Butchers (from 29 market places of 17 districts) were interviewed from the slaughterhouse of 5 city corporations (Barishal, Dhaka, Gazipur, Khulna and Rajshahi), 7 district headquarters (Bagerhat, Jashore, Munshiganj, Mymensingh, Naogaon, Satkhira and Sirajgonj) and 17 upazilas (Mongla, Chorfashon, Agailjhora, Serpur, Savar, Sarsa, Sreepur, Rupsha, Bhoirob, Sreemongol, Sirajdikhan, Muktagacha, Dhamoirhat, Bera, Poba, Tala and Tarash).

**TABLE 1** Primer used to identify the animal DNA present in cattle feeds

| Primers | Sequence | Size | Species |
|---|---|---|---|
| Common-F | 5′GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA-3′ | | |
| Cattle-B-R | 5′TAGAAAAGTGTAAGACCCGTAATATAAG-3′ | 274 | Cattle |
| Sheep-S –R | 5′CTATGAATGCTGTGGCTATTGTCGCA-3′ | 331 | Sheep |
| Goat-G-R | 5′CTCGACAAATGTGAGTTACAGAGGGA-3′ | 157 | Goat |
| Pig-P-R | 5′GCTGATAGTAGATTTGTGATGACCGTA-3′ | 398 | Pig |
| Chicken-C-R | 5′AAGATACAGATGAAGAAGAATGAGGCG-3′ | 227 | Chickens |

F: forward, R: reverse.
Source: Matsunaga et al. (1999).

*Preparation of survey schedule for rendering mill and slaughterhouse survey*

The questionnaire contained the following key information as identification and general information of the rendering mills, respondent, raw materials, specific risk materials (SRMs), product, mad cow disease. For slaughterhouse survey, the structured questionnaire contained all the information's about respondent, different cuts, meat offal and SRMs

### 2.1.4 | Feed mill survey

*Selection of feed mills*

There are 117 feed mills in Bangladesh to produce composite commercial cattle and poultry feeds. All the cattle and poultry feed mills were surveyed. Based on the findings of cattle feeding practice survey, feed samples were collected from seven randomly selected feed mills from the list of 15 composite commercial cattle feed mills whose feeds were used by the cattle farmers in the study areas.

*Laboratory investigation of feeds*

Thirty feed samples were randomly selected from BSE prone area and used for polymerase chain reaction (PCR) to check whether the MBM (presence of DNA of cattle, sheep, goat, pig and chickens) is present in the feeds or not. The oligonucleotides or primers used in this study for animal protein identification as modified protocol followed by Matsunaga et al. (1999) (Table 1).

*Sample preparation*

From each collected samples first, 1 g of composite feed was ground into a fine powder by pestle and mortar and added 5 ml of PBS. Mixture was ground strongly. The suspension was centrifuged at 5000 rpm for 5 min and the supernatant was collected in a fresh Eppendorf tube for DNA extraction and stored in −20°C.

*DNA extraction*

For the DNA extraction from the composite feed samples conventional phenol-chloroform-isoamyl alcohol method was followed. A volume of 200 $\mu$l suspension was taken in an Eppendorf tube, mixed with 200 $\mu$l of digestion buffer and 2 $\mu$l of proteinase K to prepare digestion mixer. The sample was digested overnight at 56°C with shaking on a thermo block. Then 400 $\mu$l of phenol-chloroform-isoamyl alcohol was added to the digestion mixture, mixed with vigorous vortexing and incubated on ice for 2 min. The mixture was then centrifuged at 15,000 rpm for 5 min at 4°C and the supernatant was collected in a fresh Eppendorf tube. Again 400 $\mu$l of phenol-chloroform-isoamyl alcohol was added to the tube and centrifuged as above to collect the supernatant. Then the supernatant was transferred to 95% ethanol and 3 M sodium acetate solution @ 2.5 and $^{1}/_{10}$th of the supernatant volume, respectively and centrifuged at 15,000 rpm for 5 min. The supernatant was discarded and the DNA pellet was washed twice with 500 $\mu$l of 70% ethanol by centrifugation as above. The DNA pellet was dried at 37°C on a thermoblock. Finally, the DNA pellet was resuspended in 50 $\mu$l of nuclease free water and stored at −20°C.

*Polymerase chain reaction (PCR)*

The PCR was carried out using the PCR Master Mix (Promega Corporation, W1 USA) as per manufacturer's direction. The extracted DNA was used as template. Before starting the PCR, all the surfaces of the equipment were properly wiped with 70% alcohol. All the reagents were thawed, bottom downed by brief centrifugation and placed in PCR cooler. The PCR was carried out in 25 $\mu$l reaction volume containing 5 $\mu$l of template DNA, 12.5 $\mu$l of PCR Master Mix, 0.5 $\mu$l of each primer and 6.5 $\mu$l of nuclease free water. Thirty-five cycles of amplification were run using a Gradient Mastercycler (Eppendorf, Germany) as follows: initial denaturation at 95°C for 5 min, denaturation at 94°C for 0.5 min, annealing at 60°C for 0.5 min, extension at 72°C for 0.5 min and final extension at 72°C for 5 min.

*Analysis of PCR products by agarose gel electrophoresis*

Note that, 1.5% agarose gel (w/v) was prepared by dissolving agarose powder in 1× TAE buffer. The agarose was dissolved by heating in microwave oven. After that ethidium bromide was added to the agarose solution @ 0.5 $\mu$g/ml (5 $\mu$l of stock/100 ml). The agarose solution containing ethidium bromide was poured into the gel-casting tray to which the comb was properly positioned. When the gel was completely set, the comb was removed gently and the gel was transferred into the electrophoresis tank which was filled with 1× TAE buffer. An amount of 5 $\mu$l PCR product was mixed with DNA loading buffer (5 vol. PCR products + 1 vol. DNA loading buffer) and loaded into the slots of the gel. As a size standard, a 100 bp ladder was also loaded to one slot. Electrophoresis was run at 90 V for 40 min. After the completion of electrophoresis the gel was placed on the UV transilluminator in the

**TABLE 2** Determinants or definition of BSE challenge or risk levels

| External challenge | MBM (tons) imports | | |
|---|---|---|---|
| | 1986–1990 from UK | UK – imports before 86 & 91 – 93 × 10, after 93 × 100 | Imports from other BSE countries × 10 |
| Extremely high | ≥10.000 | | |
| Very high | 1000 to <10,000 | | |
| High | 1 00 to <1000 | | |
| Moderate | 20 to <100 | | |
| Low | 10 to <20 | | |
| Very low | 5 to <10 | | |
| Negligible | 0 to <5 | | |

*Note*: The abbreviation 'MBM' refers to different animal meals (MBM, MMBM, BM and Greaves) that could carry the BSE-agent because it contains animal (ruminant) proteins. It does not refer to composite feed that could potentially contain MBM, MMBM, BM or Greaves.

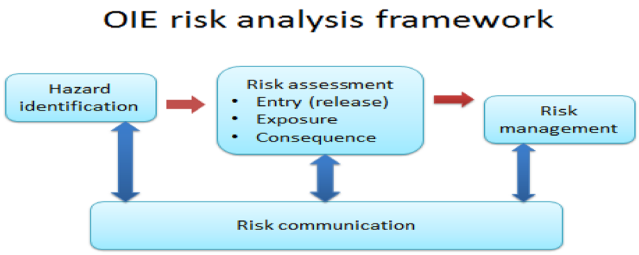


**FIGURE 1** OIE risk analysis framework (OIE, 2006)

dark chamber of the image viewing and documentation system. The result was viewed on the monitor as well as saved electronically.

## 2.2 | Risk analysis

The analysis was done by the OIE guided risk analysis model 2006 (OIE, 2006).

### 2.2.1 | Hazard identification

Hazard identification is the first step in the risk analysis. The scientific papers on BSE epidemiology and its origin were reviewed, hazards were scheduled and surveys were conducted on livestock production system, import and use of livestock commodity to identify the hazards present in Bangladesh context. The data were analysed to identify the actual hazard for the occurrence of BSE in the country as per OIE guidelines (Figure 1).

### 2.2.2 | Risk assessment

*Entry or external challenge assessment or release pathways*

The term external challenge or risk refers both the likelihood and the amount of the BSE-agent entering into a defined geographical area in a given time period through infected MBM. External challenge or risk or entry (release) challenge level was defined briefly by following the strategy proposed by Scientific Steering committee (SSC, 2003) of the European Union as Table 2.

In other countries affected by BSE and in the United Kingdom at other periods, the risk that exposed cattle were carrying the BSE-agent or that MBM was contaminated with BSE was lower. Accordingly, the challenge posed by the same amount of imports would be much lower or the same level of challenge would only occur at higher imports. To adapt the thresholds accordingly, the following multipliers were used.

Import from United Kingdom in other periods: MBM: Before 1986 and from 1991 to 1993: multiply all thresholds by 10; 1993 and after: multiply all thresholds by 100.

Import from countries other than the United Kingdom affected by BSE: regardless of period and whenever there is reason to assume that BSE was already present at time of export: MBM: multiply all thresholds by 10.

*Exposure assessment or exposure pathways or internal challenge assessment*

The extent of risk from the imported materials depends on the use and recycling of the imported BSE hazardous materials as it is estimated by released pathways. These in turn depend on the stability of the livestock production system. To assess the stability of the livestock production system, cattle feeding practices in 476 cattle farms, 117 cattle feed mills and 30 feed samples from seven feed mills for the presence of animal protein were investigated. Stability is defined as the ability of cattle husbandry system to prevent the introduction and to reduce the spread of the BSE agent within its borders. A 'stable' system would eliminate BSE over time; 'unstable' system would amplify it (SSC, 2002a, 2002b, 2003) as stated in Table 3.

The most important stability factors are those that reduce the risk of recycling of BSE

- Avoiding feeding of MBM to cattle
- Rendering system able to largely inactivate BSE-infectivity (e.g. by applying 'standard' treatment at $133^{0C}/20\ ^{min}/3\ ^{bar}$).

**TABLE 3** Exposure or internal challenge level

| Stability | Level | Effect on BSE infectivity | Most important stability factor | | |
|---|---|---|---|---|---|
| | | | Feeding | Rendering | SRM removal |
| Stable: the system will reduce BSE infectivity | Optimally stable | Very fast | OK | OK | OK |
| | Very stable | Fast | 2 of the 3 factors OK and 1 reasonably OK | | |
| | Stable | Slow | 2 or 1 OK and 2 reasonably OK | | |
| Neutrally stable | | ±Constant | 3 reasonably OK or 1 OK | | |
| Unstable: the system will amplify BSE infectivity | Unstable | Slow | 2 reasonably OK | | |
| | Very unstable | Fast | 1 reasonable OK | | |
| | Extremely unstable | Very fast | None even reasonable OK | | |

**Feeding**: OK = evidence provided that it is highly unlikely that any cattle received MBM. **Reasonably Ok** = voluntary feeding unlikely but cross contamination cannot be excluded. **Rendering**: OK = only plants that reliable operate at $133^{0}$ $20^{min}$ 3 $^{bar}$ standard. **Reasonably** Ok = all plants processing high risk material (SRM), fallen stock, material not fit for human consumption) operating at $133^{0}$ $20^{min}$ 3 $^{bar}$-standard, low risk material is processed at more gentle conditions. **SRM removal**: OK = SRM–removal from imported and domestic cattle in place, well implemented and evidence provided. Fallen stock I excluded from the feed chain. **Reasonably Ok** = SRM–removal from imported and domestic cattle in place but not well implemented of documented. If in addition to a reasonable OK SRM removal fallen-stock is excluded from rendering, the SRM removal might be considered 'OK'.

**TABLE 4** Import of MBM in Bangladesh from 2007 to 2013

| Sl.No | Name of the country | Year of import (metric tons) | | | | | | | Total import (metric tons) |
|---|---|---|---|---|---|---|---|---|---|
| | | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | |
| 1 | Australia | 1620 | 61,830 | 55,000 | 17,919 | – | 46,500 | 21,600 | 205,996 |
| 2 | Croatia | – | – | – | 40,100 | – | 10,400 | 4500 | 29,500 |
| 3 | New Zealand | – | – | 54,250 | – | – | 500 | 3000 | 14,300 |
| 4 | Norway | 400 | 25,390 | | – | – | – | – | 48,743 |
| 5 | Paraguay | – | – | – | 46,550 | 115,100 | 110,250 | 25,420 | 334,060 |
| 6 | Brazil | – | – | – | – | – | – | 22,540 | 27,900 |
| 7 | Germany | – | – | – | – | – | – | 5500 | 6300 |
| 8 | The Netherlands | 825 | 400 | – | – | – | – | – | 2525 |
| | Total import | 2845 | 87,620 | 109,250 | 104,569 | 115,100 | 167,650 | 82,560 | 669,324 |

–, no importation data.
*Source*: Office record of Department of Livestock services (DLS), Farmgate, Dhaka 1215.

## 3 | RESULTS

### 3.1 | Import of MBM, slaughtered offal and its fate

#### 3.1.1 | Import of MBM

Before 2007, MBM was not imported in Bangladesh. From 2007 to 2014, a total of 66,93,24 MT of MBM were imported from Australia, Croatia, New Zealand, Norway, Paraguay, Brazil, Germany and the Netherlands. Only 29,265 MT of MBM were imported from BSE risk countries like Brazil, Germany and the Netherlands (Table 4). These MBM were used in different poultry and fish feed industries but not used to prepare cattle feeds.

#### 3.1.2 | Import of slaughtered offal or waste

No slaughtered offal or waste were imported in Bangladesh for rendering purpose or production of processed animal protein (PAP).

#### 3.1.3 | Fate of imported MBM and laboratory investigation of feeds

The imported MBM was used directly in 117 poultry and 145 fish feed industries for the manufacturing of composite commercial feeds for monogastric animals like poultry and fish. Survey revealed that MBM was not used in cattle feed formulation in Bangladesh at any level. PCR

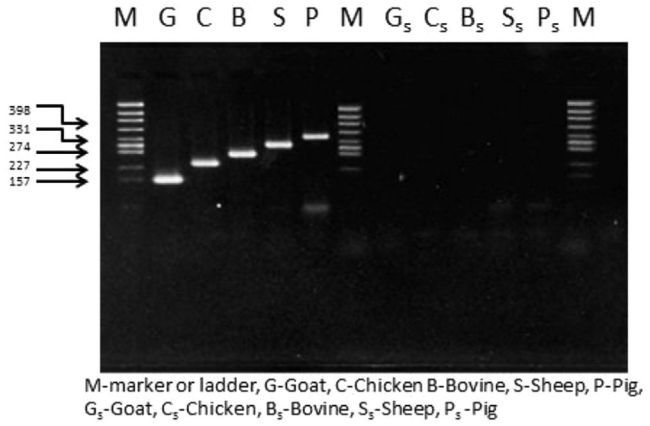


**FIGURE 2** Agarose gel electrophoresis of PCR products amplified from known specific template DNA of Goat, chicken, bovine, sheep and pig (G, goat; C, chicken; B, bovine; S, sheep; P, pig) and feed sample DNA ($G_s$, goat; $C_s$, chicken; $B_s$, bovine; $S_s$, sheep; $P_s$, pig) and M = 100 bp marker

results also showed absence of animal DNA in the composite feeds of cattle (Figure 2).

## 3.2 | Domestic production of MBM and its usages in feed mills

Survey showed absence of domestic MBM production industry in Bangladesh. Few illegal entrepreneurs produced PAP (processed animal protein) for poultry and fish feeds from tannery wastes in Hazaribag, Dhaka but government controlled it rigorously time to time (DLS, 2016).

### 3.2.1 | Results of slaughterhouse survey

The butchers expressed that the blood, hides, male and female genital organs, gall bladder, mandible, scapula, horn, hooves and ear were not sold for human consumption. Blood left in place or buried in slaughtered places. Hides were sold for industrial purpose and horns, hooves, scapula, skull (after processing of head) and mandible collected by local people. The collected materials (bones, hooves etc.) were sold to the agents of local bone crushing industry. The livelihood of local poor people depends on collecting and selling of slaughtered waste materials. The interview of butchers also revealed that the dorsal root ganglia, spinal cord, tonsils were sold with meat and brain and the lower part of intestines were sold separately for human consumption. Eyes were rejected and remained scattered in the slaughtered area or threw into dustbin. Researcher found that a meat processing industry named 'Bengal Meat' collected penis and omasums for export purpose. During the survey, researcher also found that omasums were collected for export purpose only in Dhaka city corporation area. It was observed that the true rendering is not in place in Bangladesh. However, SRMs are normally used for different purposes. None of these SRMs are entered into animal feed chain. Therefore, the system is identified as stable system that is the means of reduction of the $PrP^{sc}$ very fast, if any.

### 3.2.2 | Results of feed mills survey

There were 117 feed industries in Bangladesh to make composite commercial poultry and cattle feeds and 145 for fish feeds. Among the 117, a few industries produced a small amount of cattle feeds. It was observed that no industry produces cattle feed using MBM. Feed millers do not disclose the necessary information on packet about feed composition, ingredients and date of expiry, storage guidelines, energy levels and vitamins. About 30% of feed millers printed ingredients name and percentage on the sacks of feed and rest of the feed millers printed nutritional analysis of feed only. During the survey, no permanent nutritionist was found in composite feed mills.

### 3.2.3 | Findings of risk assessment

*Entry or external challenge assessment or release pathways*

It was observed that 29,265 MT MBM were imported from BSE risks countries (Germany, Brazil and the Netherlands) after 2007 (2007–2013). Table 5 has been generated from Table 4 (described in Materials and Methods) to calculate the risk level for Bangladesh and the risk was determined by plotting data on the specific plot. It was found that the risk for MBM was estimated as moderated risk in Bangladesh (calculated based on Tables 4 and 5). From this assessment, it was predicted that opportunity for the introduction of $PrP^{sc}$ into Bangladesh through MBM is moderate. However, the actual risk depends on the exposure of the imported live cattle and MBM to the feed chain.

## 3.3 | Exposure assessment or exposure pathways or internal challenge assessment

### 3.3.1 | Feeding practice of animals in Bangladesh

The survey on cattle feeding practice revealed that only 22.5% farmers used commercial composite ready cattle feed either of 15 brands as concentrate while 77.5% used homemade concentrate in the farms (Tables 6A, 6B and 7). At least one third of the farms used ACI cattle feeds. Our PCR analysis revealed absence of animal DNA in the tested feeds.

### 3.3.2 | Milk replacer

From the survey of this study, it was found that no milk replacer was used by the farmers in the study area.

**TABLE 5** External challenge level for MBM imports

| Level | Imports from UK (ton) | | | | Imports from other BSE infected countries (ton) | | | |
|---|---|---|---|---|---|---|---|---|
| | Before 1986 | 1986–1990 | 1991–1993 | 1994–2014 | Before 1986 | 1986–1990 | 1991–1993 | 1994–2014 |
| Extremely high | >100,000 | >10,000 | >100,000 | >1,000,000 | >1,000,000 | >100,000 | >1,000,000 | >10,000,000 |
| Very high | 10,000 to <100,000 | 1000 to <10,000 | 10,000 to <100,000 | 100,000 to <1,000,000 | 100,000 to <1,000,000 | 10,000 to <100,000 | 100,000 to <1,000,000 | 1,000,000 to <10,000,000 |
| High | 1000 to <10,000 | 100 to <1000 | 1000 to <10,000 | 10,000 to <100,000 | 10,000 to <100,000 | 1000 to <10,000 | 10,000 to <100,000 | 100,000 to <1,000,000 |
| Moderate | 200 to <1000 | 20 to <100 | 200 to <1000 | 2000 to <10,000 | 2000 to <10,000 | 200 to <1000 | 2000 to <10,000 | 20,000 to <100,000 |
| Low | 100 to <200 | 10 to <20 | 100 to <200 | 1000 to <2000 | 1000 to <2000 | 100 to <200 | 1000 to <2000 | 10,000 to <20,000 |
| Very low | 50 to <100 | 5 to <10 | 50 to <100 | 500 to <1000 | 500 to <1000 | 50 to <100 | 500 to <1000 | 5000 to <10,000 |
| Negligible | 0 to <50 | 0 to <5 | 0 to <50 | 0 to <500 | 0 to <500 | 0 to <50 | 0 to <500 | 0 to <5000 |

*Note*: Bangladesh imported 29,265 M tons MBM from Germany, Brazil and the Netherland. So far, it can be considered as negligible.

**TABLE 6A** % components of cattle diet ingredients

| Feed items | Number of users | % Of users |
|---|---|---|
| Straw | 476 | 100% |
| Green grass | 476 | 100% |
| Concentrate | 476 | 100% |
| Homemade concentrate | 369 | 77.5% |
| Ready feed | 107 | 22.5% |
| MBM | 0 | 0% |
| Milk replacer | 0 | 0% |

**TABLE 6B** Commercial ready feeds in the surveyed farms

| Sl. No | Name of ready feed | Number of users (107) | Per cent |
|---|---|---|---|
| 1 | Aftab cattle feed | 3 | 2.8% |
| 2 | ACI cattle feed | 39 | 36.4% |
| 3 | Ag feed | 1 | 0.9% |
| 4 | AIT cattle feed | 7 | 6.5% |
| 5 | Aman feed | 6 | 5.6% |
| 6 | Anchor cattle feed | 6 | 5.6% |
| 7 | BRAC cattle feed | 3 | 2.8% |
| 8 | Care cattle feed | 4 | 3.7% |
| 9 | Doctor's cattle feed | 5 | 4.7% |
| 10 | Paragon feed | 5 | 4.7% |
| 11 | Quality feed | 3 | 2.8% |
| 12 | Tamim feed | 2 | 1.9% |
| 13 | Teer cattle feed | 18 | 16.8% |
| 14 | Thailand fish feed | 3 | 2.8% |
| 15 | Unknown (anonymous) | 2 | 1.9% |

### 3.3.3 | Perception of stakeholders about BSE

The present study revealed that only 1.68% farmers had heard about the BSE. Among these, 22.2% were informed by newspaper and 22.2% from training, 33.3% from TV news and rest 22.2% were from multiple sources. It was also found that persons involved in feed mills and rendering mills (Bone crushing mills) had basic understanding about the BSE but butchers had not heard about BSE at all.

### 3.3.4 | Rendering

The present study revealed that there was no real rendering mill in Bangladesh; however, there were 27 bone crushing mills for the purpose of crushing hooves and bone. The raw materials were used in the bone crushing mills and produced different sizes of products. The products were sold to both domestic consumer and exporters. The domestic consumers produce gelatin capsule, dicalcium phosphate (DCP), fertilizer and poultry feeds (Table 7).

**TABLE 7** Fate of animal by-products at crushing mill

| By-products | Crushed products | Use | | Comments* |
|---|---|---|---|---|
| | | Domestic use (35%) | Export (65%) | |
| Bones, hooves and horns | Size $^{3}/_{8}''$ | Gelatin, DCP, fertilizer and poultry feed | Germany | Lack of BSE free certificate hindering the export |
| | Size $^{5}/_{8}''$ | | China | |
| | Size $^{3}/_{4}''$ | | India | |
| | Size $^{3}/_{32}''$ | | Spain | |
| | Size $^{3}/_{16}''$ | | UK | |

*Views of the owner of the bone crushing mills.

## 4 | DISCUSSION

MBM is considered as only vehicle for the introduction of BSE in the cattle population when it is contaminated with infectious prion protein. The contamination is possible if the BSE is prevalent in the cattle population of the country, rendering mills render ruminant offal's along with specific risk materials (SRM) and MBM is supplied to cattle repeatedly. Before 2007, MBM was not imported in Bangladesh; the period in between 1986 and 1990 is considered as crucial for risk period by OIE. Therefore, Bangladesh did not import MBM at the crucial time. After 2007, Bangladesh imported 29,265 MT MBM from Germany, Brazil and the Netherlands. OIE identified these countries as controlled BSE risk and EU identified as GBR level III countries. Therefore, our import risk analysis calculated a moderate risk for the introduction of infectious prion PrP to Bangladesh through MBM. However, controlled BSE risk means relevant control measures that were applied to the exporting country to control BSE transmission. In Bangladesh, MBM is used in the commercial poultry farms only for better production and chicken is considered as non-susceptible to BSE.

Survey on Bangladeshi cattle feeding practice revealed that farmers are usually fed their animals on a diet of rice straw, green grass, rice bran small quantity of oil cake, molasses, salt, vitamins and minerals along with other non-conventional feed items like rice gruel, kitchen waste etc. The survey result of this research showed that 100% of the farmers used straw and green grass as a primary feed or basal diet for their cattle. They also supplied some concentrate feeds such as rice bran, wheat bran, oil cake, rice gruel depending on the socio-economic condition of the farmers and available resources of feed in that area. For better performance 77.5% farmers use homemade concentrate and only 22.5% use ready feeds. During investigation, it was found that these feeds did not contain any animal DNA meaning that MBM was not added to those feeds. From the statement, we can urge that Bangladeshi cattle are vegetarian cattle. Atypical BSE has never been reported in Bangladesh. Atypical BSE is linked with animal origin feeds. Therefore, risk from the imported risk has been eliminated from cattle feed chain. Therefore, our stability and consequence assessments calculated negligible risk imposed from the imported MBM. Pakistan imported MBM from England, Belgium and Germany (Ozawa, 2003). England is considered most crucial for the BSE risk. However, after risk analysis Pakistan is considered as negligible BSE risk country by OIE (OIE, 1996). Based on the estimate of MBM, Bangladesh should stand as BSE negligible risk country.

During the feed mills survey, researcher found that the feed millers produce their composite feed in the same plant. For those reasons, there is a chance of cross contamination of feed ingredients and prepared feed during storing, processing, transportation and in vendor shop of composite commercial feed. Therefore, there should be strong regulation to control feed manufacture. Government should continue its restriction to import MBM from BSE risk country and always review its regulation as per BSE risk analysis.

The BSE surveillance system in Bangladesh is characterised by passive system. As the member state of OIE, BSE is notifiable in Bangladesh. It was stated that the notification was carried out in the field offices by the owner of the animal or a third person or official veterinarians when animals with neurological symptoms and/or dead without an apparent cause are observed. No compensation scheme has been installed for dairy farmers in case of clinically suspected or officially confirmed BSE case in Bangladesh. Although, only a limited active surveillance was done by private company, there is no nationwide active surveillance in Bangladesh (Halder et al., 2009; Ozawa, 2003). Bangladesh government should have a nationwide active surveillance for BSE. For the active surveillance, national reference laboratory for the diagnosis of BSE will be required. This study was conducted as part of PhD research for a duration of 3 years covering randomly selected 17 districts out of 64 of the country, and thus the data represent the Bangladesh. Study includes all the parameters like MBM usages, cattle feeding practice, laboratory investigations and animal importation over a long period of time, though data in regard to animal importation would be discussed in somewhere else. From the analysis, it was found that two main stability factors; feeding and rendering traditionally were controlled in Bangladesh. Therefore, the risk from the internal challenge is negligible. For those reasons, system would fully prevent the propagation of BSE infectivity and eliminate BSE infectivity from the system very fast. It was concluded that the BSE cattle system of Bangladesh was optimally stable between 1980 and 2006. In between 2007 and 2014, one of the stability factors feeding was audited as 'reasonable OK'. The system prevented the largely propagation of the BSE infectivity but the elimination of BSE infectivity from the system was slower than in an optimally stable system. Therefore, Bangladesh can be considered as BSE negligible risk country.

## 5 | CONCLUSION

Based on the European Union Scientific Steering Committee risk analyses framework 2003, the external challenge analysis revealed that MBM imported from Germany, Brazil and the Netherlands put Bangladesh in moderate risk and internal challenge analysis put Bangladesh in negligible risk for the introduction of $PrP^{sc}$ among cattle population of Bangladesh. Therefore, Bangladesh can be considered as BSE negligible risk country.

### ACKNOWLEDGEMENTS

The Bangladesh Agricultural Research Council (BARC), Ministry of Agriculture, Government of the Peoples Republic of Bangladesh [grant numbers 29, NATP Phase-1, BARC] supported this work.

### CONFLICT OF INTEREST

The authors do not have any conflict of interest.

### AUTHOR CONTRIBUTIONS

MN Islam: data collection and curation; methodology; formal analysis; writing – original draft; writing – review and editing. MSI Siddiqui: data curation; writing – review and editing. MT Islam: software; formal analysis; writing – review and editing; co-supervision. MR Islam: formal analysis; writing – review and editing; co-supervision. EH Chowdhury: conceptualisation; methodology; data curation; formal analysis; writing – original draft; writing – review and editing; supervision; funding acquisition; project administration.

### ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Since no animal experimentation was conducted, ethical approval was not required. However, written informed consent was taken from the cattle farmers, feed mill owners and butchers. Furthermore, the study protocol was approved by the Departmental Board of Studies and the Committee for Advanced Studies and Research of the university.

### CONSENT FOR PUBLICATION

All the cattle farmers, feed mill owners and butchers consented to publish their data.

### DATA AVAILABILITY STATEMENT

Data generated or analysed during this study are available and included in this article as supplementary information files.

### PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.627

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## SUPPORTING INFORMATION

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**How to cite this article:** Islam, N., Siddiqui, S. I., Islam, T., Islam, R., & Chowdhury, E. H. (2022). Usage of meat and bone meal in animal, poultry and fish feeds: A survey and risk analysis for the occurrence of Bovine Spongiform Encephalopathy in Bangladesh. *Veterinary Medicine and Science*, *8*, 377–386. https://doi.org/10.1002/vms3.627