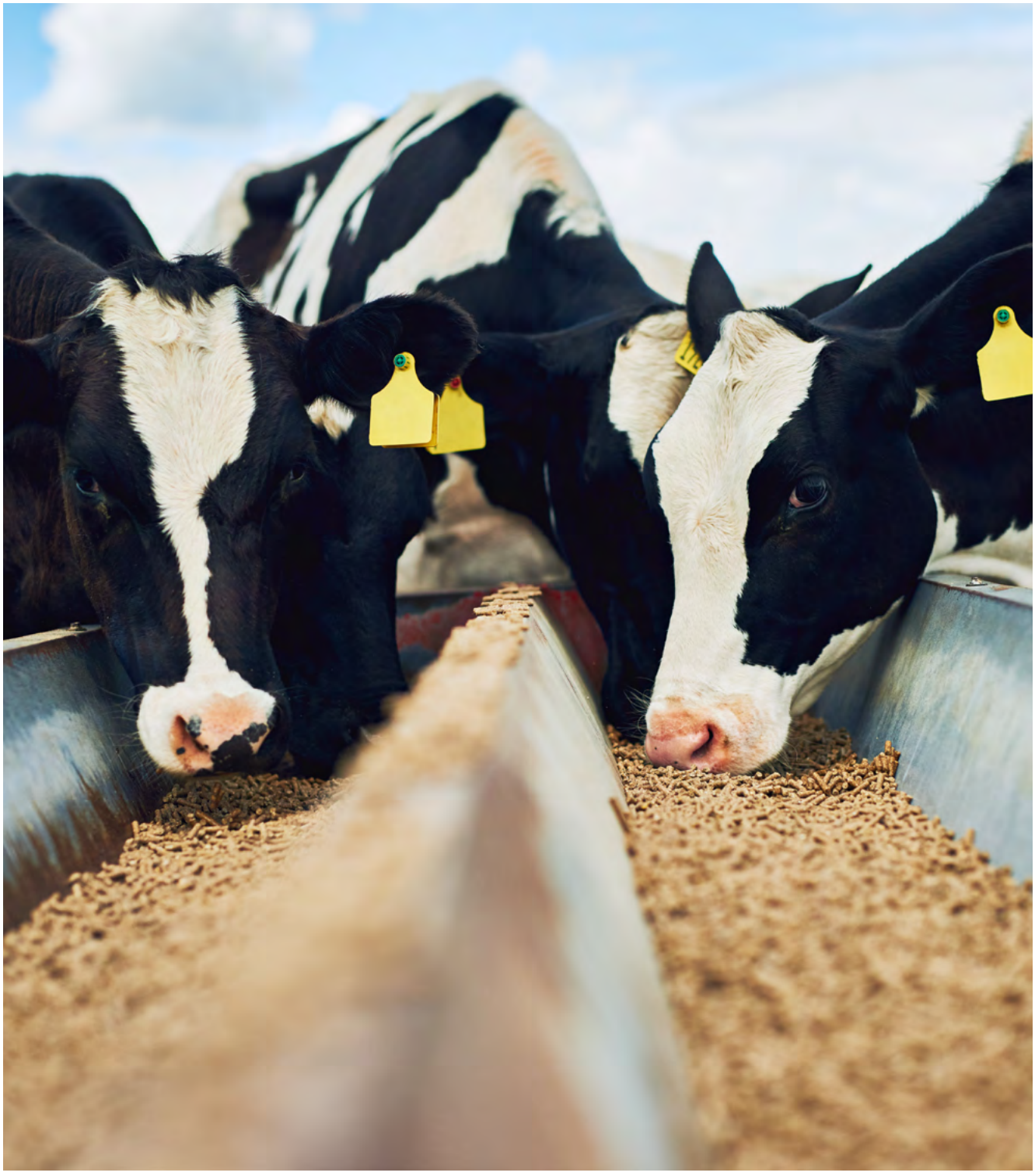
Guidelines for Targeted BSE Surveillance

July 2023



World Organisation for Animal Health
Founded as OIE

Status Department

https://www.woah.org/en/disease/bovine-spongiform-encephalopathy/

Required citation: WOAH (2023). *Guidelines for Targeted BSE Surveillance*. Paris, 28 pp., DOI [https://doi.org/10.20506/woah.3369]. Licence: CC BY-SA 3.0 IGO.

Cover image: © Getty
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Introduction

These guidelines aim to support WOAH Members in the implementation of a bovine spongiform encephalopathy (BSE) surveillance system by providing an overview of the clinical signs of BSE and the criteria for targeted BSE surveillance, as well as an overview of the components of a credible BSE surveillance system. These guidelines complement the information in Chapters 1.8 and 11.4 of the *Terrestrial Animal Health Code* (*Terrestrial Code*) and Chapter 3.4.5 of the *Manual of Diagnostics and Vaccines for Terrestrial Animals* (*Terrestrial Manual*); therefore, it is highly recommended that the reader consults these afore-mentioned WOAH standards when using these guidelines.

Supplementary information on BSE, including links and references to additional reading material, examples of clinical examination forms and disorders pertinent to the differential diagnosis of BSE, are provided in Appendices.

1.

An overview of the clinical signs associated with BSE

(Article 11.4.20. Point 1 of the *Terrestrial Code*)

BSE is a fatal neurodegenerative disease in adult bovines, with a variable incubation period of two to more than 10 years. The majority of the cases develop clinical signs five to seven years after exposure to the agent. BSE is refractory to treatment, with death occurring weeks or months after the clinical signs appear. Breed or sex are not predisposing risk factors associated with infection or presence of the disease. As a neurodegenerative disease, BSE causes progressive neurological signs, which for simplification purposes can be grouped into changes in:

- **mental status, behaviour and activity**: placid animals become aggressive, animals become more fearful, their behaviour at milking or when entering the milking parlour changes, animals become more aggressive or nervous than before towards bovines or humans.
- **sensation**: over-reactivity to stimuli (touch, light, sound).
- **posture and movement**: low head, wide-based posture and incoordination, walking or running into objects or walls with eyes appearing normal, walking aimlessly around in circles or drifting to one side when walking.

Additionally, non-specific signs may also be reported in clinical cases such as loss of body condition and weight, decrease in milk yield and a low heart rate despite excitable behaviour.

The three most typical non-specific signs of BSE are apprehension, hyperaesthesia and ataxia [1]:

- **Apprehension**: the animal appears very alert, follows every movement, startles frequently without obvious stimuli, flinches repeatedly on sudden movements, runs away when approached, tries to escape when cornered.

- **Hyperaesthesia**: hyperaesthesia can include over-reactivity to a range of external stimuli, but it is the repeatability or the progressive nature of over-reactivity that is characteristic of BSE and distinguishes it from over-reactivity that may be within the range of normal bovine behaviour, such as touching of the head or neck, which is often not tolerated, even by a healthy animal. Signs of hyperaesthesia include:

  - ***Hypersensitivity to touch**: forceful kicking when hind limbs are touched, exaggerated response to head touch/haltering, exaggerated response when approached from front.*

  - ***Hypersensitivity to sound**: startle/ flinch to sudden unexpected environmental noise, startle/ flinch on at least one or repeated auditory stimuli.*

- **Ataxia**: uncoordinated movement of limbs, swaying hind quarters, losing balance on hind quarters, high stepping (hypermetric) hind limbs or fore limbs, stiff movements of hind limbs.

Following the discovery of atypical BSE (both H and L types), there have been reports describing specific clinical signs associated with classical versus atypical BSE. Notwithstanding these reports, it is not possible to discriminate clinically between the three types of BSE. Appendix 1 cites a number of references describing clinical cases of classical and atypical BSE without trying to clinically distinguish between the types.

1.1
Clinical diagnosis

Since there are no pathognomonic signs to reliably diagnose BSE clinically a uniform case definition does not exist. Thus, the assessment of clinical signs can be subjective.

A characteristic sign of BSE is a 'startle response' to stimuli that would be perceived as normal by most healthy animals (e.g. a puddle on the floor, noise by workmen), which is why tests for over-reactivity have been used to aid in the clinical diagnosis:

- **bang test**: hitting a metal object with a hammer or hand-clap to elicit a startle response;
- **broom or flexible stick test**: touching the hind limbs with an object to elicit kicking;
- **flash test**: exposing the animal to sudden light to elicit startle;
- **clipboard test**: waving a clipboard or a hand towards the animal to elicit a startle response or even panic.

Clinically suspect bovines that respond repeatedly to these external stimuli and respond to additional tests for over-reactivity are more likely to have BSE.

Bovines progressing to end-stage of the disease will develop severe incoordination, particularly in the hind quarters, leading eventually to paresis, abnormal rising behaviour, considerable difficulty getting up and inability to get up at all. They may be unable to place their limbs correctly and may lie with one or both legs stretched out backwards. Eventually the animal will become lethargic and will die.

Appendix 1 includes references and links to reading material and visual tools on clinical protocols, clinical signs of BSE, and differential diagnosis.

1.2
Differential diagnosis

Many neurological diseases in bovines can have a similar clinical presentation as BSE [2], which makes clinical history and response to treatment important for disease differentiation. Histopathological studies on suspect cases of BSE have shown that the most common diseases on a differential list with BSE were inflammatory diseases (encephalitis, meningitis, myelitis and combinations of these, e.g. listeriosis, malignant catarrhal fever), metabolic diseases (cerebro-cortical necrosis/thiamine deficiency, hypomagnesaemia), degenerative diseases/anomalies (progressive ataxia of Charolais breed, cerebellar atrophy, myelopathy), neoplastic diseases and idiopathic diseases (idiopathic brainstem neuronal chromatolysis, idiopathic cerebral oedema) [3-9].

The most frequently identified neurological inflammatory disease is listeriosis. BSE does not cause obvious cranial nerve disorders so the presence of signs of facial paralysis (droopy ears, inability to blink, asymmetric face), which is often observed in cases of listeriosis, is unlikely to be associated with BSE. Based on discriminatory analysis to distinguish BSE from other neurological diseases listeriosis was

characterised by a shorter clinical duration, its predominant occurrence in winter and spring, and a higher frequency of the following signs: nervousness of entrances, head rubbing, blindness, circling and falling [10]. A decision tree model for clinically suspected BSE cases in Belgium showed that signs particularly associated with listeriosis were abnormal head carriage, circling and head pressing or rubbing, whereas those associated with meningoencephalitis were recumbency and blindness [9].

However, many cases of BSE (sometimes over 50% in the studies) did not present with any significant histopathological lesions in the brain and the cause for the neurological signs could not be identified. Live animal submissions have shown that there may be conditions in bovines that produce behavioural or sensory changes that may be confused with BSE, even if the origin of such conditions is not in the central nervous system [11]; in some cases there may be no macroscopic or neuropathological changes or biochemical abnormalities present in serum from these animals that would lead to an alternative diagnosis. Misdiagnosis is more likely if clinicians are not familiar with the disease, which is why proof of progression, over-reactivity and presence of neurological signs (ataxia) are important to identify animals that show signs of the clinical spectrum of BSE. A detailed neurological assessment was considered to be sufficient to exclude BSE in 96 bovines with neurological signs although diseases of metabolic or toxic origin (hypocalcaemia, nervous ketosis, hepatic encephalopathy, cerebro-cortical necrosis, botulism, septicaemia) were more diagnostically challenging [12].

A range of neurological diseases that may be considered in the differential diagnosis of BSE are outlined in Appendix 2. This is not an exhaustive list covering all diseases in every country, however.

2.

Targeting animals for BSE surveillance

(Article 11.4.20. Point 2 of the *Terrestrial Code*)

The objective of BSE surveillance is to detect the disease in the bovine population. Article 11.4.20 of the *Terrestrial Code* identifies those animals that should be reported and followed up with appropriate laboratory testing in accordance with the Terrestrial Manual to accurately confirm or rule out the presence of BSE. They are classified into four distinctive groups:

a. Bovines displaying progressive clinical signs suggestive of BSE.

b. Bovines showing behavioural or neurological signs at ante-mortem inspection at slaughterhouses or abattoirs.

c. Bovines presented as unable to rise or walk without assistance with an appropriate supporting clinical history (i.e. the presentation cannot be attributed to other common causes of recumbency).

d. Bovines found dead (fallen stock) with an appropriate supporting clinical history (i.e. the presentation cannot be attributed to other common causes of death).

These groups correspond to bovines that lie on the clinical spectrum of BSE, and have a higher probability of having BSE, if the disease was present in the country, compared to the general bovine population.

2.1 Bovines displaying progressive behavioural or neurological clinical signs suggestive of BSE

(Article 11.4.20. 2a of the *Terrestrial Code*)

Bovines displaying progressive clinical signs suggestive of BSE are those animals displaying progressive behavioural or neurological signs suggestive of BSE that are refractory to treatment. As part of the procedures and protocols in place covering all stages in the livestock production chain (Article 1.8.5.4 of the *Terrestrial Code*), an official veterinarian requires a detailed anamnesis to confirm that the bovine fits the criteria to be targeted for BSE surveillance, for example: 'an adult animal, the only one affected in the herd with a change in behaviour or temperament, sensation and/or posture/locomotion'.

A working knowledge of 'normal' bovine behaviour is required to know the range of normal behaviour, in particular in the early stages, where clear neurological abnormalities (e.g. incoordination) may not yet be manifest. If unsure and the welfare of the animal is not compromised (it is not recumbent or in obvious distress) it may be advisable to re-schedule another visit after 1-2 weeks to assess whether signs have progressed. This also enables the veterinarian to assess any effect of treatment or wait for blood test results to rule out other diseases if appropriate. It is important to note that BSE may be accompanied by other diseases (e.g. listeriosis and BSE or ketosis and BSE), although this is rare. The lack of response to treatment of any suspected disease and further disease progression may be indicative of BSE. The clinical signs should be documented, particularly if a re-visit is scheduled, using either a detailed clinical examination form (see Appendix 3 for an example) or a simple questionnaire with tick boxes for signs associated with BSE or conditions with similar signs, which is easier to analyse, to compare the frequency of signs with other conditions that may be confused with BSE (see Appendix 4 for an example).

Eventually the official veterinarian may decide to submit the animal for testing. Secondary criteria for targeting for BSE surveillance could be applied at this point, for example: 'an adult animal, the only one affected in the herd with a change in behaviour or temperament/sensation and/or posture/locomotion and/or generalised non-specific signs sustained over several weeks that is refractory to treatment and to which other common causes of behavioural or neurological signs could not be associated'. A minimum set of clinical signs should be present before the animal can be declared as displaying clinical signs suggestive of BSE.

2.2
Bovines showing behavioural or neurological signs at ante-mortem inspection at slaughterhouses or abattoirs
(Article 11.4.20.2b of the *Terrestrial Code*)

This category refers to bovines that did not pass the ante-mortem inspection at abattoirs and show behavioural and/or neurological signs suspicious of BSE. Clinical examination of bovines at abattoirs is usually limited to a short visual inspection because space and time constraints may not allow for a detailed examination without interfering with the routine slaughtering process. In addition, nothing is known of the clinical history of the inspected animals. Pre-screening bovines presented for slaughter by assessing certain behaviour and the response to tactile, acoustic, and visual stimuli was not considered to be specific enough to be useful [13]. As mentioned previously, a clear definition of clinical signs is imperative before they are used as clinical markers in order to limit the number of bovines erroneously identified as BSE suspects thus avoiding a negative impact on the specificity of the surveillance programme. (Without this clear definition of and selection criteria for suspect cases, the system is at risk of poor specificity, meaning that a large number of bovines that do not have BSE are suspected of having the disease).

Observations to assess the health status of bovines generally include assessment of the general body condition of the animal, locomotory changes, cleanliness of the animal and evident signs of injury or inflammation suggestive of a systemic disease. Not all abnormalities will lead to the suspicion of a neurological disease, let alone BSE. For example, the Swiss Veterinary Authority's guidance on carrying out an ante-mortem inspection of slaughter animals advises to check for certain BSE-associated signs in bovines over 30 months of age, which did not pass the initial inspection [14]:

- Unsteady, wobbly gait, buckling, unexplained fall
- Fear of doorways, thresholds, grooves and other obstacles on the floor
- Over-reactivity to noise, sudden light or touch, particularly of head and neck
- Unusually nervous, aggressive or jumpy, with tendency to kick
- Nose wrinkling, teeth grinding

The marked display of one of the signs in each category or signs in more than one category should raise high suspicion of BSE.

2.3
Bovine animals presented as unable to rise or walk without assistance
(Article 11.4.20. 2c of the *Terrestrial Code*)

Clinical examination of bovines presented as unable to rise or walk without assistance is limited because when animals present in recumbency, assessment of gait or testing of over-reactivity by touching the hind legs is not possible. At this stage, bovines may also be less over-reactive to touch. An appropriate supporting clinical history (previous gait abnormalities, sensory or behavioural changes, which cannot be attributed to other common causes of behavioural or neurological signs) is necessary. The clinical history may be available if the affected bovine is reported in the farm but may not be immediately available if the animal presents at an abattoir, during transport or at a cattle market. In more extensive production systems, if an appropriate supporting clinical history is not available, the surveillance system should be more inclusive when deciding whether to test.

BSE does not cause any physical changes but the bovine's increasing difficulty getting up may lead to swollen joints or lesions on the legs [15]. It should be noted that the clinical history may be unreliable, particularly if there is uncertainty about the definition of signs. For example, leg weakness may also be described as lameness.

If bovines are recumbent and treatment based on previous laboratory tests (e.g. treatment with calcium for suspected cases of milk fever) or treatment for other suspected diseases or common causes of recumbency was unsuccessful and did not result in any improvement, BSE should be considered particularly if the adult animal presents with abnormal limb position (one or both hind limbs are stretched backward) or is over-reactive (three consecutive startle responses to either hand approach or clipboard test, flash test or hand clap) [16].

2.4
Bovines found dead (fallen stock) with an appropriate supporting clinical history
(Article 11.4.20. 2d of the *Terrestrial Code*)

Fallen stock includes any animal that has died of natural causes or of disease on a farm; during transport to or while in a slaughterhouse or abattoir; or that has been killed on a farm for reasons other than for human consumption. As the animal cannot be examined alive, historical animal and clinical data from the farmer and veterinarian (if seen prior to death) is useful to decide whether this animal would qualify as a BSE surveillance candidate. An appropriate supporting clinical history (previous gait abnormalities, sensory or behavioural changes, which cannot be attributed to other common causes of behavioural or neurological signs) is necessary before deciding to test.

3.

Components of a credible BSE surveillance system

(Article 11.4.20. Point 3 of the *Terrestrial Code*)

A robust BSE surveillance programme must ensure that all the different steps, from the identification of and follow up of targeted bovines that lie on the clinical spectrum of BSE, until the results of the test done on targeted samples of such animals or their carcases have been produced and reported, can be implemented at any point in space and time. Figure 1 gives a basic overview of the flow of the components of a credible surveillance system to detect BSE cases.

According to point 3 of Article 11.4.20 of the *Terrestrial Code*, a credible surveillance system for BSE should be supported by: ongoing awareness and training programmes, a reporting system based on the notification of the disease, appropriate laboratory testing and robust documented protocols and procedures.

3.1 Ongoing awareness and training programmes

(Articles 11.4.20.3a and 1.8.5.1 of the *Terrestrial Code*)

The decline of BSE cases worldwide has undoubtedly led to a considerable reduction in reporting of clinical suspects, even in countries where the number of BSE cases was initially relatively high (e.g. Great Britain, Switzerland). This emphasises the importance of having a continuous training programme in place to maintain awareness of this notifiable disease. Information about BSE is readily available on the Internet but requires an adequate Internet connection, which may not be available for everyone. Alternative methods of communicating on the topic are to include articles on BSE in professional magazines, newsletters, or other communication material targeted to relevant stakeholders, to include presentations on BSE at agricultural or veterinary shows or other relevant gatherings where this topic could be presented. Veterinary and agricultural students should be made aware of BSE as part of their college or university education and it should also be part of their continuous professional development once they have graduated.

Surveillance relying on the reporting of disease suspicion requires a training program that will ensure all stakeholders involved in the rearing and production of livestock, including bovine breeders, owners and keepers, veterinarians, hauliers and slaughterhouse workers, are aware of the clinical signs of BSE and well as the statutory reporting requirements.

The veterinarian plays a particular role as contact person for the farmer when an animal is ill or during routine farm inspections. The official veterinarian has to make the decision on whether to submit an animal for BSE testing, either on the farm by the field veterinarian or at the slaughterhouse by the veterinary inspector, whose ante-mortem inspection is imperative to decide whether an animal is healthy and fit for human consumption.

Since the suspect diagnosis is based on clinical signs and animal and clinical history, a good knowledge of the disease is imperative for the veterinarians making the decision on the presented animals. That will ensure the system is sufficiently sensitive to detect potential BSE cases (i.e. most of the BSE cases are submitted for testing) and specific (i.e. most of the cases submitted for testing are actually positive), so that the post-slaughter destination of carcasses and financial compensation for owners are correctly determined.

Continuous professional development is often a mandatory requirement for veterinarians. Awareness may be facilitated by offering free lectures or webinars on BSE and referring to these in newsletters or other forms of communication to the

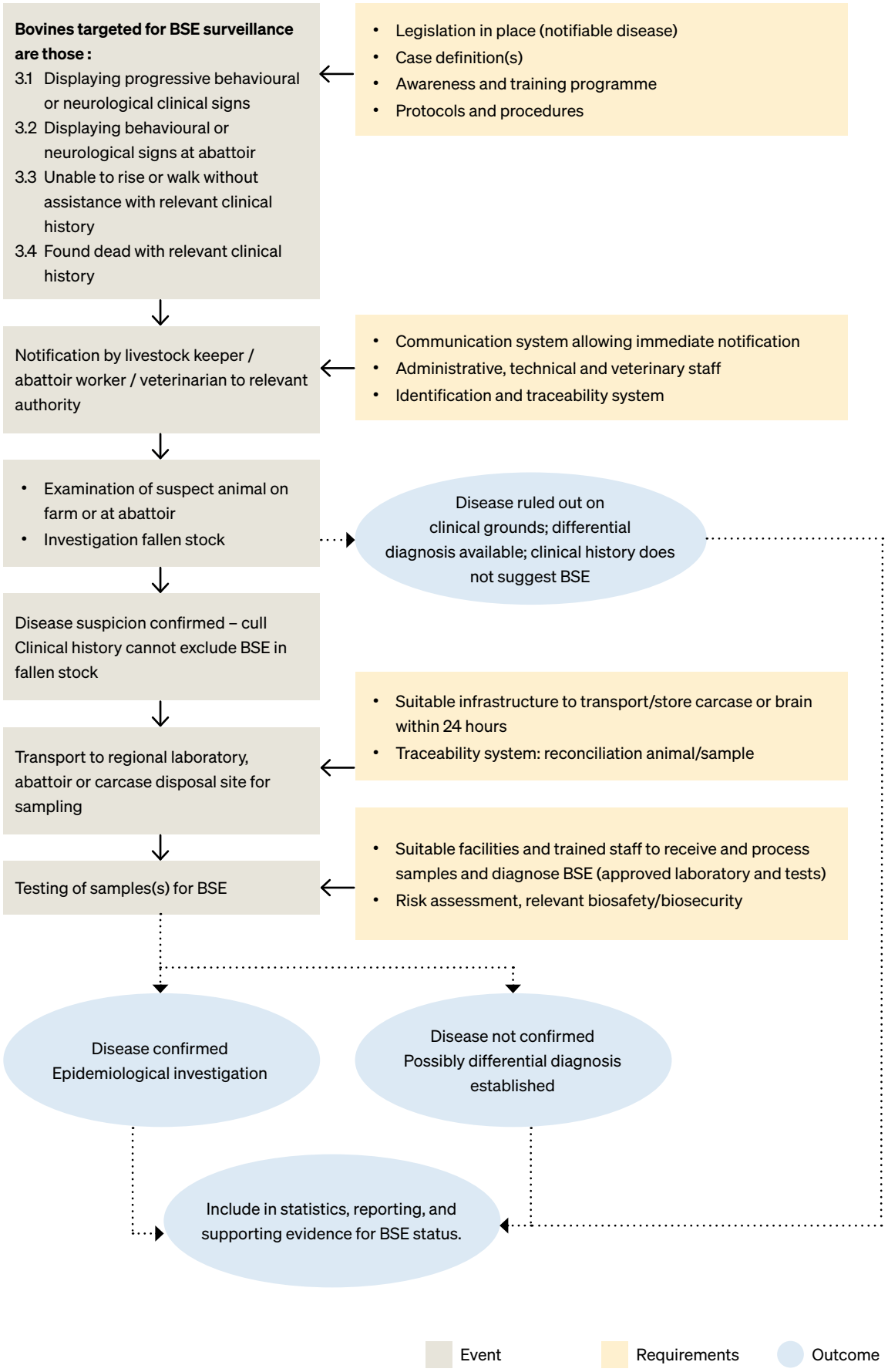

Figure 1.
Overview of the flow of the components of a credible surveillance system to detect BSE cases

veterinary community. Strategic collaboration with the Veterinary Statutory Bodies is also recommended. Websites of national surveillance centres or laboratories are usually an ideal platform to display information about BSE and actions for livestock owners when BSE is suspected.

3.2 Notification of the disease

(Articles 11.4.20.3b and 1.8.5.2a of the *Terrestrial Code*)

According to the provisions of Chapters 11.4 and 1.8 of the *Terrestrial Code* BSE must be a notifiable disease, recognised as such in the national legislation, supported by measures including incentives, compensation or penalties.

Even if stakeholders are familiar with the clinical presentation of BSE, surveillance where only bovines reported with signs of the disease are tested is prone to underreporting. This may be due to the social stigma attached to having a confirmed case, potential loss of source of livelihood or fear of the consequences of a case confirmation.

Some factors that may improve reporting of BSE cases are: financial compensation in case the animal is culled and tested and declared not fit for human consumption; routine veterinary visits (higher probability that cases are discussed and observed by a veterinarian); good relationship between farmer and veterinarian; trust in the competent authority; education and knowledge (see section 4.1); identification and training on epidemiological sensors/detectors [17-20].

3.3 Laboratory testing

(Articles 11.4.30.3c and 1.8.5.3 of the *Terrestrial Code*)

BSE surveillance generally requires a good infrastructure to sample brains before severe autolysis sets in, including adequate facilities to store samples temporarily at low temperatures, to distribute samples and process, and test them within a short turnaround time. This may not be possible in every country depending on livestock practices, climate and resources. In those situations, surveillance activities may focus on bovines in facilities that handle larger numbers of animals, such as slaughterhouses, abattoirs or carcase disposal sites. Proximity to a laboratory and availability of reliable courier or transport services will also influence surveillance.

3.4 Protocols and procedures

(Articles 11.4.20.3d and 1.8.5.4 of the *Terrestrial Code*)

A credible surveillance system for BSE must have robust and documented procedures and protocols for the identification and reporting of potential candidate animals targeted for BSE surveillance, for the determination of animals to be subjected to laboratory testing, for the collection and submission of samples for laboratory testing, and for the follow-up epidemiological investigations for BSE positive findings.

The system in place should be able to confirm the identity of the animal(s) selected for surveillance and ensure traceability through the entire process.

3.4.1
Definition of the target population for BSE surveillance

The target population for BSE surveillance should be clearly defined to ensure that the surveillance system is credible, reliable, and sufficiently specific and sensitive, as described above. The target population for BSE surveillance is described in section 3 of these guidelines and should be tailored to each country's context.

3.4.2
Reporting of bovines described in sections 3.1 to 3.4

At the planning stages, a credible surveillance system will have to be tailor-made for each country based on multiple factors, including, climate and geography, bovine population and distribution, husbandry systems, legal framework, veterinary manpower, infrastructures and resources, and cooperation between the different stakeholders, among others.

A country may consider setting some indicators to aid in designing and planning the surveillance (see Table 1). In the absence of any assigned quota on the number of bovines that should be tested in each of the four target groups, these indicators can be used to set targets for the surveillance in each group. These indicators could be reviewed annually and used to evaluate the performance of the surveillance.

Once an animal has been reported as a possible suspect case, a protocol should be in place to investigate and record it. Appendix 3 provides an example of a clinical examination form for this stage, on a live animal, which would be part of those described under 3.1. Similar templates could be developed for the other three groups.

Table 1.
Examples of indicators for planning and evaluating BSE surveillance.

Risk group	Examples of reference indicators
Bovines displaying progressive behavioural or neurological clinical signs suggestive of BSE	• List of diseases or conditions causing neurological signs in adult bovines that are present in the country • Expected prevalence of these diseases (if data available) • Percentage of notifications of bovines with neurological signs compatible with BSE in recent years • Number of adult bovines notified as BSE suspects
Bovines showing behavioural or neurological signs at ante-mortem inspection at slaughterhouses or abattoirs	• Most frequent causes of rejection at ante-mortem inspection at abattoirs or slaughterhouses • Percentage of adult bovines that did not pass the ante-mortem inspection at abattoirs or slaughterhouses
Bovines presented as unable to rise or walk without assistance	• List of diseases or conditions causing recumbency in adult bovines (over four years old) in the country • Percentage of adults unable to rise or walk without assistance, found on farm, relative to the adult bovine stock (over four years old) (if available, including suspect diagnosis)
Bovines found dead (fallen stock) with an appropriate supporting clinical history	• Percentage of adult bovines found dead in the field/on farm relative to adult bovine population • Percentage of adult bovines found dead in transport relative to transported adult bovine population. • Percentage of adult animals found dead at animal markets or abattoirs relative to the adult bovine population present at animal markets or abattoirs

3.4.3
The determination of animals to be subjected to laboratory testing

Appendix 4 provides an example of a questionnaire used for the clinical presentation of reported suspect BSE cases.

3.4.4
The collection and submission of samples for laboratory testing

Brain samples are required for a diagnosis of BSE. This is ideally achieved through the foramen magnum when the head is separated from the neck using scissors, forceps and a spoon-like instrument because it does not require opening the skull (see Chapter 3.4.5 of the *Terrestrial Manual*). The target area is the obex in the brainstem, which needs to be considered when a live animal is euthanised by shooting so as to avoid too much trauma to the brain.

3.4.5
The follow-up epidemiological investigations for BSE positive findings

In case of a classical BSE case, the epidemiological investigation should be completed as soon as possible to identify the source and take precautions to prevent the occurrence of further cases and any risk to human health, e.g. by removing bovines born around the same time (cohort) as the confirmed BSE case, which have been potentially exposed to the same food source, as well as the offspring of, the BSE case.

It is assumed that the food-borne route is the most likely source of the classical BSE cases. Determining the origin of a BSE outbreak is the ideal objective of the epidemiologic investigation of BSE cases, but also complicated due to the long incubation period because ingestion of contaminated feed typically occurs at a young age and many years will have passed before the animal develops clinical BSE.

If a case of BSE is identified, an epidemiological investigation should aim to clarify if any identified source of infection has been controlled and if the risk of BSE agents being recycled within the bovine population has continued to be negligible. The investigation is also advisable for cases of atypical BSE.

Following the detection of a case of BSE in Ireland in 2015, a questionnaire was developed to aid field-based data collection [21]. An epidemiological questionnaire should cover the following points:

- Animal details (tag or other identification, age, sex, breed, home-bred or purchased)
- Date and location of visit
- Herd details (dairy, suckler, mixed, etc.)
- Herd size
- Practising veterinarian responsible for the farm
- Age structure of herd
- Date of clinical onset, stage of lactation and/or use for embryo transfer, use for semen collection in case of bulls
- Dam/sire of the case; details of offspring and the fate of offspring
- Details of the nutritional management of the herd: supplementary feeds offered (including milk replacers), their source, dates of delivery, whether or not rations were mixed on farm, details of drinking water supply
- Feed storage and cleaning of feed storage areas

- Surgical procedures and veterinary treatments carried out on the animal
- Previous cases (contact with previous cases or any organic material from previous cases)
- Other species kept on farm and duration; contact of case with other species; exposure of case to feed from other species
- Waste management (manure, abattoir waste, placenta, etc.)
- Carcase disposal on farm
- Presence of other diseases, particularly prion diseases, on the farm (e.g. scrapie or chronic wasting disease).

4.
Appendices

Appendix 1

Bovine spongiform encephalopathy: the disease

Bovine spongiform encephalopathy (BSE), commonly referred to as 'mad cow disease', is a progressive and fatal transmissible spongiform encephalopathy affecting bovine animals (*Bos taurus* and *Bos indicus*) caused by the misfolding and subsequent accumulation of the pathogenic misfolded isoform (PrP^{Sc}) of the prion protein in the brain. BSE is a rare disease affecting single animals in a herd. It is extremely rare to find two cases in a herd with disease at the same time and when this did occur it was in countries where the incidence of BSE was relatively high.

The misfolded, disease-associated prion proteins produced by this disease are resistant to enzymatic digestion by proteinases, resulting in their lethal capabilities. However, this resistant characteristic also makes them useful disease markers in diagnostic tests, such as Western immunoblot or immunohistochemistry. This has resulted in the discovery of different BSE types: classical and atypical BSE. Classical BSE is known to be zoonotic and the cause of variant Creutzfeldt-Jakob Disease in humans [22]. Although atypical BSE isolates have been shown to be transmissible to transgenic mice carrying the human prion protein gene [23], at the time of writing atypical BSE has not been directly associated with any human prion disease.

Classical BSE is linked to feeding bovines meat and bone meal (usually in concentrate rations) contaminated with the BSE agent. It has not been conclusively determined if the BSE agent was always present in cattle populations (similar to scrapie in sheep and goats) and conditions favouring recycling of the agent allowed infection to spread, leading to its emergence as a 'new' disease in Great Britain in 1985. The occurrence of further classical BSE cases in many countries has been prevented by prohibiting the inclusion of processed animal protein in ruminant feed and subsequently in livestock feed. The ban of processed animal protein in ruminant feed also prevents recycling of an atypical BSE agent in feedstuffs.

Atypical BSE is detected most frequently in bovines over eight years of age although younger cases have been reported, for instance five years in Spain in 2019 [24]. Based on experimental studies, disease progression is generally slow, ranging from weeks to months, and determining disease onset depends on the level of observation, which is higher in dairy cows that are milked daily. An animal may also present with an apparent sudden onset of disease (e.g. unable to get up) even though its behaviour or temperament may have changed weeks or months prior to that.

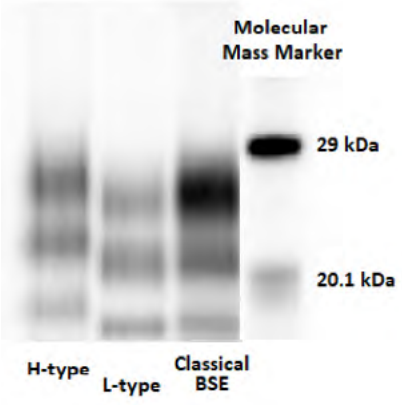

Figure 2.
Western immunoblot on brain samples from classical and atypical BSE cases. Antibody Sha31; the bottom protein band is higher in H-type BSE compared to classical BSE, whereas it is lower in L-type BSE.

Atypical BSE is not believed to be food-borne and is detected in older bovines at a frequency of about one in 1 million tested cattle, like the sporadic form of TSE in humans, which occurs spontaneously [25]. However, it has been shown experimentally that the atypical BSE agent can cause disease in bovines when given in a high dose by the oral route [26]. There are two types of atypical BSE, distinguishable by the migration pattern of digested disease-associated prion protein in a Western immunoblot. H-type atypical BSE [27] has a bottom prion protein band that is higher compared with the equivalent band for classical BSE. L-type atypical BSE [28] has a bottom prion band that is lower compared with the equivalent band for classical BSE (see Fig. 2).

Unlike other infectious diseases the confirmatory diagnosis of BSE is currently only possible after the death of an animal (post mortem) because affected bovines do not develop an

immune response to the prion protein that can be used for diagnostic purposes in live animals. In addition to this, the disease-associated prion protein is not present in accessible tissues or fluids in significant amounts to be detectable by ante mortem diagnostic tests. Suspicion of disease is therefore based on clinical presentation. However, pathognomonic signs to reliably diagnose BSE clinically do not exist. There are some helpful clinical markers, however, that assist in forming a suspected diagnosis of BSE; examples of these markers are described in the supplementary material outlined as follows.

Supplementary material

Clinical protocols

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Additional training material

- Department of Agriculture, Food and the Marine, Republic of Ireland. Neurological signs of BSE. https://www.youtube.com/watch?v=8-BIh3ZcHFc
- APHA Weybridge, Great Britain. Clinical signs of bovine spongiform encephalopathy in cattle. https://vimeopro.com/aphalearning/clinical-signs-of-bovine-spongiform-encephalopathy-in-cattle
- Department of Farm Animals, University of Zurich. Clinical findings in bovine spongiform encephalopathy. https://www.youtube.com/watch?v=V09hriOtAn4
- Webinar Vet, Great Britain. Transmissible spongiform encephalopathies in cattle. Presentation by T Konold, APHA Weybridge. https://www.thewebinarvet.com/webinar/transmissible-spongiform-encephalopathies-in-cattle (requires registration, which is free)

Differential diagnosis

- K Robinson, APHA. Neurological disease investigation in cattle. https://www.youtube.com/watch?v=XyOTEm5edhQ
- RB Kushwaha India. Rabies in cow. https://www.youtube.com/watch?v=Sl92jM59dyo
- Video resources from de Lahunta'sVeterinary Neuroanatomy and Clinical Neurology book, case studies. http://www.neurovideos.vet.cornell.edu/index.aspx

Appendix 2

Neurological disorders pertinent to the differential diagnosis of BSE

Neurological disorders of bovines that need to be differentiated from BSE (classification by major clinical signs). Disorders with multiple signs may appear in several categories

	Disorder / Disease	Disorders of behaviour and personality	Seizures	Visual dysfunction	Cranial nerve disorders (including strabismus and dysphagia)	Head tilt, circling, nystagmus and other signs of vestibular abnormalities	Opisthotonos, tetany, tremor, muscle spasm	Coma and altered states of consciousness	Incoordination of the head and the limbs: cerebellar diseases	Tetraparesis, paraparesis, ataxia of the limbs, and episodic weakness	Itching, licking, self-mutilation
	BSE	X	X				X	X	X	X	X
Familial and Congenital	Bovine ceroid lipofuscinosis	X	X	X				X		X	
	Generalised glycogenosis	X	X	X				X		X	
	Mannosidosis	X									
	Convergent strabismus				X						
	Exophthalmus				X						
	Spastic syndrome of adult bovines						X				
	Familial epilepsy		X								
	Cerebellar abiotrophy								X		
	Progressive ataxia of Charolais bovines									X	
	Bovine progressive degenerative myeloencephalopathy ('weaver')									X	
	Kyphosis of Jersey bovines									X	
	Multifocal symmetrical encephalopathy									X	
Physical	Head trauma	X	X	X	X	X	X	X	X		
	Post-calving paralysis									X	
	Spinal cord and vertebral trauma									X	
Nutritional	Thiamine responsive cerebrocortical necrosis	X	X	X	X		X	X		X	
	Vitamin A deficiency		X	X							
	Nutritional myodegeneration (white muscle disease)									X	
	Sodium deficiency	X	X	X							X
Metabolic	Hypomagnesemia	X	X				X	X		X	
	Ketosis	X						X			X
	Hepatic encephalopathy	X	X					X			
	Hypocalcaemia	X	X				X	X		X	
	Metabolic encephalopathies			X							

	Disorder / Disease	Disorders of behaviour and personality	Seizures	Visual dysfunction	Cranial nerve disorders (including strabismus and dysphagia)	Head tilt, circling, nystagmus and other signs of vestibular abnormalities	Opisthotonos, tetany, tremor, muscle spasm	Coma and altered states of consciousness	Incoordination of the head and the limbs: cerebellar diseases	Tetraparesis, paraparesis, ataxia of the limbs, and episodic weakness	Itching, licking, self-mutilation
Infectious	Bacterial meningitis and meningoventriculitis	X	X	X	X		X	X			
	Louping ill	X						X	X	X	
	Thromboembolic meningoencephalitis (TEM)	X		X	X			X		X	
	Listeriosis	X			X	X		X			
	Rabies	X	X		X			X			X
	Pseudorabies	X	X	X				X			X
	Verminous encephalitis	X			X	X		X	X		
	Myelitis	X				X		X	X	X	
	Sporadic bovine encephalomyelitis (Buss disease) and other inflammatory meningoencephalomyelitides	X	X		X			X		X	
	Encephalitis of viral bovine rhinotracheitis	X		X	X			X		X	
	Malignant catarrhal fever (MCF)	X	X	X				X			
	Botulism	X			X			X		X	
	Tetanus	X					X	X		X	
	Mycotic encephalitis	X	X	X	X			X		X	
	Babesia encephalitis	X						X		X	
	Otitis media-interna	X				X		X	X		
	Sarcocystitis					X				X	
	Clostridial polymyositis									X	
	Theileriosis					X					
	Bovine trypanosomiasis					X					
	Bovine parasitic otitis					X					
	Actinobacillosis, actinomycosis	X						X			
Idiopathic	Nervous coccidiosis	X	X	X			X	X			
	Thoracolumbar spondylosis deformans and osteoarthrosis									X	
Space occupying lesions	Abscesses	X	X	X	X	X	X	X	X	X	
	Neoplasia	X	X	X	X	X	X	X	X	X	
	Granuloma	X	X	X	X	X	X	X	X	X	
	Cysts involving the central nervous system	X	X	X	X	X	X	X	X	X	

	Disorder / Disease	Disorders of behaviour and personality	Seizures	Visual dysfunction	Cranial nerve disorders (including strabismus and dysphagia)	Head tilt, circling, nystagmus and other signs of vestibular abnormalities	Opisthotonos, tetany, tremor, muscle spasm	Coma and altered states of consciousness	Incoordination of the head and the limbs: cerebellar diseases	Tetraparesis, paraparesis, ataxia of the limbs, and episodic weakness	Itching, licking, self-mutilation
Toxic	**Lead poisoning**	X	X	X	X		X	X		X	
	Metaldehyde toxicity		X				X				
	Cyanide poisoning	X	X				X	X			
	Salt and water intoxication	X	X	X	X		X	X			
	Organophosphates	X	X		X		X	X		X	X
	Ivermectin toxicosis									X	X
	Methyl bromide intoxication									X	
	Ethylene glycol toxicosis									X	
	Chlorinated hydrocarbons	X	X				X	X			
	Urea-ammonia	X	X	X				X			
	Strabismus	X						X			
	Thiamine responsive cerebrocortical necrosis	X	X	X	X			X			
	Organomercury toxicity	X		X				X	X	X	
	Polyether antibiotics: monesin and lasalocid intoxication									X	
	Sorghum toxicity									X	
	Plant associated tremor syndromes		X			X	X				
	Miscellaneous toxic plants (e.g. locoweed)	X	X	X				X	X	X	
	Plant induced mannosidosis	X						X			
	Nitrofurazone toxicosis						X				
	Tick paralysis									X	
	***Kochia scoparia* poisoning (Mexican fireweed)**	X		X				X			

Appendix 3

Clinical examination forms with examples

Example 1

Animal No: 110110 654321 Farm: Owner:
Clinician:
Date:

- If normal: tick (✔)
- if abnormal: circle (◯ when listed) and describe in detail on following page if insufficient space in box
- if test not performed: cross out (**X**), and always indicate why if non-performance is due to the animal's reaction

ANIMAL FREE

Posture (head, neck, limbs, back)	Low head on occasions	
Walking (amount / willingness)	Stop and go	
Turning	✔	
Running (amount / willingness)	Trot: None	Gallop: None
OVERALL GAIT **Stiff/Lame** **Neurological** **Other on gait**	(No) / Yes, describe: (No) / May be / Yes, describe: (No) / Yes, describe:	
Slipping / Falling (describe if yes)	(No) / Yes _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	(No) / Yes_ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Obstacle (device: drain cover)	Hesitant to step over drain; sniffs a lot before crossing	
Acceptance of crush (going in)	Hesitant; needs to be pushed with force	

ANIMAL IN CRUSH

	Symmetry	**Left**	**Right**
Eye position (strabismus?)	✔		
Eyelid position (ptosis?)	✔		
Third eyelids (position)	✔		
Nose (sym. & movements to breath)	✔		
Menace response	Exaggerated (head toss)		
Ears (position and reaction to touch)	✔		
Blink (lateral & medial canthus)	✔		
Nose (reaction to touch)	✔		
Lips (sym. / reaction to touch: smile)	✔		
Eye movements	✔		
Sweat beads on muzzle	✔		
Salivation (✔, ↑, or ↑↑)	Increased after head tests		
Jaw position / Tongue tone	✔	✔	

HEAD RESTRAINED/ HALTER

	Symmetry	Left	Right
Optic nerve / fundus	Not examined (too bright)		
Light reaction (direct & consensual)	Not examined (too bright)		
Corneal reflex	✔		
Cutaneous trunci (CT) **& neck prick** (NP)	CT: ✔	NP: nervous (head toss), vocal	
Tail tone / anal tone	✔	✔	

OVERALL ASSESSMENT

Mental status ✔ (normal), dull, depressed, 'hyper', etc.	Hyper, seems very alert, constantly moving ears	
Behaviour & reactivity free ✔ (normal), excited, playful, fearful, nervous, friendly, boisterous, dangerous, 'hyper', active, quiet etc.	Nervous, startles frequently, e.g. when bird flew over, when sniffing crush	
Behaviour in crush ✔ (normal), quiet, restless, agitated, agitated 1st then settled down, never settled down, frantic, etc.	Head toss when approached from front; generally restless	
Behaviour Head restraint (HR) & Head tests (HT)	HR: Nervous, head tossing	HT: Nervous, head tossing
Clipboard test**	Body flinch 5x	
Bang test (BT) **/ Hand Clap** (HC)	BT: No reaction	HC: No reaction
Flash test	Not tested (too bright)	
Flexible stick test	Forceful kicking (only tried 2x)	
Tremors	No / Yes, describe: Fine head tremor when undisturbed in crush	
Scratch test	✔	

Example 2

Animal No: 110110 654321

Date:

GENERAL EXAMINATION

Temperature: 38.7°C

Mucous membranes: ✔

Heart rate: 56 bpm despite restlessness

Lymph nodes: ✔

Ruminal Contractions: ✔

Body condition: Good (3)

Dehydrated? ✔

Additional/ extraneural findings: grazes on hind legs

Behaviour in pen prior to exam: very alert, following every move; separated from others as becoming aggressive towards other cows

Status: ( ) BSE not suspected
(✔) Maybe BSE
( ) BSE suspect

TO DO:

Action	Date completed	Result
Bloods taken: EDTA, Serum	01 July 2022	
Urine taken:		
Skin scraping (location):		
Video of (describe):	01 July 2022	Behaviour in pen
Still photograph of (describe):		
Other :		Reschedule visit in 14 days to check for clinical progression

Appendix 4

Example of a questionnaire on the clinical presentation of reported suspect BSE cases

Clinical signs observed by veterinary officer.
(Please enter 'X' in appropriate box(es) if observed. If only reported by farmer and not observed by veterinary officer, enter 'R')

Disorders of behaviour and personality

Apprehension		Ear twitching		Kicking in parlour	
Hypersensitivity		Ear held at odd angles		Reluctance to go into parlouror through doorways	
Touch					
Sound					
'Maniacal'		Abnormal behaviour		Head pressing	
Panic striken		Head shyness		Head rubbing	
Temperament change		Licking of flank		Teeth grinding	
Abnormal head carriage		Licking of nose		Other	

Locomotor/Neurological signs

Blindness		Falling		Recumbency	
Circling		Paresis		Tremors	
Hindleg ataxia		Foreleg ataxia		Knuckling of fetlock	

General signs

	Decrease	No change	Increase	Not applicable
Weight				
Condition				
Milk yield				

Initial signs:

Clinical progression:

Other comments:

5.
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