Bovine spongiform encephalopathy surveillance in Argentina

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Summary: Bovine spongiform encephalopathy (BSE) is a new disease of cattle first described in the United Kingdom in November 1986. BSE belongs to the scrapie-related group of diseases. The epidemiological studies performed in the United Kingdom demonstrate that the BSE epidemic was caused by feeding cattle with ruminant-derived protein contaminated by a scrapie-like agent. Until June 1994, the disease had been detected in indigenous cattle in Ireland, Switzerland and France. Three cases reported in Germany, two in the Sultanate of Oman, and single cases in the Falkland Islands (Islas Malvinas), Denmark, Portugal and Canada occurred in animals imported from the United Kingdom. Several countries have implemented surveillance programmes analysing the risk factors involved in the epidemic. An analysis of risk factors conducted in Argentina shows that it is highly unlikely that BSE or scrapie exist in the country, or will arise via feed in the future.

As a continuation of the analysis of risk factors, a surveillance programme was implemented in the field and in abattoirs. Specialised personnel were trained in the clinical, histopathological and biochemical detection of the disease through a network of laboratories which covered 85% of the total cattle population and 100% of the high-risk group (dairy cows over five years of age). By using a statistical procedure with reference to the bovine population in nine provinces, 1,019 brains from animals belonging to the high-risk group were selected and studied by histopathological and biochemical analyses for BSE detection. The results were negative in all cases.

It can be concluded from this analysis (with a sensitivity of detection of 2.95 per 1,000, and 95% statistical confidence) that Argentina may be regarded as BSE-free, and that the importation of infected animals or by-products may represent the sole potential source of introduction of BSE infection into the country in the future.

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INTRODUCTION

Bovine spongiform encephalopathy (BSE) is a recently-detected disease of cattle, first described in the United Kingdom in November 1986 (30). Retrospective diagnosis showed that the first cases occurred as early as April 1985 (34). Until July 1993, the epidemic involved 104,417 cases on more than 25,000 farms (33).

BSE belongs to the group of 'scrapie-like' diseases, which affect the brain and spinal cord, inducing vacuolar ('spongiform') lesions (30, 32) and accumulation of disease-specific fibrils (SAF: scrapie-associated fibrils), derived from a native sialoglycoprotein called the prion protein (PrP) (8, 21).

Epidemiological studies conducted in the United Kingdom have demonstrated that the vehicle of the infection was meat-and-bone meal containing ruminant-derived protein contaminated with a scrapie-like agent. This meat-and-bone meal was incorporated into bovine feedstuffs and fed to cattle, resulting in an increase in exposure to the agent which was sufficient to cause disease (34, 35).

Cattle were exposed to the infection abruptly and simultaneously in several parts of the United Kingdom in 1981-1982 (35). The major reason for this phenomenon was a change in the processing of meat-and-bone meal, which was originally produced using hydrocarbon solvents for the extraction of tallow. As a result of this change, the scrapie-like agent present in the ruminant waste was not fully inactivated by at least some of the replacement methods of rendering.

Until September 1993, 73 histopathologically-confirmed cases of BSE had been detected in Ireland (4), some of which occurred among cattle imported from the United Kingdom. The two cases reported in the Sultanate of Oman (2), the three cases in Germany, and the single cases in the Falkland Islands (Islas Malvinas), Denmark, Canada and Portugal (until June 1994; figures reported to the Office International des Epizooties [OIE]) occurred in bovines imported from the United Kingdom.

Forty cases have been reported in Switzerland (9) and nine in France (OIE figures). The origin of these cases and some of the cases in Ireland is not known, but they could have derived from the import and use in cattle feed of infected meat-and-bone meal from the United Kingdom. In addition, five cases of ovine scrapie have been detected in Italy and five cases in Belgium since the beginning of the surveillance programme (28).

Knowledge of the epidemiology of BSE led to the elaboration of analyses of risk factors for BSE in France (19), Spain (18) and Germany (24). A detailed analysis has been performed in the United States of America (USA) (26, 29). A similar study was conducted in Argentina, analysing factors related to ovine and bovine populations, feeding and slaughter conditions, structure and functioning of the animal health services, and importation of cattle, semen and embryos from susceptible species. This analysis established that both the existence and the development of BSE in Argentina are highly improbable, provided that the present conditions and controls are maintained (1, 22).

Although clinical signs of BSE are readily discernible (more than 85% agreement between clinical detection and histopathological diagnosis: 14), no ante-mortem
diagnostic assay exists for either the aetiological agent or the by-products of infection. Only post-mortem histopathological examination, electron microscopy and biochemical analysis of nervous tissues are available as confirmatory tests (31, 32). Good correlation exists between histopathological and biochemical diagnosis (21). Biochemical analysis is necessary when histopathological observation is inconclusive or when there is significant autolysis of the sample (12).

In view of the possibility that BSE may be an endemic infection which has remained undetected due to a very low incidence of clinical disease (14), several countries of the European Union (28) and the USA (16) have initiated surveillance programmes based on the histopathological examination of brains from cattle suspected of having disease of the central nervous system (CNS).

As a follow-up to the analysis of BSE risk factors in Argentina (1, 22) – which showed that the existence of BSE in the country was highly unlikely and that the importation of infected animals was the only potential source of infection (meat-and-bone meal is not fed to cattle, sheep or other ruminants in Argentina) – this country has enforced several regulations to prevent the introduction of BSE. These measures were elaborated according to the OIE recommendations for prevention of BSE (17). A surveillance programme was also initiated by establishing a network of private and public diagnostic laboratories. The programme works through the official system of animal health control in the major risk area (dairy cattle range), and in abattoirs subjected to federal inspection. This system was organised for the detection and sampling in the field of bovines suspected of having disease of the CNS, and/or sampling in abattoirs of animals belonging to the high-risk population (dairy cows over five years of age).

This programme included the following:
- information for veterinary surgeons and cattle owners on the subject of BSE;
- mandatory notification of suspected neurological disease in bovines;
- training of specialised personnel in the diagnosis of spongiform encephalopathies, collection and forwarding of material;
- examination of more than 1,000 bovine brains in one year by histopathological and biochemical analysis.

The purpose of this report is to present the results obtained after analysis of 1,019 bovine brains from nine provinces, corresponding to the higher-risk bovine population. Eleven samples were collected from the market in Liniers, where animals from a number of different provinces are present. No data are available regarding the precise origin of these samples.

**MATERIALS AND METHODS**

**Surveillance system**

A surveillance system was established, employing technical personnel from the *Servicio Nacional de Sanidad Animal* (SENASA), the *Instituto Nacional de Tecnología Agropecuaria* (INTA) and a private diagnostic laboratory. The system began operating in the field and in abattoirs in November 1992 (Fig. 1; Table I).
Samples and sampling design

Bovine brain samples were collected from animals over five years of age, and belonged to one of the following categories:

\[ a \] 882 brains extracted deliberately from dairy cows over five years of age selected at random in abattoirs;

\[ b \] 91 brains extracted deliberately from clinically-suspect animals detected in abattoirs;

\[ c \] 40 brains from animals suspected of neurological disease which were sent from the field to the laboratory.

Eighty-five percent of the bovine population of the country (100% of dairy cattle) reside in the geographical region covered by the sampling network (Figs 1 and 2).

The number of samples was 1,019, which enables detection of infection levels greater than 2.95 per 1,000, with 95% statistical confidence (23).
TABLE I

<table>
<thead>
<tr>
<th>Institution</th>
<th>Neurological field cases</th>
<th>Suspect (a)</th>
<th>Inconclusive (b)</th>
<th>Normal (c)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTA Castelar</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>SIPA General Pacheco</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>128</td>
<td>135</td>
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<tr>
<td>SIPA Pontevedra</td>
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<td>6</td>
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<td>259</td>
</tr>
<tr>
<td>Laboratorio Azul</td>
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<td>-</td>
<td>-</td>
<td>13</td>
</tr>
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<td>SAC Producción</td>
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<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>INTA Anguil</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>INTA Bariloche</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>INTA Villa Mercedes</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>SELSA Esperanza</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>SELSA Reconquista</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
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<td>1</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>SIPA Rosario</td>
<td>-</td>
<td>47</td>
<td>-</td>
<td>496</td>
<td>543</td>
</tr>
<tr>
<td>Sub-total</td>
<td>40</td>
<td>91</td>
<td>6</td>
<td>882</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,019</td>
</tr>
</tbody>
</table>

a) observed at the abattoir as suspected of having disease of the central nervous system
b) inconclusive histopathological diagnosis
c) no clinical evidence of disease

INTA: Instituto Nacional de Tecnología Agropecuaria
SIPA: Servicios de Luchas Sanitarias
SAC: Secretaría de Agricultura de Catamarca
SELSA: Servicios de Inspección de Productos Animales

Sample collection and forwarding

In all cases (clinical cases or animals detected in abattoirs), the materials collected were as follows:

- (for histopathological analysis) thalamus and brain stem (including two-thirds of the medulla oblongata and one cerebral hemisphere) fixed in 10% formol saline;
- (for biochemical analysis) remaining third of the medulla oblongata, part of remaining brain hemisphere, and cerebellum (immediately frozen at -20°C).

The samples were labelled according to a previously-established code. Farm of origin, breed, sex and age were recorded. Specimens were sent to the laboratory within 24 h.

Histopathological processing of samples

The procedures employed were those established in the Spongiform Encephalopathy Diagnosis Workshop III at the Central Veterinary Laboratory, Weybridge, United Kingdom.

Extreme care was taken to avoid the possible introduction of artifacts during the fixation procedure. Macroscopic observation of the fixed tissue was performed, after
Geographical distribution of cattle and numbers of samples

Numbers on the map indicate the numbers of samples collected and analysed by histopathology (upper figure) and the number of samples also analysed by biochemistry (lower figure)

making coronal cuts 5 mm apart. In all cases, the selected sites were as follows:
- medulla at the obex
- medulla through the caudal cerebellar peduncles
- midbrain at two levels (to include the superior colliculus and the red nucleus).

When analysing samples from clinical field cases, the following additional sections were taken:
- cerebellum
- frontal cortex and basal ganglia
- temporo-parietal cortex and rostral to the thalamus
- occipital cortex including hippocampus.

Bacteriological, virological and/or biochemical examinations were performed as required.

The diagnostic criteria recommended by Wells et al. (30) were applied. The results were grouped into three categories: positive, negative or inconclusive. The
histopathological examination was directed towards the detection of the following characteristics, typical of BSE lesions:

**a)** vacuolating spongiform lesion of the neuropil and discrete, spherical vacuoles (microcystic vacuolation) which make the grey matter appear to be porous. This must not be confused with oedema, post-mortem changes and fixation artifacts.

**b)** intracytoplasmic vacuolation of the neurons of some nuclei, showing large vacuoles which can be single or multiple; these vacuoles may even change the neuronal morphology. The following nuclei may be involved:
- dorsal nucleus of the vagus nerve
- solitary tract
- spinal tract of the trigeminal nerve
- reticular formation
- vestibular nuclei
- central grey matter of the mesencephalon.

Vacuolation of the red nucleus (and sometimes the oculomotor nucleus) was regarded as non-specific, in accordance with reports describing the presence of such vacuolation as incidental and not related to CNS pathology (5).

**c)** anatomical distribution and symmetrical location of lesions (5, 30).

In some clinical cases, lesions were characterised by microscopy, and it was possible to establish the aetiology of the disease accordingly. In the remaining cases, complementary analyses (bacteriological, virological, toxicological and biochemical analyses of blood) were necessary.

### Biochemical processing of samples

The basis of biochemical diagnosis of BSE is the detection of PrP, the infectious isoform of which (PrP 27-30 or PrP<sup>Sc</sup>) is resistant to proteinase K digestion.

Extraction of PrP was performed in accordance with the method described by Hilmert and Diringer (7), modified according to the protocol of the Central Veterinary Laboratory, Weybridge, United Kingdom. The extracted proteins were separated in a 15% polyacrylamide gel, in accordance with the method described by Laemmli (15). Purified PrP 27-30 was included as positive control. The proteins were transferred to a nitrocellulose membrane in accordance with the method described by Towbin et al. (25). An enzyme-linked immunosorbent assay (ELISA) was conducted, employing an anti-PrP antiserum raised in rabbits (i.e. alkaline-phosphatase-conjugated anti-rabbit serum) and the system naphtol-as-mix-phosphate/fast red TR salt as colour reagent.

A sample was considered positive for the infectious isoform of PrP when a band with the electrophoretic mobility of this protein was detected by the ELISA.

All 1,019 samples were analysed by histopathology (Table II). All samples from clinical cases and from suspect animals detected in abattoirs, and those with inconclusive histopathological diagnosis (a total of 137) were processed for biochemical detection of PrP<sup>Sc</sup>. This study was adopted as a confirmatory analysis, since at least one report has stated that PrP<sup>Sc</sup> may be detected in the absence of histopathological lesions (12).
**TABLE II**

*Geographical distribution of field, suspect and inconclusive samples*

<table>
<thead>
<tr>
<th>Province/State</th>
<th>No. of samples</th>
<th>Neurological field cases</th>
<th>Suspect (a)</th>
<th>Inconclusive (b)</th>
<th>BSE diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liniers*</td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>0/11</td>
</tr>
<tr>
<td>Buenos Aires</td>
<td>396</td>
<td>19</td>
<td>44</td>
<td>4</td>
<td>0/396</td>
</tr>
<tr>
<td>Catamarca</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>0/2</td>
</tr>
<tr>
<td>Córdoba</td>
<td>203</td>
<td>5</td>
<td>12</td>
<td>–</td>
<td>0/203</td>
</tr>
<tr>
<td>Corrientes</td>
<td>7</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>0/7</td>
</tr>
<tr>
<td>Entre Ríos</td>
<td>96</td>
<td>–</td>
<td>10</td>
<td>–</td>
<td>0/96</td>
</tr>
<tr>
<td>La Pampa</td>
<td>7</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>0/7</td>
</tr>
<tr>
<td>Río Negro</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>0/1</td>
</tr>
<tr>
<td>Santa Fe</td>
<td>285</td>
<td>7</td>
<td>23</td>
<td>1</td>
<td>0/285</td>
</tr>
<tr>
<td>San Luis</td>
<td>11</td>
<td>5</td>
<td>1</td>
<td>–</td>
<td>0/11</td>
</tr>
<tr>
<td>Sub-total</td>
<td>1,019</td>
<td>40</td>
<td>91</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>137 (c)</td>
<td></td>
<td>0/1,019</td>
</tr>
</tbody>
</table>

* samples taken from the market in Liniers (province of origin unknown)

a) observed at the abattoir as suspected of disease of the central nervous system

b) inconclusive histopathological diagnosis
c) histopathological observation and PrPSc detection

BSE: bovine spongiform encephalopathy

**RESULTS**

Pathological and biochemical findings

*Histopathological diagnosis*

Fourteen of the 40 clinical cases detected in the field had been diagnosed preliminarily as suffering from encephalitis due to infection with bovine herpesvirus 1 (BHV1). Six of these cases were confirmed by histopathological diagnosis and viral isolation (Table III). The signs were observed mostly in animals aged 1.0-1.5 years; only one animal was aged five years. Morbidity was in the range of 0.5-5% and mortality was 90-100%. The affected animals were from different breeds, raised under different management methods, and grazed on natural or introduced pastures. The symptoms were hyperexcitability with teeth grinding, hyperaesthesia, fever, opisthotonus, walking in circles and blindness. The typical histopathological observation was non-suppurative encephalitis, with perivascular cuffs, infiltration of mononuclear cells, diffuse or focal gliosis, active microglia, satellitosis and neuronophagy. In two cases (nos 612 and 846), necrosis was observed at the cortical level, involving Gitter cells and inclusion bodies.

Two of the eight remaining cases (nos 843 and 844) presented suppurative meningitis, with bacteriological isolation of *Pseudomonas aeruginosa* and *Streptococcus* spp. Case no. 610 had thromboembolic meningoencephalitis with extensive vasculitis, fibrinous thrombi in blood vessels, bacterial colonies and perivascular infiltration of polymorphonuclear cells also affecting adjacent zones of the cerebral parenchyma with areas showing parenchymatous haemorrhages. Two samples (nos 845 and 109) showed
no significant lesions. Sample no. 109 presented focal vasogenic oedema in the white matter of the cerebellum.

Animal no. 839 did not show microscopic lesions, and the anamnesis and biochemical analysis revealed hypocalcaemia/hypomagnesaemia. No conclusive histopathological diagnosis could be made in cases nos 837 and 613; however, BHV1 was isolated from the brain of the latter animal.

Three animals (nos 847, 848 and 873) from Tapalqué and General Viamonte (Buenos Aires province) had severe non-suppurative encephalitis, with foci of infiltrating polymorphonuclears. In all three cases, *Chlamydia* sp. was detected by direct immunofluorescence.

Case no. 840 was a three-year-old Aberdeen Angus which had suppurative encephalitis with infiltration of polymorphonuclear leukocytes, and microabscesses with perivascular and meningeal infiltration of mononuclear cells. The lesions were compatible with cerebral listeriosis, but no bacterial isolation was achieved.

Fifteen cases of hypocalcaemia or hypocalcaemia/hypomagnesaemia were observed; in these cases, the diagnosis was based on the anamnesis and biochemical analysis of blood. No significant CNS lesions were observed.

Cases nos 26, 645, 841, 842, 874, 876 and 877 did not show histopathological lesions. Case no. 26 had probably been intoxicated with *Cynodon dactilon*.

During this period, the programme for rabies surveillance detected 17 suspected cases of rabies. Fifteen cases were confirmed. The two remaining cases (nos 876 and 877) were not confirmed. Histopathological examination for BSE and biochemical analysis for PrPSc also gave negative results.

The histopathological findings are summarised in Table III.

For six of 1,019 samples, histopathological analysis was inconclusive, due to minimal changes in non-significant nuclei. Biochemical examination for PrPSc in these six samples gave negative results.

None of the samples obtained from suspect animals or random selection in abattoirs presented histopathological lesions compatible with BSE.

### Table III

<table>
<thead>
<tr>
<th>Observations</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-suppurative encephalitis</td>
<td>7</td>
</tr>
<tr>
<td>Suppurative encephalitis</td>
<td>1</td>
</tr>
<tr>
<td>Non-suppurative necrotising encephalitis</td>
<td>2</td>
</tr>
<tr>
<td>Suppurative meningitis</td>
<td>2</td>
</tr>
<tr>
<td>Thromboembolic meningoencephalitis</td>
<td>1</td>
</tr>
<tr>
<td>Focal vasogenic oedema</td>
<td>1</td>
</tr>
<tr>
<td>No significant lesions</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
</tbody>
</table>

Histopathological findings in the forty clinical field cases

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For six of 1,019 samples, histopathological analysis was inconclusive, due to minimal changes in non-significant nuclei. Biochemical examination for PrPSc in these six samples gave negative results.

None of the samples obtained from suspect animals or random selection in abattoirs presented histopathological lesions compatible with BSE.
**Biochemical diagnosis**

The 137 samples analysed for PrP\text{Sc} detection – clinical field samples (40 cases), samples from animals observed in abattoirs as being suspected of CNS disease (91 cases) and those samples which were inconclusive by histopathological diagnosis (6 cases) – were also negative for PrP\text{Sc}.

**Statistical analysis**

As the diagnostic methodology for BSE involves performing histopathological and biochemical controls of post-mortem brain samples, the decision was taken to use material obtained at slaughter (in addition to the forty clinical cases). The statistical criteria used were based on the following:

a) Random selection was performed as the animals were observed in the abattoir, taking into account the place of origin of the animals.

b) Each animal was representative of a geographical region, to ensure that the whole country was covered. The farm of origin was also considered. Thus, in the nine major cattle-raising provinces, the sampling covered 120 districts, 197 localities, 901 farms and 1,019 animals (Fig. 2; Table IV).

c) In view of the pathogenesis of BSE, the animals selected were over five years of age, with the exception of those cases which were specially significant as pathological references. The latter numbered 137 of 1,019, and originated from nine provinces.

d) Statistically, the sample of 1,019 animals provided an effective level of detection of disease prevalence greater than 2.95 per 1,000, with 95% confidence (23). As all results were negative, it is concluded – with 95% confidence and for a prevalence level greater than 2.95 per 1,000 – that BSE does not exist in Argentina.

This conclusion is drawn from the relationship presented below (derived from the binomial distribution for probabilistic models), according to which at least one case should have been detected if the detection level had been exceeded:

\[
\frac{\log \text{error level}}{\log \text{control level}} = \frac{0.05}{0.99705} = 1.30103 \approx 1,014 \text{ animals.}
\]

An error level of 0.05 (5%) represents 0.95 (95%) confidence, and a control level of 0.99705 represents a maximum level of non-detectable prevalence of 0.00295 (i.e. 2.95 per 1,000).

The size of the sample (1,019) was greater than the size required to fulfil the established levels of detection (95% confidence and 2.95 per 1,000 as the maximum prevalence level).

e) Taking into consideration the representative sample of animals investigated with respect to the 901 farms of origin, the statistical analysis shows that the negative results correspond to a level of detection greater than 3.3 per 1,000 farms (0.0033) with 95% (0.95) confidence:

\[
\frac{\log \text{error level}}{\log \text{control level}} = \frac{0.05}{0.9967} = 1.30103 \approx 906 \text{ farms.}
\]

An error level of 0.05 (5%) corresponds to 0.95 (95%) confidence, and a control level of 0.9967 corresponds to a non-detectable prevalence level smaller than 0.0033 (3.3 per 1,000).
**Table IV**

*Distribution of samples by province, city and farm*

<table>
<thead>
<tr>
<th>Province/State</th>
<th>No. of districts</th>
<th>No. of cities</th>
<th>No. of farms</th>
<th>No. of animals</th>
<th>Animals/farm</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Analyzed cases</th>
<th>Field cases</th>
<th>Suspect (a)</th>
<th>Inconclusive (b)</th>
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<td>Buenos Aires</td>
<td>67</td>
<td>73</td>
<td>342</td>
<td>396</td>
<td>1.15/1</td>
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<td>Ho</td>
<td>13</td>
<td>19</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td>Catamarca</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
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<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Córdoba</td>
<td>12</td>
<td>38</td>
<td>195</td>
<td>203</td>
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<td>1</td>
<td>10</td>
<td>192</td>
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<td>5</td>
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<td>6</td>
<td>–</td>
</tr>
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<td>74</td>
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<td>96</td>
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</tr>
<tr>
<td>La Pampa</td>
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<td>6</td>
<td>7</td>
<td>7</td>
<td>1/1</td>
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<td>1</td>
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<td>1</td>
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<td>1</td>
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<td>9</td>
<td>11</td>
<td>1.22/1</td>
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<td>6</td>
<td>1</td>
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<td>285</td>
<td>1.07/1</td>
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<td>8</td>
<td>272</td>
<td>0</td>
<td>2</td>
<td>283</td>
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<tr>
<td>Liniers *</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>Sub-total</td>
<td>20</td>
<td>50</td>
<td>941</td>
<td>8</td>
<td>1,019</td>
<td>9</td>
<td>1,010</td>
<td>23</td>
<td>996</td>
<td>40</td>
<td>91</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>195</td>
<td>901</td>
<td>1,019</td>
<td>1.11/1</td>
<td>1,019</td>
<td>1,019</td>
<td>1,019</td>
<td>137</td>
<td>–</td>
<td>–</td>
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</tr>
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</table>

*Animals from several provinces are present at the market in Liniers, and no data were available regarding province or farm of origin. It was assumed that each animal belonged to a different farm*

- a) observed at the abattoir as suspected of disease of the central nervous system
- b) inconclusive histopathological diagnosis

C: cross-breed
AA: Aberdeen Angus
Ho: Holstein
He: Hereford
f) As negative results were found at both levels (2.95 per 1,000 for animals and 3.3 per 1,000 for farms), there is a reasonable statistical basis for considering that BSE is highly unlikely to exist in Argentina.

g) In addition, with regard to the selection requirements relating to farm of origin, breed, sex and age of the animals, the following observations can be made (Table IV):

- the ratio between animals and farms is 1.11 animals per farm (i.e. in 10% of cases there were duplicate controls)
- of the 1,019 animals, 941 were Holstein (92.3%), 50 were Aberdeen Angus (49%), 20 were cross (1.5%) and 8 were Hereford (0.8%)
- 1,010 (99%) of 1,019 cattle tested were cows
- 996 (97.6%) of the 1,019 cattle were over five years of age, and only 23 (2.25%) were under five years of age.

The ratio of the number of cases of BSE per year in the United Kingdom (around 30,000 in 1992) to the total bovine population was 1 affected animal per 400. This ratio is coincident with the ratio between the number of sampled animals (1,019) in this study and the number of animals in the higher-risk group (dairy cows over five years of age) which are slaughtered annually in Argentina (approximately 400,000), i.e. 1 to 400.

The absence of the disease among the potentially more susceptible population is demonstrated experimentally here, and these data therefore confirm the hypothesis that BSE does not exist in Argentina.

**DISCUSSION**

In October 1987, Wells *et al.* reported a new disease of cattle in southern Great Britain. In view of the similarity of this disease to scrapie in sheep and other spongiform encephalopathies, the disease was called bovine spongiform encephalopathy (BSE) (30). The first diagnoses were made in November 1986, but retrospective studies showed that the first clinical case had occurred as early as April 1985. Subsequent epidemiological research performed in the United Kingdom demonstrated that the epidemic was a consequence of three major factors (14):

- a relatively large ovine population with endemic scrapie;
- a change in the method used for rendering ovine and bovine waste, allowing the survival of a scrapie-like agent in meat-and-bone meal;
- the use of ruminant meat-and-bone meal as an additive for bovine feedstuffs.

These factors determined the magnitude of the epidemic in the United Kingdom. Although affected animals have been detected among the native cattle in other countries, none of these countries has experienced the high incidence recorded in the United Kingdom, probably due to the absence of one or more of the above factors.

An analysis of risk factors for BSE in Argentina (1, 22) identified the importation of infected bovines as the sole probable source of entry of BSE into the country. The same study showed that it was very unlikely that scrapie or BSE had been introduced during the previous ten years, and that it is virtually impossible for cattle to be infected through concentrated feedstuffs.
Briefly, the analysis demonstrated the following:

a) The ratio of cattle to sheep in Argentina is 32 to 1, and the two species are farmed in separate geographical areas.

b) No scrapie cases have been detected in the country.

c) For economic reasons, rendering is only a small part of the disposal system for animal waste.

d) Concentrated feeds are never used for sheep, and only very rarely for beef cattle. Small quantities of concentrates containing only plant protein are fed to dairy cattle, and this occurs only seasonally if there is insufficient pasture. Of the meat-and-bone meal produced, 43% is exported, and the remainder is used as a dietary supplement for chicken, poultry and pigs.

In addition, the rules laid down by SENASA allow live animals to be imported only from countries considered by the OIE to be scrapie- and BSE-free. Animals imported from countries which reported BSE cases after the importation are under continuous surveillance. The importation of meat-and-bone meal from countries in which BSE has been reported is also banned.

However, the possible existence of some form of BSE cannot be excluded. For this reason, a special surveillance programme was designed, with the aim of searching for cases among the bovine population of higher potential risk (dairy cattle over five years of age). This programme involves analysing every suspected case of neurological disease and establishing the presence or absence of BSE using current diagnostic methods. The programme was designed according to a statistical system based on the bovine population of higher risk, in a random distribution enabling detection of a prevalence smaller than 3 per 1,000, with 95% confidence, in the higher-risk population. This level is considered acceptable in relation to the epidemic in the United Kingdom.

In Argentina, several aetiologies are recognised for neurological disease in cattle, as follows:
- tetanus
- hypocalcaemia
- hypomagnesaemia and sporadic cases of rabies (in the north of the country)
- tremorgenic symptoms caused by fungus and plant intoxication (27)
- neurological disease attributed to listeriosis (3), *Chlamydia* spp. (11) or *Haemophilus somnus* (3)
- polioencephalomalacia attributed to metabolic alterations (3, 6, 11)
- infections with neurotropic BHV1 (frequently observed [20], although subsequently the diagnostic investigation of neurological disease suspected to be caused by BHV1 has been intensified [3]).

CNS disease may also be due to other viral infections, i.e. pseudorabies, bovine virus diarrhoea or malignant catarrhal fever (11, 13).

Following the analysis of 36 cases of clinically-suspected neurological disease in bovines from the major cattle-breeding area in Argentina, which had previously been subjected to virological, bacteriological, biochemical and toxicological diagnosis, the
most frequently identified agents were found to be BHV-1, bovine virus diarrhoea virus, *Haemophilus somnus* and *Listeria monocytogenes* (3). No histopathological lesions suggestive of BSE were observed, and definitive diagnoses were obtained in 52% of the cases (3). These results agree with the results presented above, in which oedema, tetanus, hypocalcaemia, hypomagnesaemia and sporadic bovine encephalitis were detected, in addition to some of the aforementioned agents. None of the 91 suspect cases detected in the abattoir had histopathological damage compatible with BSE, nor was the characteristic BSE protein detected by biochemical analysis.

These observations are in full agreement with those reported by Jeffrey (10) in Scotland, who observed no lesions in 97 of 225 BSE-negative bovine brains and detected histopathological features attributable to infectious and/or degenerative disease matching those described above. Similar observations were made by Miller *et al.* (16) in the USA.

The *Secretaría de Agricultura, Ganadería y Pesca* in Argentina has adopted the necessary measures for exclusion of the risk of introduction of BSE into Argentina. A risk assessment has been conducted to establish the critical points to be considered, and a surveillance programme has been undertaken for the early detection of BSE, should an outbreak of the disease occur. A surveillance programme for clinical detection of BSE in bovines suspected of neurological disease and analysis of brains from suspected animals detected in abattoirs commenced in November 1992. To date, the results have been negative. These measures currently ensure freedom from BSE in Argentina and should enable this status to be maintained.

The early detection of BSE is important, should the disease occur in Argentina, as this would allow the immediate implementation of control and eradication measures. Effective surveillance depends on the technical abilities of veterinary practitioners and laboratory diagnosticians within the animal health system.

The potential economic impact of BSE on the beef or bovine by-products industry in Argentina would be very significant, and although the disease has not been detected in the country to date, the clinical signs are familiar to clinical veterinarians, diagnosticians and pathologists, who are prepared for recognition of this disease.

In the United Kingdom, the incidence of BSE in adult cattle in 1993 was 10 per 1,000 animals. If similar conditions existed in Argentina, the authors would have detected clinical and/or histopathological cases, because 1,019 brains were sampled randomly and account was taken of the size of the target population and the desired level of certainty (95% confidence, and a level of certainty of 2.95 per 1,000 for animals and 3.3 per 1,000 for farms). The negative results obtained support the conclusions of the risk assessment (1, 22), that BSE is not present in Argentina. However, only continuation of the control measures and surveillance programme will make it possible to sustain this status.

**CONCLUSIONS**

Research conducted on animals suffering from nervous disorders and dairy cattle over five years of age has provided no evidence of BSE occurrence in Argentina.
In view of the 100% negative results obtained in the histopathological and biochemical observations performed, it may be concluded that BSE does not occur in Argentina as an emerging or a yet undetected pathology.

The absence of the disease is confirmed by the magnitude and characteristics of the statistical design applied, which consisted of random coverage of 1,019 animals from 901 farms belonging to the areas and populations of highest risk. This sample size gives a sensitivity of detection of 2.95 per 1,000, and 95% statistical confidence.

From the analysis of risk factors and the results of the surveillance programme currently being implemented, it is concluded that Argentina is free from BSE at present, and that the single potential source of the disease is the importation of infected animals or by-products.

The continuity of the surveillance programme will secure early detection of BSE, and it is hoped that this will ensure permanent maintenance of the present animal health situation in the country with regard to this disease.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the professional staff at SENASA and INTA, and to others who actively collaborated in the detection of animals with BSE-compatible symptoms in the field and in abattoirs, namely the following: D. Bedotti (Estación Experimental Agropecuaria [EEA]-Anguil), F. Uzal (EEA-Bariloche), R. Soni (EEA-Mercedes, Corrientes), R. Sager (EEA-Mercedes, San Luis), J.C. Bardón and G. Combesies (Laboratorio Azul), O. Tosticarelli (Servicios de Luchas Sanitarias [SELSA]-Rosario), J. Carbajales (SELSA-Santa Fe), J. Loureyro (SELSA-Esperanza), C. Mattos (SELSA-Reconquista), F. Pellegrino, P. Torres and P. Clos (SELSA-Capital), A. Robinson and J.C. Bonzo (SELSA) and the personnel of the Servicios de Inspección de Productos Animales at the abattoirs visited for collection of materials.

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* * *


France. Trois cas ont été signalés en Allemagne, deux dans le Sultanat d'Oman et des cas isolés dans les îles Malouines, au Danemark, au Portugal et au Canada, qui portaient sur des animaux importés du Royaume-Uni. Plusieurs pays ont mis en œuvre des programmes de surveillance et d’analyse des risques d’épidémie. Une analyse des facteurs de risque, effectuée en Argentine montre que la présence de l’encéphalopathie spongiforme bovine ou de la tremblante du mouton est très improbable et qu’il y a fort peu de risques que l’une et l’autre se manifestent à l’avenir à l’occasion de la consommation d’aliments.

Dans le cadre de l’analyse des facteurs de risque, un programme de surveillance a ensuite été mené sur le terrain et dans les abattoirs. Un personnel spécialisé a été formé à la recherche de la maladie par des analyses cliniques, histopathologiques et biochimiques grâce à un réseau de laboratoires couvrant 85 % du cheptel bovin total et 100 % du groupe à haut risque. Conformément à un protocole statistique portant sur la population bovine de neuf provinces, 1 019 encéphales d’animaux appartenant au groupe à haut risque (vaches laitières de plus de cinq ans) ont été sélectionnés et soumis à un examen histopathologique et biochimique en vue du diagnostic de l’encéphalopathie spongiforme bovine. Dans tous les cas, les résultats ont été négatifs.

Cette analyse (avec une sensibilité de 2,95 % et 95 % de fiabilité statistique) permet de conclure que l’Argentine peut être considérée comme un pays indemne de l’encéphalopathie spongiforme bovine et que, à l’avenir, l’importation d’animaux ou de produits d’origine animale infectés serait la seule source potentielle d’introduction de la maladie dans le pays.


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* *

REFERENCES


