Decision Support Tools for Clinical Diagnosis of Disease in Cows with Suspected Bovine Spongiform Encephalopathy

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Bovine spongiform encephalopathy (BSE) was first recognized and defined as a pathological entity in the United Kingdom in November 1986 (41). Initial epidemiological investigations and examination of archived brains indicated that the first clinical cases occurred around April 1985. The disease affects adult animals, with a peak age-at-onset of 4 to 5 years (16, 18, 42). The age range of clinical, confirmed cases is very wide (between 20 months and almost 20 years) (14), although BSE is rarely confirmed in animals younger than 30 months. The specific origin is not clear, but the marker of the disease is the prion protein PrPres (28). In the late 1970s, a reduction in the use of hydrocarbon solvents in the production of meat-and-bone meal coincided with the accepted start of exposure of the cattle population in Great Britain (2, 3, 44, 45, 46); the first BSE confirmed cases were born at the time of this change (15).

Most BSE cases are suspected to result from the recycling of infected cattle tissues, via meat-and-bone meal, within the cattle population (43, 46). The duration of clinical signs is on average 1 to 2 months, but it can be less than 2 weeks (23, 24, 31, 47) and as long as a year (2, 23). Currently, BSE can only be confirmed postmortem by pathological examination of brain tissue. The histological changes are typical: microscopic lesions in the central nervous system consisting of bilaterally symmetrical, noninflammatory vacuolization of neuronal perikarya and gray matter neuropil (42). The new variant of Creutzfeldt-Jakob disease was identified in the United Kingdom in 1996 (48). Subsequently, several investigations have indicated a possible link with BSE (7, 19).

In Europe, clinical studies were performed, either in confirmed cases of BSE (2, 13, 20, 23, 24, 42, 44) or in BSE suspected and subsequently confirmed or unconfirmed cases (4, 10, 39). BSE is characterized clinically by apprehension, hyperesthesia, gait ataxia, and loss of body condition (11, 20, 34, 44, 47, 49). The differential diagnosis of BSE is complex (29). Diagnostic procedures include thorough clinical and neurological examinations with emphasis on the assessment of behavior, locomotion, and sensitivity (5). Pattern-matching models for the differential diagnosis of BSE were also developed recently (9, 10).

The present clinical study applies a generalized approach that is independent of BSE prevalence. The present study is the first veterinary application of classification and regression trees (CARTs) (6). The method is applied to a data set consisting of the suspected clinical BSE cases in Belgium. The aim of the present study was to identify important indicators for the classification of clinically suspected cases as a “BSE highly suspected case,” allowing the development of clinical decision support tools. The conclusions are compared to previously published results.

MATERIALS AND METHODS

Target population. The statistics of the Belgian cattle population are given in Table 1, and its age pyramid by sex is shown in Fig. 1.

Laboratory analyses. Confirmatory diagnosis of all suspected BSE cases consists of (i) a histological examination of the central nervous system, demonstrating the spongiform aspect of the brain; (ii) extraction and examination of the fibers associated with scrapie (scrapie-associated fibers [SAF]); and (iii) an immunohistochemical examination (34, 38, 40, 41).
RESULTS AND DISCUSSION

Distribution in space and time. No statistically significant differences in spatial ($\chi^2 = 0.98; df = 1; P = 0.322$) and temporal ($\chi^2 = 3.57; df = 2; P = 0.168$) distributions were found between the sample retained in the present study and the total number of suspected clinical BSE cases during the period from 1998 to 2000 (Fig. 2). Animals over 48 months were encountered significantly more in the suspected cases ($\chi^2 = 17.02; df = 1; P < 0.001$). For bovines older than 24 months, the annual cumulated incidence of clinical BSE confirmed cases for the period studied were, respectively, $3.94/10^6$ animals in 1998 (95% confidence interval [CI] = 1.5/10^6 to 8.6/10^6 animals), $1.9/10^6$ animals in 1999 (95% CI = 0.4/10^6 to 5.6/10^6 animals), and $5.5/10^6$ animals in 2000 (95% CI = 2.5/10^6 to 10.5/10^6 animals). The ratios between the clinical BSE confirmed and suspected cases were low: 3.7% in 1998, 1.6% in 1999, and 3.2% in 2000. Fewer suspected cases were reported between June and October, when the animals are kept outdoors (average per month = 14.4), compared to the period from November to May (average per month = 31.1, Poisson regression likelihood ratio $\chi^2 = 36.02; df = 1; P < 0.001$), but no such departure from a uniform distribution could be detected for confirmed BSE cases (1.6 cases per month during period from June to October versus 1.4 cases per month during period from November to May, Poisson regression likelihood ratio $\chi^2 = 0.06; df = 1; P = 0.812$). This observation suggests that the BSE rapid tests carried out in accordance with European decision 2000/764/EC (1) play an important role in the global BSE epidemiomonitoring network.

Duration of illness. The mean duration of illness (defined as the time between the onset of clinical signs and the veterinary officer’s visit) is an important parameter taking account of a BSE suspicion (23, 39, 42) and could be determined for 23 confirmed BSE cases: the median length was 30 days (average = 44 days, minimum = 7 days, maximum = 143 days). This observed median duration is considerably shorter than the 40 to 80 days reported for other European countries (13, 39, 44). No precise information was available for the seven confirmed cases: all four abattoir cases were included, one case was diagnosed a few days after the animal was purchased, one case was diagnosed when the animals were turned out onto their pastures, and for the last case a period of a few months was reported without further information. This underlines the difficulty in obtaining reliable information when dealing with concealed disorders or when the animals are outdoors (33). An increase in precision of reported duration was, however, noted in the present study after training sessions of the government officers involved, indicating that a similar awareness campaign of farmers and private veterinary practitioners would lead to more precise data.

Major predictor variables. The age distribution of suspected BSE cases for the period 1998 to 2000 is shown in Fig. 3. BSE confirmed cases were significantly more observed in animals in the age bracket of 54 to 112 months ($\chi^2 = 9.86; df = 1; P = 0.002$). The distribution of year of birth of the suspected BSE cases for the same period is shown in Fig. 4. BSE confirmed cases were more frequently observed in animals born between 1990 and 1995 ($\chi^2 = 13.17; df = 1; P < 0.001$).
Other signs

Most common signs of meningitis and/or encephalitis

**Listeria monocytogenes (LIS)**

Alcoholism and/or encephalopathy (ME signs)

Other signs

**Neurohistopathological findings.** Listeriosis and meningitis and/or encephalitis were the most frequent neurohistopathological findings in the period 1998 to 2000 (Table 3). Four groups of cattle were considered in the following sections of the present study: group A (BSE), group B (listeriosis), group C (meningitis and/or encephalitis), and group D (no specific neurohistopathological lesions). The absence of significant neurohistopathological lesions in 56% of clinically suspected but afterward unconfirmed BSE cases is comparable to those previously published (10, 25, 27, 39). However, the neurohistopathological findings observed in Belgium are not identical to those reported in the differential diagnosis of BSE in United Kingdom (21, 22, 39). This observation suggests that the prevalence of different diseases taken into account in the differential diagnosis of BSE would differ between the two countries.

Clinical findings. In function of results of neurohistopathological findings (groups A to D), the BSE suspected cases, subsequently confirmed or unconfirmed, were compared for each clinical sign separately and for all clinical signs combined.

(i) **Comparison of each clinical sign.** The comparison from each clinical sign was performed on the basis of the neurohistopathological-finding groups (Table 2). When BSE confirmed cases were compared to all BSE suspected but unconfirmed cases, the adjusted odds ratios (adjORs) were significantly higher than 1 for 10 clinical signs (BSE signs, in decreasing order): kicking in the milking parlor, hypersensitivity to touch and/or sound, head shyness, panic-stricken response, reluctance to enter in the milking parlor, abnormal ear movement or carriage, increased alertness behavior, reduced milk yield, teeth grinding, and change in temperament. Maximum efficacy in a receiver-operating-characteristic curve was obtained for a score of between 2 and 4 BSE signs (Fig. 5). The same clinical signs were recorded more frequently in BSE cases compared to group D, except for kicking in the milking parlor (no significant adjOR). When BSE cases were compared to group B, six signs were statistically frequent in BSE cases (these were, in decreasing order: circling, head pressing or rubbing, and abnormal head carriage). In addition, listeriosis occurs more frequently in winter and spring (39), and the mean duration of illness was found to be shorter, varying from 4 to 14 days (35, 39). Compared to group C, six signs were reported more frequently in BSE cases (these were, in decreasing order: head shyness, reduced milk yield, hypersensitivity to touch and/or sound, panic-stricken response, abnormal ear movement or

### Table 2. Adjusted odds ratio, calculated for 25 clinical signs in 30 cows with BSE and 272 bovines with suspected but unconfirmed BSE

| Sign category and specific sign | All other cases (n = 272) | Listeriosis (n = 79) | Meningitis and/or encephalitis (n = 34) | Cases without specific lesions (n = 153) |
|--------------------------------|-------------------------|---------------------|----------------------------------------|-----------------------------------------|
| **Most common signs of BSE confirmed cases (BSE signs)** | | | | |
| Kicking in the milking parlor | 6.75 (1.27–35.82)* | 4.59 (0.58–36.35) | 6.05 (0.28–131.26) | 5.32 (0.88–32.10) |
| Hypersensitivity to touch or/and sound | 5.82 (2.67–12.75)* | 4.08 (1.70–9.76) | 5.29 (1.83–15.29) | 6.87 (3.00–15.74)* |
| Head shyness | 5.82 (2.67–12.60)* | 4.92 (2.03–11.93) | 6.49 (2.13–19.77) | 5.99 (2.64–13.60)* |
| Panic-stricken response | 5.63 (2.50–11.76)* | 4.59 (1.90–11.08) | 3.45 (1.25–9.56) | 6.52 (2.86–14.87)* |
| Reluctance to enter in the milking parlor | 5.39 (1.78–16.34) | 3.62 (0.96–13.60) | 14.88 (0.79–281.52) | 4.89 (1.46–16.43)* |
| Abnormal ear movement or carriage | 3.80 (1.77–8.17) | 1.76 (0.76–4.09) | 3.97 (1.41–11.18) | 5.99 (2.64–13.60)* |
| Increased alertness behavior | 3.75 (1.76–8.02) | 2.43 (1.04–5.68) | 2.33 (0.86–6.31) | 5.23 (2.31–11.85)* |
| Reduced milk yield | 3.65 (1.55–8.57) | 3.28 (1.18–9.10) | 5.74 (1.29–25.04) | 3.24 (1.31–8.00)* |
| Teeth grinding | 2.54 (1.15–5.58) | 3.18 (1.24–8.20) | 1.84 (0.64–5.32) | 2.49 (1.08–5.72)* |
| Temperament change | 2.49 (1.17–5.28) | 1.62 (0.70–3.75) | 2.33 (0.87–6.29) | 3.19 (1.45–7.03)* |
| **Most common signs of listeriosis (LIS signs)** | | | | |
| Abnormal head carriage | 0.68 (0.32–1.44) | 0.35 (0.15–0.83) | 0.38 (0.14–1.02) | 1.07 (0.49–2.36) |
| Circling | 0.65 (0.23–1.84) | 0.18 (0.06–0.55) | 0.62 (0.17–2.25) | 2.58 (0.78–8.54) |
| Head pressing or rubbing | 0.59 (0.19–1.87) | 0.24 (0.07–0.81) | 0.47 (0.12–1.84) | 1.44 (0.41–5.05) |
| **Most common signs of meningitis and/or encephalitis (ME signs)** | | | | |
| Recumbence | 0.57 (0.27–1.21) | 0.73 (0.31–1.69) | 0.33 (0.12–0.90) | 0.57 (0.26–1.25) |
| Blindness | 0.10 (0.01–1.67) | 0.09 (0.01–1.54) | 0.05 (0.00–0.93) | 0.13 (0.01–2.19) |
| **Other signs** | | | | |
| Loss of condition | 2.02 (0.95–4.26) | 1.91 (0.82–4.43) | 2.33 (0.85–6.40) | 1.99 (0.91–4.34) |
| Increased kicking of the muzzle and/or flank | 2.01 (0.73–5.48) | 1.81 (0.57–5.81) | 14.88 (0.79–281.52) | 1.80 (0.63–5.15) |
| Hindleg and/or foreleg ataxia | 1.83 (0.80–4.17) | 1.63 (0.66–4.05) | 1.87 (0.66–5.28) | 1.91 (0.81–4.47) |
| Weakness of legs | 1.61 (0.77–3.40) | 1.25 (0.55–2.88) | 1.59 (0.60–4.24) | 1.77 (0.82–3.86) |
| Abnormal behavior | 1.59 (0.75–3.59) | 1.12 (0.48–2.61) | 3.02 (1.11–8.27) | 1.71 (0.78–3.74) |
| Tremors | 1.52 (0.72–3.22) | 1.47 (0.64–3.39) | 1.83 (0.69–4.87) | 1.50 (0.69–3.25) |
| Maniacal excitement | 1.40 (0.48–4.09) | 0.71 (0.23–2.22) | 1.15 (0.28–4.70) | 2.32 (0.71–7.55) |
| Loss of wt | 1.25 (0.52–3.00) | 2.11 (0.74–6.02) | 0.99 (0.32–3.07) | 1.11 (0.45–2.73) |
| Paresis | 1.12 (0.47–2.68) | 1.57 (0.57–4.31) | 0.74 (0.25–2.33) | 1.03 (0.42–2.53) |
| Falling | 0.95 (0.44–2.05) | 0.64 (0.27–1.49) | 0.83 (0.31–2.25) | 1.21 (0.54–2.70) |

* Values statistically significant at \( P = 0.05 \) are indicated by an asterisk.
FIG. 2. Clinical BSE in Belgium. A diagram shows spatial distribution of the investigated cases, i.e., suspected cases with full clinical evaluation record. (A) BSE suspected cases and subsequently unconfirmed cases from 1998 to 2000 ($n = 272$); (B) all BSE confirmed cases before October 2002 ($n = 30$).
carriage, and abnormal behavior), and two signs were encountered more frequently in group C (ME signs, in decreasing order: blindness and recumbence). In all comparisons, the most common signs of BSE were behavioral signs (especially head shyness and panic-stricken response), hyperesthesia, and reduced milk yield. Ataxia did not appear as a specific sign. It must be stressed again that these results were obtained on the basis of odds ratios, i.e., a statistic that is independent of sample size and disease prevalence. The observation that the behavioral changes and hyperesthesia predominate can be related to the preferred localization of the neuronal vacuolization in cattle (23, 34, 36). This relationship between clinical sign and pathology considerably increases the appropriateness to use the signs in a decision-support tool. Nevertheless, a complete clinical examination and a detailed case anamnesis remain essential to arrest BSE (24). No single clinical sign is pathognomonic for BSE, and recourse to complementary confirmatory examinations is needed (29, 32).

(ii) Comparison of all clinical signs (patterns). The clinical patterns observed in individual groups (A to D) were compared by using a Spearman rank correlation. All six pairwise comparisons (A versus B, A versus C, A versus D, B versus C, B versus D, and C versus D) yielded significant correlations, ranging in magnitude from 0.51 to 0.88. Using entire sets of patterns did not permit statistical discrimination, and neurohistopathological findings therefore have no predictive power for making clinical decisions. The consistent differences observed in comparing overall clinical pictures indicate that suspected BSE cases form a distinct clinical entity.

**Construction of a clinical decision tree.** The following predictor variables were introduced in CART: age (in months), year of birth, each of the 25 clinical signs described in Table 2, and a statistic that is independent of sample size and disease prevalence. The observation that the behavioral changes and hyperesthesia predominate can be related to the preferred localization of the neuronal vacuolization in cattle (23, 34, 36). This relationship between clinical sign and pathology considerably increases the appropriateness to use the signs in a decision-support tool. Nevertheless, a complete clinical examination and a detailed case anamnesis remain essential to arrest BSE (24). No single clinical sign is pathognomonic for BSE, and recourse to complementary confirmatory examinations is needed (29, 32).
identified all confirmed cases (sensitivity = 1, but this simple model had a specificity of only 0.61). Inclusion of all predictor variables produced a model that still had sensitivity equal to 1 (30 BSE confirmed cases in a final node), but the specificity was increased to 0.85 (42 of 272 nonconfirmed cases in a final node). The BSE confirmed cases were found in three final nodes: (i) animals between 54 and 57 months, born after 1993; (ii) animals between 54 and 112 months of age with at least four BSE signs; and (iii) animals between 68 and 112 months, born after 1993 with fewer than two typical listeriosis signs (Table 2, LIS signs).

The originality of the current approach lies in the fact that, first, it offers an explorative and interactive tool and, second, the results and conclusions arrived at are independent of BSE prevalence, through its use of odds ratios. The second feature is especially appealing for rare events. A similar decision tree, allowing the distinction of “highly suspected BSE cases” from all other suspected BSE cases, could be applied in other countries, with or without the use of rapid tests. The continued addition of clinical data would permit further improvement of the current model tree, even if the clinical BSE pattern would be modified in time. The same methodology can also be applied to other afflictions and diseases, e.g., scrapie.

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FIG. 6. CART decision tree for clinically suspected BSE cases in Belgium.
