JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2004, p. 172–178 Vol. 42, No. 1
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.1.172–178.2004

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

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Received 6 June 2003/Returned for modification 13 September 2003/Accepted 5 October 2003

Reporting of clinically suspected cattle is currently the most common method for detecting cases of bovine spongiform encephalopathy (BSE). Improvement of clinical diagnosis and decision-making remains crucial. A comparison of clinical patterns, consisting of 25 signs, was made between all 30 BSE cases, confirmed in Belgium before October 2002, and 272 suspected cases that were subsequently determined to be histologically, immunohistochemically, and scrapie-associated-fiber negative. Seasonality in reporting suspected cases was observed, with more cases being reported during wintertime when animals were kept indoors. The median duration of illness was 30 days. The 10 most relevant signs of BSE were 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 temperament change. Ataxia did not appear to be a specific sign of BSE. A classification and regression tree was constructed by using the following four features: age of the animal, year of birth, number of relevant BSE signs noted, and number of clinical signs, typical for listeriosis, noted. The model had a sensitivity of 100% and a specificity of 85%. This approach allows the use of an interactive decision-support tool, based entirely on odds ratios, a statistic independent of disease prevalence.

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 PrP^{res} (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 clinical 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 fibres [SAF]); and (iii) an immunohistochemical examination (34, 38, 40, 41).

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TABLE 1. Belgian cattle population in the period from 1998 to 2000[a]

Yr	No. of herds	No. of live animals aged:	
		<24 mo	≥24 mo
1998	48,126	1,402,685	1,522,944
1999	51,776	1,341,152	1,571,563
2000	50,666	1,490,775	1,626,981

[a] Source: SANITEL (Belgian databank for registration and movement of cattle).

Inclusion criteria. All clinical BSE cases, reported before October 2002 (n = 30), and all other suspected clinical cases identified during the period from 1998 to 2000 were included in the present study (30, 31). The period from 1998 to 2000 covers the time between the first confirmed case in Belgium in October 1997 (38) and the introduction of the rapid diagnostic tests in January 2001 (26). Cases were only included if they concerned animals over 20 months of age and if a complete clinical evaluation record form was available (n = 272).

Clinical evaluation record. Suspected clinical BSE cases are discovered on the farm by the owner and/or private veterinary practitioner and in the abattoir by a state veterinary officer during antemortem examinations. Owners and veterinarians are regularly updated through all possible channels. A specifically assigned state veterinary officer reexamines all suspected cases. The officer completes a clinical evaluation record form indicating presence or absence of 25 clinical signs (Table 2). The clinical signs include behavioral change, sensory problems, locomotion problems, posture anomalies, and general problems.

Scoring of clinical signs. A value of 1 was attributed for each observed clinical sign, and the score of an individual animal is defined as the sum (i.e., the number of clinical signs observed for that animal). Depending on the cases, scores can be total scores, or scores for groups of signs, but this will be made clear in the text.

Database. The following data were entered for every case fulfilling the above inclusion criteria: (i) year, month, method of detection (farm or abattoir), and geographical localization; (ii) month and year of birth and age of the animal; (iii) results of laboratory analyses; and (iv) presence or absence of the signs listed in Table 2 and scores of the most frequent clinical signs for each of the neurohistopathological diagnosis categories.

Statistical analysis. Statistical analyses were carried out with STATA/SE 7 (Stata Statistical Software, release 7.0; Stata Corp., College Station, Tex.) and CART 4.0 (6). The former was used to prescreen the signs to be entered into CART, using adjusted odds ratios, as defined by Grenier (17). CART was developed by Breiman et al. (6) and Clark and Pregibon (8) and was evaluated previously in medical science studies (12, 37). This methodology is particularly useful for classification and regression problems where one has a set of classification or predictor variables and a single response variable. The predictor variables can be a mixture of factors and numeric variables. The initial construction of a tree involves three elements: (i) selection of binary splits of the measurement space, (ii) the decision of whether to declare a node as terminal or to continue splitting, and (iii) the assignment of each terminal node to a class (12). In the present study the Gini index was used as the splitting method and a 10-fold cross-validation was used as the method for testing the obtained trees. The ultimate aim was to produce a decision tree with sensitivity equal to 1 (all confirmed cases are included in the final nodes) and with a specificity as high as possible (the number of unconfirmed cases in the final nodes is minimized).

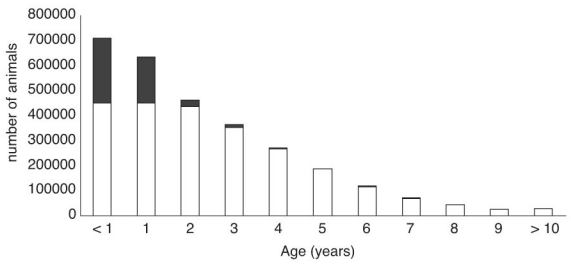

FIG. 1. Age pyramid of Belgian cattle in 2000. Bars: □, females; ■, males.

RESULTS AND DISCUSSION

Distribution in space and time. No statistically significant differences in spatial (χ^2 = 0.98; df = 1; P = 0.322) and temporal (χ^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 (χ^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 χ^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 χ^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 epidemiosurveillance 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 other 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 (χ^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 (χ^2 = 13.17; df = 1; $P < 0.001$).

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	$_{adj}$OR (95% CI)[a]			
	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	5.83 (2.67–12.75)*	4.08 (1.70–9.76)*	5.29 (1.83–15.29)*	6.87 (3.00–15.74)*
Head shyness	5.80 (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	5.43 (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 (1.29–25.64)*	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 licking 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 (0.75–3.39)	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 (0.25–2.23)	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)

[a] Values statistically significant at $P = 0.05$ are indicated by an asterisk.

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 ($_{adj}$ORs) 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 $_{adj}$OR). When BSE cases were compared to group B, six signs were statistically frequent in BSE cases (these were, in decreasing order: head shyness, panic-stricken response, hypersensitivity to touch and/or sound, reduced milk yield, teeth grinding, and increased alert behavior), whereas three others were significantly more often recorded in group B (LIS signs, 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

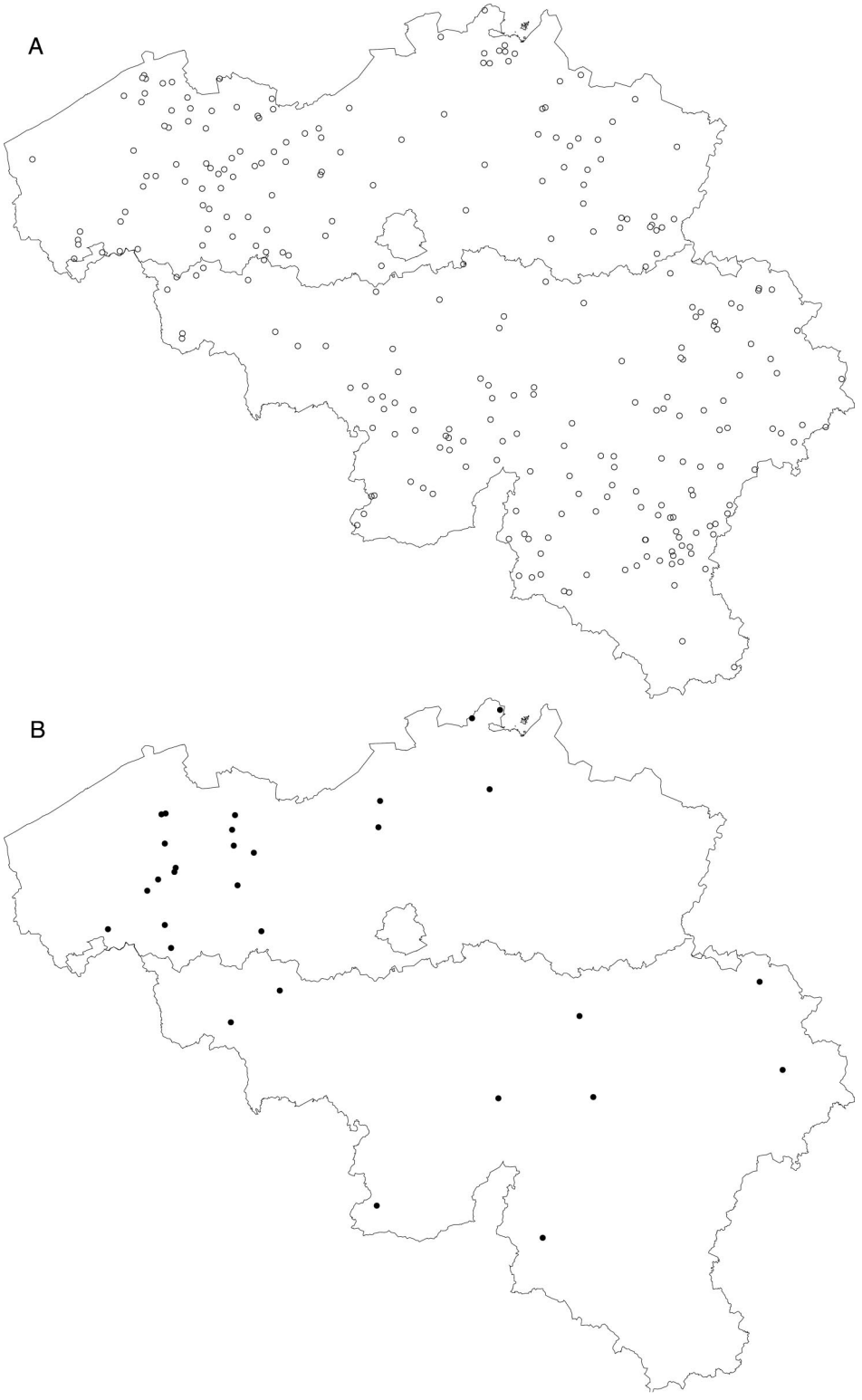

FIG. 2. Clinical BSE in Belgium. A diagram shows spatial distribution of the investigated cases, i.e., suspected cases with full clinical evaluation record. (A) BSE suspected cases and subsequently unconfirmed cases from 1998 to 2000 ($n = 272$); (B) all BSE confirmed cases before October 2002 ($n = 30$).

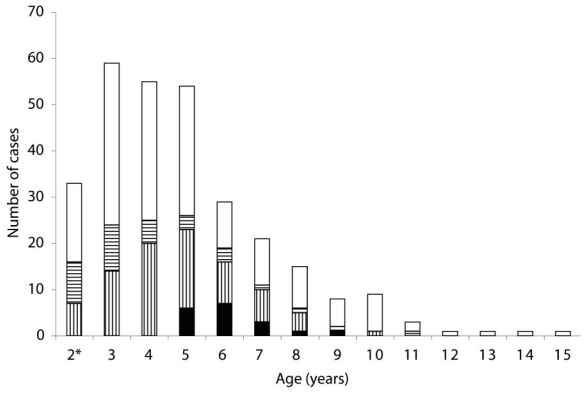

FIG. 3. Age distribution of clinically suspected BSE cases in Belgium between 1998 and 2000 (n = 290), Bars: ■, BSE; ▤, listeriosis; ▥, meningitis and/or encephalitis; □, others; ❋, bovines between 20 and 24 months.

TABLE 3. Frequency of the neurohistopathological findings in 290 BSE suspected cases older than 20 months with a full clinical evaluation record (1998 to 2000)[a]

Neurohistopathology	Frequency
Listeriosis	79
Suppurative encephalitis and/or meningitis[b]	21
BSE	18[a]
Nonsuppurative encephalitis and/or meningitis[b]	13
Suppurative thromboembolic encephalitis	2
Tumors	2
Putrefaction	2
No specific neurohistopathological lesions	153
Total	290[a]

[a] Twelve confirmed BSE cases between January 2001 and September 2002 were added when these these results were compared with data in Table 2 and Fig. 6.

[b] Combined with the meningitis and/or encephalitis group in Table 2.

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 of BSE, but locomotor signs alone were noted in one BSE case and together with general disorders in another case (a pattern also reported by McElroy and Weaver) (24), but nevertheless ataxia as such was not 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, combination of hypersensitivity to touch and/or sound and/or increased alertness, the score of 10 clinical signs that were encountered significantly more frequently in BSE confirmed cases (Table 2, BSE signs), the score of the three clinical signs that were significantly more frequent in listeriosis (Table 2, LIS signs), and the score of the two clinical signs that were significantly more frequent in meningitis and/or encephalitis (Table 2, ME signs). The clinical decision tree obtained in CART is shown in Fig. 6. Using only the 54-to-112-month age bracket

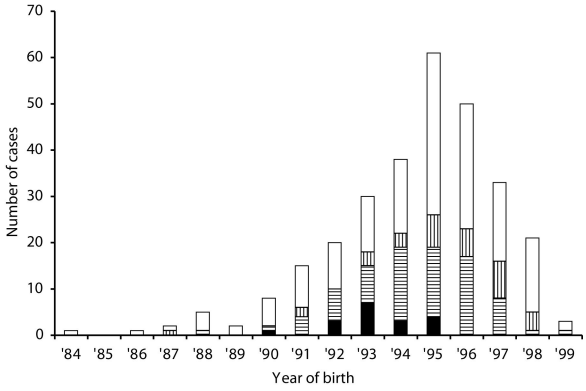

FIG. 4. Clinically suspected BSE cases in Belgium between 1998 and 2000 by birth cohort (n = 290). Bars: ■, BSE; ▤, listeriosis; ▥, meningitis and/or encephalitis; □, others.

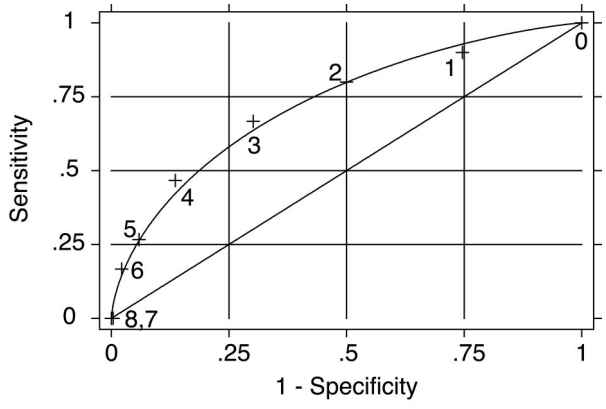

FIG. 5. Receiver-operating-characteristic curve of the score of the 10 most relevant signs of BSE (area under the curve = 0.7290 s.e. [area] = 0.0503; 0, 1, . . ., 7, 8 = BSE sign score).

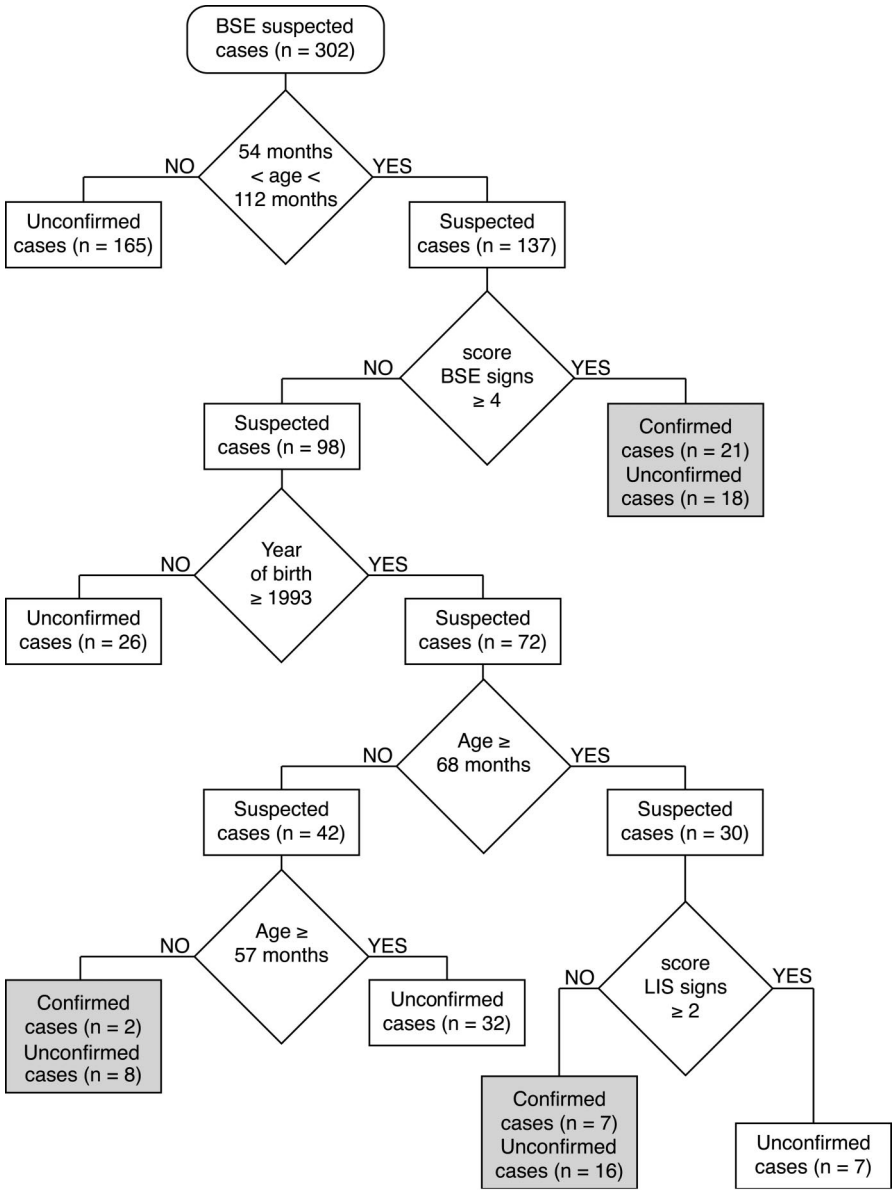

FIG. 6. CART decision tree for clinically suspected BSE cases in Belgium.

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.

ACKNOWLEDGMENTS

We thank all members of the Federal Agency for the Safety of the Food Chain (FASFC), the Veterinary Agrochemical Research Centre, the Central Animal Health Organization (CAHO), and the many farmers and veterinarians who cooperated in this study. We particularly thank P. Dechamps (FASFC) for access to all clinical evaluation records, H. Deblander (Institut Scientifique Louis Pasteur, Brussels, Belgium) for the mapping, and I. V. Monfort (CAHO) for providing the cattle population data.

REFERENCES

1. **Anonymous.** 2000. European Commission Decision 2000/764/EC of 29 November 2000 on the testing of bovine animals for the presence of bovine spongiform encephalopathy and amending decision 98/272/EC on epidemiosurveillance for transmissible spongiform encephalopathies. Eur. Commun. Off. J. **L305:**35–38.
2. **Bradley, R.** 1994. Les encéphalopathies spongiformes animales en Grande Bretagne. Bull. Soc. Vet. Prat. France **78:**339–385.
3. **Bradley, R., and J. W. Wilesmith.** 1991. Epidémiologie des encéphalopathies spongiformes en Grande-Bretagne. Epidemiol. Sante Anim. **19:**27–48.
4. **Braun, U., E. Schicker, and B. Hörnlimann.** 1998. Diagnostic reliability of clinical signs in cows with suspected bovine spongiform encephalopathy. Vet. Rec. **143:**101–105.
5. **Braun, U., U. Kihm, N. Pusterla, and M. Schönmann.** 1997. Procédure d'examen clinique en cas de suspicion d'encéphalopathie spongiforme bovine. Schweiz. Arch. Tierheilk. **139:**35–41.
6. **Breiman, L., J. H. Friedman, R. A. Olshen, and C. J. Stone.** 1984. Classification and regression trees. Wadsworth, Pacific Grove, Calif.
7. **Bruce, M. E., R. G. Will, J. W. Ironside, I. McConnell, D. Drummond, A. Suttie, L. McCardle, A. Chree, J. Hope, C. Birkett, S. Cousens, H. Fraser, and C. J. Bostock.** 1997. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. Nature **389:**498–501.
8. **Clark, L. A., and D. A. Pregibon.** 1992. Tress-based models, p. 377–419. *In* Chambers J. A. and T. J. Hastie (ed.), Statistical models. Wadsworth, Pacific Grove, Calif.
9. **Cockcroft, P. D.** 2000. Clinical sign profile likelihood ratios for bovine spongiform encephalopathy suspects. Res. Vet. Sci. **68:**285–290.
10. **Cockcroft, P. D.** 1999. Pattern-matching models for the differential diagnosis of bovine spongiform encephalopathy. Vet. Rec. **144:**607–610.
11. **Cranwell, M. P., R. S. Hancock, J. R. Hindson, S. A. Hall, N. J. Daniel, A. R. Hopkins, B. Wonnacott, M. Vivian, and P. Hunt.** 1988. Bovine spongiform encephalopathy. Vet. Rec. **122:**190.
12. **Crichton, N. J., J. P. Hinde, and J. Marchini.** 1997. Models for diagnosing chest pain: is CART helpful? Stat. Med. **16:**717–727.
13. **Denny, G. O., and W. D. Hueston.** 1997. Epidemiology of bovine spongiform encephalopathy in Northern Ireland 1988 to 1995. Vet. Rec. **140:**302–306.
14. **Department for Environment, Food, and Rural Affairs.** 2002. Bovine spongiform encephalopathy in Great Britain: youngest and oldest cases by year of onset (passive surveillance only). [Online.] http://www.defra.gov.uk/animalh/bse/bse-statistics/bse/yng-old.html.
15. **Department for Environment, Food, and Rural Affairs.** 2000. Bovine spongiform encephalopathy in Great Britain: confirmed cases by year of birth. [Online.] http://www.defra.gov.uk/animalh/bse/bse-statistics/bse/ybirth.html.
16. **Ferguson, N. M., C. A. Donnelly, M. E. I. Woolhouse, M. E. I., and R. M. Anderson.** 1997. The epidemiology of BSE in cattle herds in Great Britain. II. Model construction and analysis of transmission dynamics. Philos. Trans. R. Soc. Lond. **352:**803–838.
17. **Grenier, B.** 1990. Utilisation des ODDS dans les tests associés ou séquentiels, p. 102–106. *In* Décision médicale: analyse et stratégie de la décision dans la pratique médicale. Edition Masson. Paris, France.
18. **Heim, D., and U. Kihm.** 1999. Bovine spongiform encephalopathy in Switzerland: the past and the present. Rev. Sci. Tech. Off. Int. Epiz. **18:**135–144.
19. **Hill, A. F., M. Desbruslais, S. Joiner, K. C. L. Sidle, J. Gowland, L. Collinge, L. J. Doey, and P. Lantos.** 1997. The same prion strain causes vCJD and BSE. Nature **389:**448–450.
20. **Hörnlimann, B., and U. Braun.** 1993. Bovine spongiform encephalopathy (BSE): clinical signs in Swiss BSE cases, p. 289–299. *In* R. Bradley and B. Marchant (ed.), Transmissible spongiform encephalopathy. Proceedings of a Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Communities, Brussels, Belgium.
21. **Jeffrey, M., M. M. Simmons, and G. A. H. Wells.** 1993. Observations on the diffential diagnosis of bovine spongiform encephalopathy in Great Britain, p. 347–362. *In* R. Bradley and B. Marchant (ed.), Transmissible spongiform encephalopathy. Proceedings of a Consultation on BSE with the Scientific Committee of the European Communities, Brussels, Belgium.
22. **Jeffrey, M., and J. W. Wilesmith.** 1992. Idiopathic brainstem neuronal chromatolysis and hippocampal sclerosis: a novel encephalopathy in clinically suspect cases of bovine spongiform encephalopathy. Vet. Rec. **131:**359–362.
23. **Kimberlin, R. H.** 1992. Bovine spongiform encephalopathy. Rev. Sci. Tech. Off. Int. Epiz. **11:**347–390.
24. **McElroy, M. C., and E. D. Weavers.** 2001. Clinical presentation of bovine spongiform encephalopathy in the Republic of Ireland. Vet. Rec. **149:**747–748.
25. **McGill, I. S., and G. A. H. Wells.** 1993. Neuropathological findings in cattle with clinically suspect but histologically unconfirmed bovine spongiform encephalopathy. J. Comp. Pathol. **108:**241–260.
26. **Pastoret, P. P., M. Gouffaux, C. Saegerman, S. Roels, P. Dechamps, E. Thiry, and E. Vanopdenbosch.** 2001. Le diagnostic immunologique rapide des encéphalopathies spongiformes transmissibles. Ann. Med. Vet. **145:**164–173.
27. **Pollin, M. M., I. S. McGill, and G. A. H. Wells.** 1992. The differential neurohistological diagnoses of clinically suspect but unconfirmed BSE. Neuropathol. Appl. Neurobiol. **18:**638.
28. **Prusiner, S. B.** 1998. Prions. Proc. Natl. Acad. Sci. USA **95:**13363–13383.
29. **Saegerman, C., L. Claes, A. Dewaele, D. Desmecht, F. Rollin, J. Hamoir, P. Gustin, G. Czaplicki, J. Bughin, J. Wullepit, J. Laureyns, S. Roels, D. Berkvens, E. Vanopdenbosch, and E. Thiry.** 2003. Differential diagnosis of neurologically expressed disorders in Western European cattle. Rev. Sci. Tech. Off. Int. Epiz. **22:**83–102.
30. **Saegerman, C., P. Dechamps, S. Roels, K. Petroff, R. Geeroms, G. Torck, J. Dufey, R. Fourez, M. Hamelryckx, A. Cormann, P. Viatour, V. De Coninck, F. Lomba, J. P. Vermeersch, L. Hallet, O. Lhost, M. Leemans, A. Vandersanden, D. Peharpre, B. Brochier, F. Costy, P. P. Pastoret, E. Thiry, and E. Vanopdenbosch.** 2001. Epidémiosurveillance de l'encéphalopathie spongiforme bovine en Belgique: bilan de l'année 1999. Ann. Med. Vet. **145:**47–58.
31. **Saegerman, C., P. Dechamps, E. Vanopdenbosch, S. Roels, K. Petroff, J. Dufey, G. Van Caeneghem, D. Devreese, H. Varewyck, H. De Craemere, I. Desmedt, A. Cormann, G. Torck, L. Hallet, M. Hamelrijckx, M. Leemans, A. Vandersanden, D. Peharpre, B. Brochier, F. Costy, P. Muller, E. Thiry, and P. P. Pastoret.** 1999. Epidémiosurveillance de l'encéphalopathie spongiforme bovine en Belgique: bilan de l'année 1998. Ann. Med. Vet. **143:**423–436.
32. **Saegerman, C., L. Claes, E. Vanopdenbosch, P. Bironт, H. Deluyker, and E. Thiry.** 1999. Etude rétrospective de l'incidence des troubles neurologiques rapportés et suspects d'encéphalopathie spongiforme transmissible chez les bovins en Belgique. Epidemiol. Sante Anim. **35:**31–42.
33. **Schelcher, F., O. Andreoletti, P. Cabanie, and G. Tabouret.** 2001. Démarche diagnostique dans les maladies nerveuses des bovins, 229–240. *In* Proceedings of SFB. Société Française de Buiâtrie, Paris, France.
34. **Scott, A. C., G. A. H. Wells, M. J. Stack, H. White, and M. Dawson.** 1990. Bovine spongiform encephalopathy: detection and quantitation of fibrils, fibril protein (PrP), and vacuolation in brain. Vet. Microbiol. **23:**295–304.
35. **Stöber, M.** 1987. Symptomatologie différentielle de quelques affections du système nerveux des bovins. Ann. Med. Vet. **131:**401–410.
36. **Swanson, L. W.** 2000. Cerebral hemisphere regulation of motivated behavior. Brain Res. **886:**113–164.
37. **Thwaites, G. E., T. T. Chau, K. Stepniewska, N. H. Phu, L. V. Chuong, D. X. Sinh, N. J. White, C. M. Parry, and J. J. Farrar.** 2002. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. Lancet **360:**1287–1292.
38. **Vanopdenbosch, E., P. Dechamps, C. Saegerman, J. Dufey, S. Roels, P. Mullier, L. Hallet, B. Brochier, F. Costy, G. Charlier, R. Fourez, and P. P. Pastoret.** 1998. Le premier cas d'encéphalopathie spongiforme bovine diagnostiqué en Belgique. Ann. Med. Vet. **142:**111–118.
39. **Wells, G. A. H., A. R. Sayers, and J. W. Wilesmith.** 1995. Clinical and epidemiological correlates of the neurohistology of cases of histologically unconfirmed, clinically suspect bovine spongiform encephalopathy. Vet. Rec. **136:**211–216.
40. **Wells, G. A. H., R. D. Hancock, W. A. Cooley, M. S. Richards, R. J. Higgins, and G. P. David.** 1989. Bovine spongiform encephalopathy: diagnostic significance of vacuolar changes in selected nuclei of the medulla oblongata. Vet. Rec. **125:**521–524.
41. **Wells, G. A. H., A. C. Scott, C. T. Johnson, R. F. Gunning, R. D. Hancock, M. Jeffrey, M. Dawson, and R. Bradley.** 1987. A novel progressive spongiform encephalopathy in cattle. Vet. Rec. **121:**419–420.
42. **Wilesmith, J. W.** 1998. Manual on bovine spongiform encephalopathy. Food and Agriculture Organization of the United Nations, Rome, Italy.
43. **Wilesmith, J. W., J. B. M. Ryan, and W. D. Hueston.** 1992. Bovine spongiform encephalopathy: case-control studies of calf feeding practices and meat and bone meat inclusion in proprietary concentrates. Res. Vet. Sci. **52:**325–331.
44. **Wilesmith, J. W., L. J. Hoinville, J. B. M. Ryan, and A. R. Sayers.** 1992. Bovine spongiform encephalopathy: aspects of the clinical picture and analyses of possible changes 1986–1990. Vet. Rec. **130:**197–201.
45. **Wilesmith, J. W., J. B. M. Ryan, W. D. Hueston, and L. J. Hoinville.** 1992. Bovine spongiform encephalopathy: epidemiological features 1985 to 1990. Vet. Rec. **130:**90–94.
46. **Wilesmith, J. W., J. B. M. Ryan, and M. J. Atkinson.** 1991. Bovine spongiform encephalopathy: epidemiological studies on the origin. Vet. Rec. **128:** 199–203.
47. **Wilesmith, J. W., G. A. H. Wells, M. P. Cranwell, and J. B. M. Ryan.** 1988. Bovine spongiform encephalopathy: epidemiological studies. Vet. Rec. **123:** 638–644.
48. **Will, R. G., J. W. Ironside, M. Zeidler, S. N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiari, A. Hofman, and P. G. Smith.** 1996. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet **347:**921–925.
49. **Winter, M. H., B. M. Aldridge, P. R. Scott, and M. Clarke.** 1989. Occurrence of 14 cases of bovine spongiform encephalopathy in a closed dairy herd. Br. Vet. J. **145:**191–194.