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Population-Level Retrospective Study of Neurologically Expressed Disorders in Ruminants before the Onset of Bovine Spongiform Encephalopathy (BSE) in Belgium, a BSE Risk III Country

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A retrospective epidemiological study ($n = 7,875$) of neurologically expressed disorders (NED) in ruminants before the onset of the bovine spongiform encephalopathy epidemic (years studied, 1980 to 1997) was carried out in Belgium. The archives of all veterinary laboratories and rabies and transmissible spongiform encephalopathy (TSE) epidemiosurveillance networks were consulted. For all species, a significantly higher number of NED with virological causes (rabies) was reported south of the Sambre-Meuse Valley. During the period 1992 to 1997, for which the data were complete, (i) the predicted annual incidence of NED varied significantly as a function of species and area (higher numbers in areas where rabies was present) but was always above 100 cases per million, and (ii) the mean incidence of suspected TSE cases and, among them, those investigated by histopathological examination varied significantly as a function of species and area. The positive predictive value of a presumptive clinical diagnosis of NED ranged from 0.13 (game) to 0.63 (sheep). Knowledge of the positive predictive value permits the definition of a reference point before certain actions (e.g., awareness and training campaigns) are undertaken. It also shows the usefulness of a systematic necropsy or complementary laboratory tests to establish an etiological diagnosis. TSE analysis of a small, targeted historical sampling ($n = 48$) permitted the confirmation of one case and uncovered another case of scrapie. The results of the present study help to develop and maintain the quality of the worldwide clinical epidemiological networks for TSE, especially in countries that in the past imported live animals, animal products, and feedstuffs from countries with TSE cases.

In affected ruminants, transmissible spongiform encephalopathies (TSE) cause neurological signs that can be classified into three categories: disturbances in behavior, sensitivity, and locomotion. In addition, some general clinical signs are also observed (4, 8, 44, 71, 78, 80). The course of the disease is progressive and always fatal. The lesions are restricted to the central nervous system, although the pathogenesis of infection implies a primary replication step of TSE agents in the lymphoid organs, followed by a neuroinvasive phase (32, 45, 64, 68).

Scrapie occurs in sheep, goats (49), and mouflons (80). Scrapie in sheep has been an endemic disease for more than 250 years (18, 49). Scrapie affects adult animals, with a peak age at onset of 2 to 3 years (8, 15). Not a single clinical case of scrapie has been diagnosed in animals younger than 6 months (52). The first description of the natural disease in goats dates back to 1942 (12). Subsequently, only a few cases of scrapie have been reported for this species (8, 18). In France, a clinically suspected case of spongiform encephalopathy in a cow was described in 1883, but no brain block was conserved (57). This case is still an enigma. Currently, scrapie is considered an infectious disease with maternal and horizontal contagious transmission, where host genetic factors play a central role (1, 19, 62).

Chronic wasting disease (CWD) has emerged as an important wildlife disease in North America since the 1970s (79), and over the past 5 years, the known distribution has expanded to free-ranging cervids and to game-farming industries (77). CWD is horizontally transmitted, and environmental contamination may play an important role in local maintenance of the disease (78).

Bovine spongiform encephalopathy (BSE) was first recognized and defined as a pathological entity in the United Kingdom in November 1986 (70). Initial epidemiological investiga-

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TABLE 1. Estimation of the most frequently reported NEDs[a] by species of ruminants in Belgium, 1980 to 1997

Cause	No. of reported cases			
	Cattle[b]	Game[c]	Sheep[d]	Goats[d]
Total reported cases	5,261	446	1,642	528
Reported cases with determination of cause[e]	3,080	104	1,005	314
Biological causes				
Parasitological				
Cenurosis			2	
Coccidiosis	2		5	4
Estrosis		3		
Bacteriological				
Abscess at the base of the brain	18		5	
Borulism	20			
Enterotoxemia	225	53	332	95
Haemophilus somnus	27			
Keratitis	1		1	
Listeriosis	67	1	66	46
Otitis				2
Purulent meningoencephalitis	55	2	30	12
Sinusitis	3		2	
Tetanus	17		4	3
Virological				
Aujeszky's disease	45		9	
Bovine enzootic leukemia	4		1	1
Bovine viral diarrhea	1			
Caprine arthritis encephalitis virus				4
Malignant catarrhal fever	1			
Maedi-visna			2	1
Rabies	1,088	22	305	21
Unconventional transmissible agent				
Bovine spongiform encephalopathy	1			
Scrapie			9	1
Unspecified biological causes	98	8	50	47
Nonbiological causes				
Mechanical causes				
Cerebral or cerebellar tumor	7		1	
Compression of the medulla	10	2	5	2
Foreign body	5			
Fracture	4	1	3	
Intracranial bleeding			7	
Postpartum paraplegia	3			
Postpartum paresis or paralysis	6		3	1
Physical causes—electrocution (lightning)	7			
Chemical causes				
Belladonna		1		
Copper poisoning			2	
Cyanogenetic plants	4		6	8
Ergot		1		
Glyphosate			1	
Lead	44	1	5	2
Nitrates	77		1	1
Organochlorates	3			1
Organophosphates, carbamates	3			
Poisoning by ripe fruit			1	2
Rhododendron, azalea			2	4
Strychnine		1		
Taxus baccata	90	1	17	11
Metabolic and nutritional causes				
Acute ruminal acidosis	10	2	9	10
Acetonemia	31		5	
Cerebrocortical necrosis	3	1	3	5
Hepatic encephalosis	5		5	
Milk fever	848		3	
Photosensitization			1	
Pregnancy toxemia		1	73	18
Tetany	161		1	
Uremia	36		3	1
Vitamin A deficiency	1			
Genetic causes	2			
Immumological causes	1			
Unspecified nonbiological causes	46	3	25	11

[a] Inclusion criteria used: (i) laboratory diagnosis; (ii) necropsy diagnosis; (iii) clinical diagnosis.
[b] Older than 12 months.
[c] All ages.
[d] Older than 6 months.
[e] According to the work of Saegerman et al. (54).

tions and examination of archived brains indicated that the first clinical cases occurred around April 1985 (44). The disease affects adult animals, with a peak age at onset of 4 to 5 years (2, 30, 35, 71). The age range of clinically confirmed cases is very wide (from 20 months to almost 20 years) (16), although BSE is rarely confirmed in animals younger than 30 months (54). The specific origin is not clear, but the marker of the disease is the prion protein PrP^{Sc} (PrP scrapie) (50). 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, 72, 73, 74). The first cattle with confirmed BSE cases were born at the time of this change (17). Most BSE cases resulted from the recycling within the cattle population of infected cattle tissues via meat and bone meal (71, 74). The duration of clinical signs is 1 to 2 months on average, but it can be less than 2 weeks (44, 48, 56, 75) or as long as 1 year (44). Currently BSE can be confirmed only postmortem, by pathological examination of brain tissue. The histological changes are typical: microscopic lesions in the central nervous system consist of bilaterally symmetrical, noninflammatory vacuolization of neuronal perikarya and grey-matter neuropil (71). The new variant of Creutzfeldt-Jakob disease was first identified in the United Kingdom in 1996 (76). Subsequently, several investigations have indicated a link with BSE (5, 37, 61).

Before the onset of BSE during the second half of the 1980s, there were no specific surveillance programs for TSE in ruminants in most countries. In Belgium, notification of rabies has been compulsory since 1967 and notification of ruminant TSE has been compulsory since 1990. The first scrapie case in Belgium was recognized in 1963, i.e., before compulsory notification. It appeared in a Suffolk ram, which was imported from the United Kingdom (40). The first BSE case was diagnosed in October 1997 (66). No case of CWD has ever been reported in Europe.

In the literature little information has been available on the definition of reported neurologically expressed disorder (NED) and on those NED cases that were suspected of TSE. Almost no information on the incidence of NED in wild ruminants has been found. In the United States, the National Animal Health Monitoring System (NAHMS) has registered an incidence of 4,000 per million sheep above the age of 1 year that were culled or died with "behavioral faults" in 1995 (10). For cattle, some information is available but comparison of the different studies is not feasible. According to Heim et al. (36), neurological pathologies have a preferential distribution in bovines under the age of 1 year (29.3%) and in those aged 4 to 5 years (18.9%). The VIALINE network (Vigilance, Alerte, Intervention, et Évaluation) in Haute Normandie (France) mentions a total mortality rate of 5.1% in 1993, and 13.5% of these animals were above the age of 2 years. Of these, 8% had neurological diseases (23). Combining these figures yields a mortality rate from neurological diseases of 550 per million cattle above the age of 2 years. Between 1992 and 1999, an annual average of 90 clinically suspected BSE cases per million cattle above the age of 2 years was reported to the veterinary authorities in Switzerland (passive surveillance); 43% of these cases were confirmed (20). In the United States, NAHMS recorded a mortality rate of 1,000 dairy cows per million due to "lack of coordination or severe depression" in 1995 and a morbidity rate in beef herds of 1,000 breeding females with "neurological problems" per million in 1996 (9, 11). In beef

TABLE 2. Predicted mean incidence rates of reported NEDs in Belgium during the period 1992 to 1997

Species	Predicted mean incidence rate (per 10^5 animals) north or south of the SMV	
	North	South
Cattle[a]	10	40
Game[b]	70	20
Goats[c]	390	500
Sheep[c]	70	160

[a] Animals older than 12 months.
[b] Animals of all ages.
[c] Animals older than 6 months.

herds, this rate, expressed as affected bovines per thousand, doubles when the herd size is below 100 head and is nil when the herd size is more than 300 head (9). Finally, a limited retrospective study in New Zealand, based on histopathological diagnoses made for 28 cattle suspected of BSE, is available (38).

Information on ruminant neurological cases is very scarce. However, it is extremely important to get epidemiological estimates of these cases, because most of the neurological disorders enter into the differential diagnosis of TSE in ruminants.

The present study had three goals: first, to determine the annual incidence of NED in Belgium, a country classified as level III for geographical BSE risk; second, to determine whether TSE were present in a country before the first report; and third, once TSE had been detected, to monitor the evolution of incidence in space and time.

The present epidemiological retrospective study constitutes the first validation, at a population level, of the requirements of the Office International des Epizooties (OIE), which are based on expert opinion (81).

MATERIALS AND METHODS

Population at risk. The inclusion criteria depended on the species: cattle older than 12 months, small ruminants older than 6 months, and game of all age. Ruminant population size data in Belgium were extracted from the annual registration for the period of 1980 to 1997 (available from the National Institute of Statistics), more specifically called the "15 May census."

Evolution in time. The period studied started on 1 January 1980 and ended on 31 December 1997 (the year of the first indigenous BSE case in Belgium). The starting date was selected on the basis of the trade of live animals, animal products, and meat and bone meal from the United Kingdom, where BSE occurs, to other European countries (41, 58, 59, 67) and to countries outside Europe (33, 34) and on the basis of an average BSE incubation time of 4 to 5 years (71).

Spatial distribution. Belgium has been free of rabies since July 2001 (83). Rabies was enzootic during the period under investigation. Belgium can be divided into three regions according to previous rabies prevalence. One region, the Sambre-Meuse Valley (SMV), forms a natural border that separates the other two: the area north of the SMV, where rabies was absent, and the area south of the SMV, where rabies was present. The data from the SMV itself are of limited importance and are not presented here.

Definitions. For the purpose of this study, a reported NED case is defined as a case for which either nervous clinical signs were reported to the veterinary laboratory, or a diagnosis of neurological disorder was made at autopsy or through another laboratory test, or, in the absence of the above information, analyses were performed on the central nervous system. A reported NED case with suspected TSE is defined as a reported NED case for which TSE could not be excluded (which could not be explained by any other cause) or a reported NED case, whatever the nature, for which either the survival time was 7 days or more, or the animal was euthanized before 7 days. To meet the objectives of the present study, this definition was deliberately made broader than the definitions of suspected cases proposed by the European Parliament and Council (28) and by the OIE (81, 82). The annual incidence rate is the ratio of the number of reported NED cases, or of the number of reported NED cases suspected of TSE, to the population at risk.

Database. The rabies and TSE epidemiosurveillance networks, as well the veterinary diagnostic network, report cases of NED. In the first two networks, game of all ages, all cattle above the age of 20 months, and all small ruminants above the age of 12 months, suspected of rabies but testing negative, have been examined for TSE since 1990. The veterinary diagnostic network consists of the provincial veterinary laboratories, the National Reference Laboratory for Veterinary TSE (CODA/CERVA), and the Veterinary Medicine Faculties at the University of Liège (UL) and the University of Ghent (UG).

A database with detailed information about all these cases was established. It contained the following information: laboratory, reference number, date of entry, locality of origin, race, sex, age, clinical signs, results of laboratory tests and/or autopsy, date of result, and indication of whether biological material was forwarded to other laboratories (in order to avoid double entries). The archive search began with those for 1 January 1980. The archives were complete for the period 1992 to 1997. An exhaustive list of NED cases per species was constructed, with the goal of standardizing the data independently of the source (54).

Pathological examination. (i) Targeted samples. Brain blocks have been produced by the TSE networks from 1990 on and have been available in the Department of Pathology of the Faculty of Veterinary Medicine of UL since 1980 and in the corresponding department of UG since 1990. Thirty animals were analyzed at UL, and 18 animals were analyzed at UG. The samples were taken between 1983 and 1997 and came from 17 bovines (1 below, and 16 above, the age of 24 months), 24 sheep (2 below, and 22 above, the age of 12 months), and 7 goats (1 below, and 6 above, the age of 12 months).

(ii) Histology. For histological examination, all tissues were fixed in 4% phosphate-buffered formalin, routinely processed, embedded in paraffin wax, and sectioned at a thickness of 5 μm. Sections were stained with hematoxylin and eosin stains.

(iii) Immunohistochemistry. All immunohistochemical staining was carried out on 5-μm-thick dewaxed sections. Rehydrated sections were placed in a bath of 98 or 100% formic acid at room temperature (RT) for 30 min. After being rinsed with dematerialized water, sections were sterilized at 125°C for 30 min. Endogenous peroxidase was inactivated by covering the sections with a bath of 0.3% hydrogen peroxide in methanol for 30 min at RT. Incubations with primary antibodies were performed at room temperature for 1 h. The antibody used was R524-7/IDDLOMH-AA7 (ID-Lelystad, Lelystad, The Netherlands), a polyclonal rabbit anti-PrP peptide serum diluted 1/1,500. As a secondary antibody, a biotinylated goat anti-rabbit antibody (EO432; DAKO, Glostrup, Denmark) was applied for 10 min at RT, followed by a 5-min incubation in peroxidase-conjugated streptavidin (PO397; DAKO), both diluted 1/500 at RT. 3,3′-Diaminobenzidine tetrahydrochloride (DAB) (Sigma, St Louis, Mo.) was used as a chromogen in the presence of hydrogen peroxide. Sections were counterstained with hematoxylin (Gill 3; Prosan N.V., Merelbeke, Belgium) for 30 s, mounted with DPX mountant (BDH Laboratory Supplies, Poole, England), and covered with a coverglass. This immunohistochemistry procedure has been performed in Belgium since 1996.

(iv) Transmission electron microscopy. For examination of the fibers associated with TSE (scrapie-associated fibers [SAF]), the fresh brain stem material was dissected and discarded, and 1-g aliquots were homogenized in 10% (wt/vol) *N*-lauroylsarcosine and processed by the Hilmert and Diringer technique (39). After proteinase K digestion, the final pellet was resuspended in 50 μl of distilled water and negatively stained for electron microscopy as described by Scott et al. (60). Examinations were carried out with a Philips EM 208S transmission electron microscope at a magnification of ×22,000.

Statistical analysis. Statistical analyses were carried in STATA/SE 8 (63). Unless otherwise indicated, negative binomial regression was used for analysis of the annual incidence rates of reported NED cases, suspected rabies cases, and suspected TSE cases, by considering three independent variables for the first two rates (species, originating area, and year) and four for the suspected TSE cases (species, originating area, year, and number of suspected rabies cases). Predicted values were used for the annual incidence rates of reported NED cases and suspected TSE cases in order to better highlight the results of the statistical analysis in the respective table. This was not done for the incidence rate of suspected or histologically examined TSE cases, in order to allow comparison of the results with the OIE requirements.

The cattle population south of the SMV was taken as the reference population. The following modification of the formula of Cannon and Roe was used to estimate the number of brains to be examined in order to detect BSE, if present

in at least 1% of NED cases, with a 99% probability (7, 22, 46), as recommended by the OIE (81):

$$n = \left[1 - (1 - a)^{\frac{1}{D}}\right] \cdot \left[N - \frac{(D - 1)}{2}\right]$$

where n is the sample size needed to detect one or more BSE-affected animals in the sample, a is the confidence level of observing at least one affected animal in the sample (in our case, the confidence level is 99%), D is the number of TSE-affected animals in a population with the selected minimum annual incidence, and N is the parent population size.

RESULTS

Etiological classification of the reported NED cases. The numbers of reported NED cases in Belgium between 1980 and 1997 were 5,261 for cattle, 1,642 for sheep, 528 for goats, and 446 for game. Table 1 shows the distribution by type of etiology according to Saegerman et al. (54). Laboratory diagnosis was not always performed because of the cost of investigation. Among the reported cases with determination of the cause, 80% or more (depending on species) were included on the basis of a laboratory diagnosis, 7 to 19% (depending on species) were based on necropsy diagnosis, and 5% or less (depending on species) were based on clinical diagnosis. In all species, the biological-cause group was the most common during the period studied. Among cattle, a virological cause was most frequently encountered, followed by a bacteriological cause. This order was reversed for the other species, especially for goats and game. A parasitological cause was rarely put in evidence. Among nonbiological causes, metabolic and nutritional causes were more frequently found for cattle, sheep, and goats, and a chemical cause was most commonly recorded for game. An unconventional transmissible agent was diagnosed in sheep (nine cases of scrapie), goats (one case of scrapie), and cattle (one case of BSE). Except for cattle ($\chi^2 = 5.51$; df = 2; $P = 0.064$), the proportions of cause groups in the areas north and south of the SMV were significantly different ($\chi^2 = 78$; df = 2; $P < 0.001$). Significantly different proportions of etiological causes were found north and south of the SMV for cattle ($\chi^2 = 1{,}111$; df = 7; $P < 0.001$), sheep ($\chi^2 = 739$; df = 7; $P < 0.001$), goats ($\chi^2 = 155$; df = 6; $P < 0.001$), and game ($\chi^2 = 193$; df = 3; $P < 0.001$). A significantly higher number of cases with virological causes (rabies) was reported south of the SMV ($P < 0.003$ by Fisher's exact test). No etiological cause was found in 41.5% (cattle), 39% (sheep), 40.5% (goats), and 77% (game) of the cases.

Predicted annual incidence rate. (i) Predicted annual incidence rate of reported NED. Table 2 shows the predicted incidence rate for reported NED (PIR-NED) for the period 1992 to 1997. No difference could be demonstrated between different years, and the final model included only the variables "species" and "area of origin." PIR-NED in cattle was significantly higher south of the SMV, and PIR-NED was significantly higher in sheep and goats than in cattle. Game had a higher PIR-NED than cattle in the area north of the SMV. South of the divide, the situation was reversed.

(ii) Predicted annual incidence rate of suspected TSE cases. The predicted annual incidence rates of suspected TSE cases (PIR-TSE) are given in Table 3. PIR-TSE was lower north of the SMV, irrespective of the species. This difference was constant over time. PIR-TSE was lowest in cattle and game, higher in sheep, and significantly higher in goats. There was a negative trend over time in all species, except for cattle, where there was a significant increase. PIR-TSE was furthermore positively correlated with the predicted annual incidence rate of suspected rabies cases (PIR-rabies), again in all species. PIR-rabies for the period 1992 to 1997 is shown in Table 4. The following trends were observed: there was no significant effect of time, except for cattle, for which there was a significant increase in the number of suspected rabies cases over time both north and south of the SMV; PIR-rabies was always higher south of the SMV, irrespective of species; and PIR-rabies was higher for sheep and goats than for cattle and game, which had similar rates.

(iii) Incidence rate of suspected and histologically examined TSE cases. Table 5 presents the observed annual incidence rate of histologically examined TSE cases (IR-TSEHE). The statistical analysis revealed that significantly more records were obtained for goats, both north and south of the SMV. The incidence decreased significantly over time in all species, and there was a correlation between IR-TSEHE and PIR-NED in both regions, although the relationship was stronger in the north.

Positive predictive value of presumptive clinical diagnosis. The level of agreement between the presumptive clinical diagnosis (actually made or written in the anamnesis) and the necropsy findings was calculated for the Faculties of Veterinary Medicine of UL and UG for 224 cattle older than 12 months, 112 sheep and 49 goats older than 6 months, and 8 game animals of all ages. The obtained positive predictive values of presumptive clinical diagnosis versus necropsy were as follows: 0.50 for cattle (95% confidence interval [95% CI], 0.43 to 0.56), 0.63 for sheep (95% CI, 0.53 to 0.71), 0.49 for goats (95% CI, 0.34 to 0.64), and 0.13 for game (95% CI, 0.003 to 0.53).

Pathological examination of targeted samples. On the basis of the present study, a supplementary targeting of the available brain blocks from suspected TSE cases (e.g., presence of suspected clinical signs and/or presence of vacuoles in reports) was carried out.

Among the samples examined by immunohistochemistry and histopathology, three were positive for TSE. A first brain block from a sheep that was presented at UL in March 1983 was confirmed as a case of scrapie. For this case, the immunohistochemistry confirmed the original histopathological diagnosis (neuron with pathognomonic cytoplasmic vacuolization). A second brain block from the TSE network was classified as negative for scrapie in November 1992, because the result of histopathology was not conclusive and the SAF result was negative. For this sample also, the result of immunohistochemistry was positive (multiple sites with brownish positive staining). This sample originated from a 5-year-old Hampshire ewe imported from the United Kingdom. The third brain block from UG, from a 3-year-old goat, was histologically diagnosed as scrapie in August 1992(neuron with pathognomonic multiple cytoplasmic vacuolization), but this diagnosis was not confirmed by immunohistochemistry in our study. All other samples tested remained negative.

TABLE 3. Predicted mean incidence rates of reported NED cases with suspected TSE in Belgium during the period between 1992 and 1997[a]

Region[b] and species	PIR-TSE (per 10^5 animals)					
	1992	1993	1994	1995	1996	1997
North						
Cattle[c]	2.7	2.7	2.8	3.0	3.2	3.7
Game[d]	NA[e]	5.1	4.6	4.2	4.0	4.0
Goats[f]	117.4	92.3	73.0	58.5	49.0	43.4
Sheep[f]	20.9	19.1	17.6	16.4	15.9	16.4
South						
Cattle[c]	11.5	10.2	11.9	18.9	13.3	12.6
Game[d]	NA	19.1	19.4	26.6	16.3	13.4
Goats[f]	507.5	343.0	308.2	373.4	201.9	146.6
Sheep[f]	90.4	71.0	74.1	104.4	65.6	55.3

[a] The definition of suspected TSE cases was deliberately broadened (see the text).
[b] North or south of the SMV.
[c] Animals older than 12 months.
[d] Animals of all ages.
[e] NA, not available.
[f] Animals older than 6 months.

DISCUSSION

The present study had three goals: (i) to determine the annual incidence of NED; (ii) to determine whether TSE were present in a country before the first report; and (iii) once TSE had been detected, to monitor the evolution of incidence in space and time. Our study allowed us (i) to identify the strengths and weaknesses of the organization of the network, (ii) to choose the appropriate awareness and training campaigns for the participants in the epidemiological network, and (iii) to devise appropriate measures, implement them, and monitor their effectiveness. The geographical TSE risk assessment (25, 26, 27) and TSE risk mapping methodologies would enable identification of the countries and areas where TSE emerge and of the animal groups at risk (21, 24, 33, 65). Targeted passive and active surveillance might be performed in these geographical areas and for these animal groups at risk.

Etiological classification of the reported NEDs. Even though the laboratory archives were incomplete before 1992, all laboratories processed a sufficient number of samples to make them representative of the bovine population in their vicinity. Moreover, the same types of causes were found in every region before and after 1992. It is therefore assumed that the percentages of the morbidity causes calculated for every area during the period 1992 to 1997 were indeed representative for the population at risk. The fairly high percentage of cases for which no etiological cause could be established (around 40% of reported NED cases for domestic ruminants to 77% for game) is in line with previous observations elsewhere: a similar percentage (39%) was found in a Swiss study of cattle with nervous signs and suspected of rabies (29). This result could be due partly to a metabolic or toxicological pathology producing no lesion (47). Improvements in examination techniques and acquisition of more experience could probably reduce this percentage by half (36).

During the period of this study, the role of the SMV as a dividing line for the distribution of causes of NED in Belgium was pivotal, essentially due to the rabies prevalence in the south. Differences in causes between countries had already been noted previously (42, 43, 53, 69). Knowledge about the distribution of NED causes and their associated risk factors should be improved, because the development of decision support tools is based on such knowledge (see, e.g., references 13, 14, and 53).

Predicted annual incidence rates of NED and suspected TSE cases. The annual incidence rates of NED and suspected TSE cases are higher south of the SMV than north of the SMV, and the PIR-TSE is correlated with the annual incidence rate of suspected rabies. Thus, the main hypothesis to explain this observation is that south of the SMV, where rabies is prevalent, farmers and veterinarians have historically been more aware (56). Moreover, PIR-NED is underestimated because many NED cases are not reported if the cause and/or therapy is known. In addition, the IR-TSEHE is directly correlated with the PIR-NED. Any measure resulting in an increase in NED reporting has a nonspecific knock-on effect on

TABLE 4. Predicted mean incidence rates of suspected rabies cases in Belgium during the period 1992 to 1997

Region[a] and species	PIR-rabies (per 10^5 animals)					
	1992	1993	1994	1995	1996	1997
North						
Cattle[b]	0.1	0.2	0.2	0.3	0.5	0.7
Game[c]	NA[d]	0.3	0.3	0.3	0.3	0.3
Goats[e]	5.7	4.9	4.2	3.6	3.1	2.7
Sheep[e]	2.5	2.1	1.7	1.4	1.2	1.0
South						
Cattle[b]	6.5	9.1	12.7	17.8	25.0	35.1
Game[c]	NA	16.9	16.3	15.6	15.1	14.5
Goats[e]	290.8	249.8	214.6	184.4	158.5	136.1
Sheep[e]	129.7	107.2	88.6	73.2	60.5	50.0

[a] North and south of the SMV.
[b] Animals older than 12 months.
[c] Animals of all ages.
[d] NA, not available.
[e] Animals older than 6 months.

TABLE 5. Mean incidence rates of reported NED cases with suspected TSE and with complete histological examination in Belgium during the period between 1992 and 1997

Region[a] and species	IR-TSEHE (per 10^5 animals)					
	1992	1993	1994	1995	1996	1997
North						
Cattle[b]	2.3	2.5	2.6	2.8	3.0	3.4
Game[c]	NA[d]	6.0	5.6	5.3	4.8	4.4
Goats[e]	127.1	106.3	87.2	68.8	57.3	46.0
Sheep[e]	22.7	22.3	20.8	19.5	18.6	17.5
South						
Cattle[b]	9.9	12.3	17.0	16.3	13.7	13.4
Game[c]	NA	21.6	19.9	18.5	17.0	15.5
Goats[e]	451.7	360.5	292.2	241.2	194.7	159.3
Sheep[e]	79.7	72.5	69.5	71.4	63.1	57.8

[a] North and south of the SMV.
[b] Animals older than 12 months.
[c] Animals of all ages.
[d] NA, not available.
[e] Animals older than 6 months.

reporting of suspected and histologically examined TSE cases. Progressive increases in numbers reported through the specific TSE network were noted toward the end of the study period, particularly for cattle (data not shown). Overall, PIR-NED is higher, and there are more suspected TSE cases, in small ruminants. Several hypotheses can be put forward to explain this observation. In Belgium, a considerable number of livestock holders have a few small ruminants, and research into the cause of morbidity is more frequent with hobby farmers, because they consider their animals to be pets. The "true" number of registered small ruminants has increased in Europe following the episode of food-and-mouth disease in Great Britain (31). Even when these facts are taken into account, the rate in small ruminants remains higher. In the United States, the NAHMS network has registered a NED incidence rate around fourfold higher for sheep than for cattle.

According to the OIE requirement, based on expert opinion, the annual incidence rate of NED for cattle in all countries is 100 cases per million animals, irrespective of their BSE status (81, 82). The present retrospective study provides the first external validation of this requirement at the population level. Because no clinical sign is pathognomonic for BSE (54, 82), laboratory examination of brains is essential for an efficient BSE epidemiological network. OIE (81) calculates the minimum number of samples that must be examined in order to have a probability of 99% to detect at least one case, if the disease is present in 1% of the cattle with NED. According to this OIE requirement and the number of suspected BSE cases for which a complete histopathological examination has been carried out, the power of the epidemiosurveillance effort for the period 1992 to 1997 was on average 59, 28, and 33%, respectively, for the area south of the SMV, the area north of the SMV, and Belgium as a whole. If all suspected BSE cases were analyzed (by using a definition deliberately broader than the definition of suspected cases proposed by the OIE or the European Commission [28, 82]), the power would become 99, 28, and 52%, respectively. This suggests the necessity of organizing awareness and training campaigns to improve presumptive BSE clinical sign detection for all participants in the epidemiosurveillance network, especially in countries that in the past imported live animals, animal products, and feedstuffs from countries where TSE occurs. This need particularly is addressed by the methodology for geographical BSE risk (GBR) assessment, developed by the Scientific Steering Committee of the European Commission to classify countries according to their BSE risks (25, 26, 27). Thus, in Belgium, with the onset of the BSE epidemic and one information campaign, the power of the BSE epidemiosurveillance network increased to 99% (for Belgium and the area south of the SMV) and to around 70% (north of the SMV) in 1998 and 1999 (55, 56).

The mean numbers of histologically investigated cases of NED during the period 1992 to 1997 (51 and 30 per million cattle south and north of the SMV, respectively) compare well with those for other countries at the same GBR level, level III: 10 per million in France (6) and 90 per million in Switzerland (20) during the same period.

Positive predictive value of presumptive clinical diagnosis. Determination of the positive predictive value of presumptive clinical diagnosis permits us to obtain a reference point before undertaking actions (e.g., awareness and training campaigns). In fact, it offers the possibility of following up and evaluating these actions continuously. It also shows the added benefits of systematically turning to necropsy examinations or complementary tests to establish an etiological diagnosis and above all to dispel uncertainty over the identification of suspected TSE cases (54, 82). The main purpose of a clinical TSE epidemiosurveillance network is to attain as high a sensitivity as possible (in order to identify every BSE case). This goal has to be promoted by permanent awareness and training campaigns for veterinarians, farmers, and other actors so that a higher number of nervous-disorder cases are reported and analyzed (28, 81). The low predictive value for game (0.13) can probably be explained by the low level of clinical observation and anamnesis possible for wildlife.

Pathological examination of targeted samples. The present study confirms that neuronal vacuolization can occasionally be observed in cattle in the absence of BSE (2). The additional importance of immunohistochemical staining in the control of TSE in ruminants is also proved. In fact, immunohistochemical staining revealed one extra TSE case and confirmed one suspected TSE case. The negative result upon histopathological examination is possibly an indication of a (sub)clinical case without clear signs. However, due to the lack of tonsillar tissue, confirmation could not be established (51). The absence of SAF in electron microscopy can be explained by the difficulty this technique encounters at such a large magnification. The fact that no BSE case was detected before October 1997 may be due to the low sample size.

The present retrospective study constitutes the first validation, at a population level, of the OIE requirements. The predicted mean incidence rate of NED in a GBR level III country is sufficiently high to detect confirmed BSE cases only when a minimal number of samples has been examined. The results of the present study help to develop and maintain the quality of the clinical epidemiological TSE networks worldwide, especially in countries that in the past imported live animals, animal products, and feedstuffs from countries where TSE occurs.

Finally, our study also revealed the two main limiting factors for this type of study: (i) standardization of the definitions and list of NEDs and (ii) archiving of data and brain blocks over time. The use of an identification and registration system and a laboratory information management system would undoubtedly make it easier to analyze data.

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REFERENCES

1. **Belt, P. B. G. M., I. H. Muileman, B. E. C. Schreuder, J. Bos-De Ruijter, A. L. J. Gielkins, and M. A. Smits.** 1995. Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. J. Gen. Virol. **76:**509–517.
2. **Bradley, R.** 1991. Bovine spongiform encephalopathy (BSE): the current situation and research. Eur. J. Epidemiol. **7:**532–544.
3. **Bradley, R., and J. W. Wilesmith.** 1993. Epidemiology and control of bovine spongiform encephalopathy (BSE). Br. Med. Bull. **49:**932–959.
4. **Braun, U.** 2002. Clinical signs and diagnosis of BSE. Schweiz. Arch. Tierheilk. **144:**645–652.
5. **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.
6. **Calavas, D., and C. Ducrot.** 2003. L'ESB en France. Synthèse sur l'évolution de l'épizootie à partir des données disponibles au 1er janvier 2003. Agence Française de Sécurité Sanitaire des Aliments, Paris, France.
7. **Cannon, R. M., and R. T. Roe.** 1982. Livestock disease surveys: a field manual for veterinarians. Australian Bureau of Animal Health, Canberra, Australia.
8. **Capucchio, M. T., F. Guarda, N. Pozzato, S. Coppolino, S. Caracappa, and V. Di Marco.** 2001. Clinical signs and diagnosis of scrapie in Italy: a comparative study in sheep and goats. J. Vet. Med. **48:**23–31.
9. **Centers for Epidemiology and Animal Health.** 1997. Beef '97. National Animal Health Monitoring System, U.S. Department of Agriculture, Fort Collins, Colo.
10. **Centers for Epidemiology and Animal Health.** 1996. Reference of 1996 U.S. sheep health and management practices. National Animal Health Monitoring System, U.S. Department of Agriculture, Fort Collins, Colo.
11. **Centers for Epidemiology and Animal Health.** 1996. Dairy '96. National Animal Health Monitoring System, U.S. Department of Agriculture, Fort Collins, Colo.
12. **Chelle, P. L.** 1942. Un cas de tremblante chez une chèvre. Bull. Acad. Vét. Fr. **15:**294–295.
13. **Cockroft, P. D.** 2000. Clinical sign profile likelihood ratios for bovine spongiform encephalopathy suspects. Res. Vet. Sci. **68:**285–290.
14. **Cockroft, P. D.** 1999. Pattern-matching models for the differential diagnosis of bovine spongiform encephalopathy. Vet. Rec. **144:**607–610.
15. **Department for Environment, Food and Rural Affairs.** 2004. Age distribution of confirmed scrapie cases (in sheep and goats) from 1998 to 2002. [Online.] http://www.defra.gov.uk/animalh/bse/bse-science/scrapie/scrapie_age.PDF.
16. **Department for Environment, Food and Rural Affairs.** 2004. 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/statistics/bse/yng-old.html.
17. **Department for Environment, Food and Rural Affairs.** 2004. Bovine spongiform encephalopathy in Great Britain: confirmed cases by year of birth. [Online.] http://www.defra.gov.uk/animalh/bse/statistics/bse/yrbirth.html.
18. **Detwiler, L. A.** 1992. Scrapie. Rev. Sci. Tech. Off. Int. Epizoot. **11:**491–537.
19. **Dickinson, A. G., P. J. T. Stamp, and C. C. Renwick.** 1974. Maternal and lateral transmission of scrapie in sheep. J. Comp. Pathol. **84:**19–25.
20. **Doherr, M. G., D. Heim, R. Fatzer, C. H. Cohen, M. Vandevelde, and A. Zurbriggen.** 2001. Targeted screening of high-risk cattle populations for BSE to augment mandatory reporting of clinical suspects. Prev. Vet. Med. **51:**3–16.
21. **Doherr, M. G., A. R. Hett, J. Rüfenacht, A. Zurbriggen, and D. Heim.** 2002. Geographical clustering of cases of bovine spongiform encephalopathy (BSE) born in Switzerland after the feed ban. Vet. Rec. **151:**467–472.
22. **Durand, B., M. Savey, and F. Moutou.** 1998. Etude critique de la surveillance de l'encéphalopathie spongiforme bovine dans le monde. Epidémiol. Santé Anim. **34:**29–39.
23. **Durand, F.** 1995. Le réseau VIALINE. Epidémiol. Santé Anim. **27:**31–43.
24. **European Commission Scientific Steering Committee.** 2003. Assessment of the geographical risk of bovine spongiform encephalopathy carried out worldwide by the European Commission's Scientific Steering Committee. [Online.] http://europa.eu.int/comm/food/fs/sc/ssc/out363_en.pdf.
25. **European Commission Scientific Steering Committee.** 7–8 November 2002. Opinion on the geographical BSE risk for sheep and goats (GBR-S): adaptation of the cattle GBR methodology to small ruminants, in case BSE in small ruminants would become probable or evident under field conditions. [Online.] http://europa.eu.int/comm/food/fs/sc/ssc/out294_en.pdf.
26. **European Commission Scientific Steering Committee.** 11 January 2002. Update of the opinion on the geographical risk of bovine spongiform encephalopathy (GBR). [Online.] http://europa.eu.int/comm/food/fs/sc/ssc/out243_en.pdf.
27. **European Commission Scientific Steering Committee.** 6 July 2000. Final opinion on the geographical risk of bovine spongiform encephalopathy (GBR). [Online.] http://europa.eu.int/comm/food/fs/sc/ssc/out113_en.pdf.
28. **European Parliament and Council of the European Union.** 2001. Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Off. J. Eur. Communities *L***147:** 1–40.
29. **Fatzer, R., and F. Steck.** 1974. Histologische Differentialdiagnose bei tollwurverdächtigen Rindem. Schweiz. Arch. Tierheilkd. **116:**347–356.
30. **Fergusson, N. M., C. A. Donnely, M. E. I. Woolhouse, 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.
31. **Gibbens, J. C., C. E. Sharpe, J. W. Wilesmith, L. M. Mansley, E. Michalopoulou, J. B. M. Ryan, and M. Hudson.** 2001. Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. Vet. Rec. **149:**729–743.
32. **Hadlow, W. J., R. C. Kennedy, and R. E. Race.** 1982. Natural infection of Suffolk sheep with scrapie virus. J. Infect. Dis. **146:**657–664.
33. **Heim, D., and J. Kreysa.** 2002. Risk assessment as an indicator for the distribution of BSE in the world. Schweiz. Arch. Tierheilkd. **144:**710–715.
34. **Heim, D., L. Detwiler, E. Williams, and U. Kihm.** 2001. Update on bovine spongiform encephalopathy, scrapie, and chronic wasting disease. [Online.] Office International des Epizooties, Paris, France. ftp://ftp.oie.int/69SG_2001/A_69_SG_12_CS3C.pdf.
35. **Heim, D., and U. Kihm.** 1999. Bovine spongiform encephalopathy in Switzerland: the past and the present. Rev. Sci. Tech. Off. Int. Epizoot. **18:**135–144.
36. **Heim, D., R. Fatzer, B. Hornlimann, and M. Vandevelde.** 1997. Häufigkeit neurologischer Erkrankungen beim Rind. Schweiz. Arch. Tierheilkd. **139:** 354–362.
37. **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.
38. **Hill, F.** 1994. Neurological diseases of cattle where BSE has been included in the differential diagnosis. Surveillance **21:**25.
39. **Hilmert, H., and H. Diringer.** 1984. A rapid and efficient method to enrich SAF-protein from scrapie brains of hamsters. Biosci. Rep. **4:**165–170.
40. **Hoorens, J., and W. Oyaert.** 1966. Scrapie bij het shaap. Vlaams Diergeneeskd. Tijdschr. **35:**313–317.
41. **Hornlimann, B., D. Guidon, and C. Griot.** 1994. Risikoeinschätzung für die Einschleppung von BSE. Dtsch. Tierärztl. Wochenschr. **101:**295–298.
42. **Jeffrey, M., M. M. Simmons, and G. A. H. Wells.** 1993. Observations on the differential diagnosis of bovine spongiform encephalopathy in Great Britain, p. 342–362. *In* R. Bradley and B. Marchant (ed.), Transmissible spongiform encephalopathies. Proceedings of a Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Communities. Commission of the European Communities, Brussels, Belgium.
43. **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.
44. **Kimberlin, R. H.** 1992. Bovine spongiform encephalopathy. Rev. Sci. Tech. Off. Int. Epizoot. **11:**347–390.
45. **Lasmézas, C. I.** 2003. The transmissible spongiform encephalopathies. Rev. Sci. Tech. Off. Int. Epizoot. **22:**23–36.
46. **Martin, S. W., A. H. Meek, and P. Willeberg.** 1987. Veterinary epidemiology. Principles and methods. Iowa State University Press, Ames.
47. **Mayhew, I. G.** 1989. Large animal neurology: a handbook for veterinary clinicians, p. 3–69. Lea & Febiger, Philadelphia, Pa.
48. **McElroy, M. C., and E. D. Weavers.** 2001. Clinical presentation of bovine spongiform encephalopathy in the Republic of Ireland. Vet. Rec. **149:**747–748.
49. **Parry, H. B., and D. R. Oppenheimer.** 1983. Scrapie disease in sheep, p. 31–51. Academic Press, London, United Kingdom.
50. **Prusiner, S. B.** 1998. Prions. Proc. Natl. Acad. Sci. USA **95:**13363–13383.
51. **Roels, S., E. Vanopdenbosch, J. P. Langeveld, and B. E. Schreuder.** 1999. Immunohistochemical evaluation of tonsillar tissue for preclinical screening of scrapie based on surveillance in Belgium. Vet. Rec. **145:**524–525.
52. **Russo, P., C. Ducrot, P. Belli, J.-J. Fontaine, and C. Peyrouse.** 1999. Tremblante ovine: bilan de six années d'épidémiosurveillance dans le Sud de la France (etude de 173 cas). Point Vét. **28:**667–670.
53. **Saegerman, C., N. Speybroeck, S. Roels, E. Vanopdenbosch, E. Thiry, and D. Berkvens.** 2004. Decision support tools for clinical diagnosis of disease in cows with suspected bovine spongiform encephalopathy. J. Clin. Microbiol. **42:**172–178.
54. **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. Epizoot. **22:**83–102.
55. **Saegerman, C., P. Dechamps, S. Roels, K. Petroff, R. Geeroms, G. Torck, J. Dufey, R. Fourez, M. Hamerijckx, A. Cormann, P. Viatour, V. De Connick, 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 spongi-

forme transmissible chez les bovins en Belgique: bilan de l'année 1999. Ann. Med. Vét. **145:**47–58.
56. **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. Hamerijckx, M. Leemans, A. Vandersanden, D. Peharpre, B. Brochier, F. Costy, P. Mullier, E. Thiry, and P.-P. Pastoret.** 1999. Epidémiosurveillance de l'encéphalopathie spongiforme transmissible chez les bovins en Belgique: bilan de l'année 1998. Ann. Med. Vét. **143:**423–436.
57. **Sarradet, M.** 1883. Un cas de tremblante sur un bœuf. Rev. Vet. Toulouse **7:**310–312.
58. **Savey, M., P. Belli, and M. Coudert.** 1993. L'encéphalopathie spongiforme bovine en Europe. Présent et avenir. Vet. Res. **24:**213–225.
59. **Schreuder, B. E. C., J. W. Wilesmith, J. B. M. Ryan, and O. C. Straub.** 1997. Risk of BSE from the import of cattle from the United Kingdom into countries of the European Union. Vet. Rec. **141:**187–190.
60. **Scott, A. C., S. H. Done, C. Venables, and M. Dawson.** 1987. Detection of scrapie-associated fibrils as an aid to the diagnosis of natural sheep scrapie. Vet. Rec. **120:**280–281.
61. **Scott, M. R., R. Will, J. Ironside, H.-O.B. Nguyen, P. Tremblay, S. J. DeArmond, and S. B. Prusiner.** 1999. Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc. Natl. Acad. Sci. USA **96:**15137–15142.
62. **Smits, M. A., A. Bossers, and B. E. C. Schreuder.** 1997. Prion protein and scrapie susceptibility. Vet. Q. **19:**101–105.
63. **StataCorp.** 2003. Stata statistical software, release 7.1. Stata Corporation, College Station, Tex.
64. **Terry, L. A., S. Marsch, S. J. Ryder, S. A. C. Hawkins, G. A. H. Wells, and Y. I. Spencer.** 2003. Detection of disease-specific PrP in the distal ileum of cattle exposed orally to the agent of bovine spongiform encephalopathy. Vet. Rec. **152:**387–392.
65. **Vanopdenbosch, E., S. Roels, and C. Saegerman.** 2000. Animal TSE epidemiosurveillance and diagnosis in Belgium and BSE risk assessment, 29–30. *In* Proceedings of the First Scientific Day on Transmissible Spongiform Encephalopathies: Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy. Scientific Institute of Public Health, Brussels, Belgium.
66. **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. Méd. Vét. **142:**111–118.
67. **Vicari, A., B. Hornlimann, and L. Audigé.** 1996. Appréciation du risque de contamination des aliments concentrés suisses pour bovins par l'agent de l'encéphalopathie spongiforme bovine. Epidémiol. Santé Anim. **30:**77–84.
68. **Wells, G. A. H., S. A. C. Hawkins, R. B. Green, A. R. Austin, I. Dexter, Y. I. Spencer, M. J. Chaplin, M. J. Stack, and M. Dawson.** 1998. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. Vet. Rec. **142:**103–106.
69. **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.
70. **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.
71. **Wilesmith, J. W.** 1998. Manual on bovine spongiform encephalopathy. Food and Agriculture Organization of the United Nations, Rome, Italy.
72. **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 meal inclusion in proprietary concentrates. Res. Vet. Sci. **52:**325–331.
73. **Wilesmith, J. W., J. B. M. Ryan, W. D. Hueston, and L. J. Hoinville.** 1992. Bovine spongiform encephalopathy: epidemiological features 1985-1990. Vet. Rec. **130:**90–94.
74. **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.
75. **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.
76. **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:**264–267.
77. **Williams, E. S., and M. W. Miller.** 2003. Transmissible spongiform encephalopathies in non-domestic animals: origin, transmission and risk factors. Rev. Sci. Tech. Off. Int. Epizoot. **22:**145–156.
78. **Williams, E. S., and M. W. Miller.** 2002. Chronic wasting disease in deer and elk in North America. Rev. Sci. Tech. Off. Int. Epizoot. **21:**305–316.
79. **Williams, E. S., and S. Young.** 1980. Spongiform encephalopathy of Rocky Mountain elk. J. Wildl. Dis. **18:**465–471.
80. **Wood, J. N. L., L. J. Lund, and S. H. Done.** 1992. The natural occurrence of scrapie in moufflon. Vet. Rec. **130:**25–27.
81. **World Animal Health Organization.** 2004. Terrestrial animal health code, 12th ed., appendix 3.8.4. Surveillance systems for bovine spongiform encephalopathy. [Online.] http://www.oie.int/eng/normes/mcode/en_chapitre_3.8.4.htm.
82. **World Animal Health Organization.** 1997. Guidelines for continuous surveillance and monitoring of bovine spongiform encephalopathy. January 1997 Meeting of the International Animal Health Code Commission, appendix VIIIb. Document 65 SG/12/CS 1. Office International des Epizooties, Paris, France.
83. **World Health Organization.** 2001. Rabies surveillance report. Center for Rabies Surveillance and Research. Rabies Bull. Eur. **3:**4–8.