# Lesion profiles and gliosis in the brainstem of 135 Swiss Cows with Bovine Spongiform Encephalopathy (BSE)

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## Summary

Lesion profiles are considered to be an important tool for the comparison of the various animal and human spongiform encephalopathies and to obtain information upon prion strain variations. Histological and immunohistochemical reactions ($PrP^{sc}$, GFAP) in 13 brain areas at 4 levels in the brainstem from 135 BSE-positive and 45 BSE-negative cases were retrospectively evaluated. In this retrospective study a lesion profile based on histological features was worked out on the basis of BSE cases originating from Switzerland over a period of ten years. They were confirmed post mortem by histology and immunohistology. Our findings were reviewed in comparison with lesion profiles published in England. No striking differences comparing type and quality of lesions in the relevant areas between the Swiss and the English cases were evident. Moreover, the lesion profiles and the character of the lesions did not differ between animals born before or after the offal feeding ban, which supports the hypothesis that the Swiss epidemic is sustained by the same single, stable strain of the BSE agent, which is probably the same as in the English epidemic. There was a good correlation between $PrP^{sc}$ accumulation and spongiform changes, in particular in those areas which were morphologically most affected. Astrocytosis in BSE was quantified. A significant rise in GFAP-positive cells could be shown comparing the brain stem nuclei of BSE affected with BSE-unaffected cattle, despite considerable variation between the cases and between the nuclei. The observed astrocytosis did correlate with vacuolation of the neuropil and of perikarya as well as with $PrP^{sc}$ accumulation.

Keywords: BSE, lesion profiles, gliosis, immunohistochemistry

## Lariousprofile und Gliose im Hirnstamm von 135 Braunviehkühen mit spongioformer Enzephalopathie (BSE)

Läsionsprofile sind wichtig für den Vergleich von verschiedenen tierischen und menschlichen spongiformen Enzephalopathien und der beteiligten Prionstämme. Für unsere retrospektive Studie wurden von 135 BSE positiven und 45 BSE negativen Fällen, die in einem Zeitraum von 10 Jahren diagnostiziert worden sind, je 13 verschiedene Gehirnkerngebiete von 4 verschiedenen Ebenen/Querschnitten im Hirnstamm sowohl histologisch als auch immunhistologisch ($PrP^{sc}$, GFAP) untersucht und es wurde ein Läsionsprofil erstellt. Unsere Ergebnisse wurden mit denen aus England verglichen. Schweizer und Englische Fälle unterschieden sich nicht bezüglich der Läsionstypen und -qualität in den relevanten Gehirnarealen. Darüber hinaus zeigten Tiere, welche vor oder nach dem Fütterungsverbot geboren wurden, die gleichen Veränderungen mit den gleichen Läsionsprofilen. Dies unterstützt die Hypothese, dass die Schweizer Epidemie vom gleichen stabilen Stamm des BSE Erregers verursacht wurde, welcher die Epidemie in England verursacht hat. In dieser Untersuchung war eine gute Korrelation zwischen $PrP^{sc}$ Ansammlung und spongiformen Veränderungen sichtbar, vor allem in den Gehirnkerngebieten, welche morphologisch am stärksten betroffen waren. In den BSE Fällen wurde die Astrozytose quantifiziert. Ein signifikanter Anstieg der GFAP positiven Zellen konnte, trotz grosser Variabilität innerhalb der Fälle und der untersuchten Kerngebiete, gezeigt werden, wenn die Hirststammkerngebiete von BSE positiven mit BSE negativen Rindern verglichen wurden. Die Astrozytose korreliert sehr gut mit der Vakuolisierung des Neuropils und der Perikaryen wie auch mit der $PrP^{sc}$ Ansammlung bei BSE positiven Tieren.

Schlüsselwörter: BSE, Läsionsprofile, Gliose, Immunhistochemie


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## Introduction

Transmissible spongiform encephalopathies (TSE's) agent strains can be distinguished by seven criteria (Groschup and Kuczius, 2001). The agents don't necessarily have to be different in all of these criteria.

1. Clinical symptoms
2. Incubation period
3. Transmissibility
4. Histopathological lesion profiles
5. Inactivation properties
6. Proteinase K (PK) resistance of $PrP^{sc}$ (Prion Protein)
7. Glycosylation sites of $PrP^{sc}$

The main criteria though used in strain typing studies are the incubation period and the distribution of pathological changes seen in the brains of inbred mouse strains, expressed in the form of a "lesion profile" (Bruce, 1998). In the sixties and seventies different Scrapie strains which were different in their incubation period were systematically transmitted into laboratory animals and criteria for the evaluation of the histological changes were developed (Fraser and Dickinson, 1968). According to the following criteria they established a semiquantitative method to distinguish the agent strains. The intensity, the quality and the localization of spongiform changes were studied and graded in different brain areas. Plotting the grades against the brain areas resulted in "lesion profiles", which allowed to distinguish the strains clearly and to reproduce the results. By this it could be demonstrated that there are numerous strains of Scrapie that can be distinguished on the basis of their disease characteristics in panels of inbred mouse strains. The agent strains were different comparing localization, quality and quantity of spongiform change. The lesions did not depend on the route and the dose of infection (Carp et al., 1997), but the lesion profiles can be influenced by the *Sinc* gen. Additionally no overlap could, so far, be found in mouse-passaged strains isolated from BSE and Scrapie (Bruce et al., 1991; Bruce, 1998), but the same type of transmission studies indicated that there may be a link between BSE and the new variant Creutzfeldt-Jakob Disease (nvCJD) (Bruce et al., 1997).

Lesion profiles are an important tool for the comparison of TSE agent strains. To compare the lesions from Swiss BSE cases with those from England was the aim of the present study. Lesion profiles in the brainstem of BSE-affected Swiss cattle which had been diagnosed at the Institute of Veterinary Pathology, University of Zürich, within a period of ten years were characterized, and the results compared with those published in England (Hawkins et al., 1996; Simmons et al., 1996). Similar criteria were used to compare bab (born after the ban) with nonbab cases.

Apart from spongiform lesions, gliosis is considered to be a characteristic lesion in spongiform encephalopathies. Gliosis usually consists in an astrogliosis with astrocytic hypertrophy. The extent may vary considerably. In BSE, silver staining or immunohistochemistry for the demonstration of glial fibrillary acidic protein (GFAP) is needed. If the extent of vacuolar change in BSE really correlates with astrocytosis still requires confirmation (Wells et al., 1991). Astrogliosis in TSE is probably not only a reactive change to spongiform tissue lesions, but, according to some authors, it might even be the primary event (Kretzschmar et al., 1998). Using immunohistochemistry for glial fibrillary acidic protein (GFAP) astrocytosis (astrocytic gliosis) was evaluated.

## Material and Methods

### Origin of cases

The present study was based on the paraffin embedded CNS material from 135 cows with BSE and 45 cows with other CNS disorders as negative controls, all of which were screened at the Institute of Veterinary Pathology, University of Zurich, since 1991. The positive cases were confirmed both by histology and $PrP^{sc}$ immunohistochemistry. 47 of the positive cases were bab (born after ban, that is the 1$^{th}$ of December 1991 in Switzerland) cases. Since April 1999 all cases were additionally tested for the presence of $PrP^{sc}$ by the Prionics® western blot technique (Schaller et al., 1999) and confirmed.

### Selection of brain areas

Thirteen different brain areas respectively nuclei at four different levels of the brainstem (Mesencephalon, Pons region with Cerebellum, Medulla oblongata in the Obex region and Medulla cervicalis) were examined (Fig. 1). All mentioned cases were included for histology and $PrP^{sc}$ immunohistochemistry. For the GFAP immunohistochemistry samples of 16 BSE-positive cases and 15 negative cases of the years between 1999 and 2001 were selected.

### Histology

After a pretreatment with 4% formaline and 98% formic acid, the CNS tissue blocks of about 5 mm thickness were dehydrated and paraffin embedded. Further processing consisted in routine hemalaun-eosine (HE) staining of 4 to 6 µm thick sections.

Each of the selected nuclei was graded for vacuolation of the perikarya and for spongiform change of the neuropil. The somal vacuolation was graded 0 to 3 (0 = no vacuolation, 1 = 1–2 vacuoles, 2 = 3–4 vacuoles, 3 = > 4 vacuoles) and the spongiform change

 

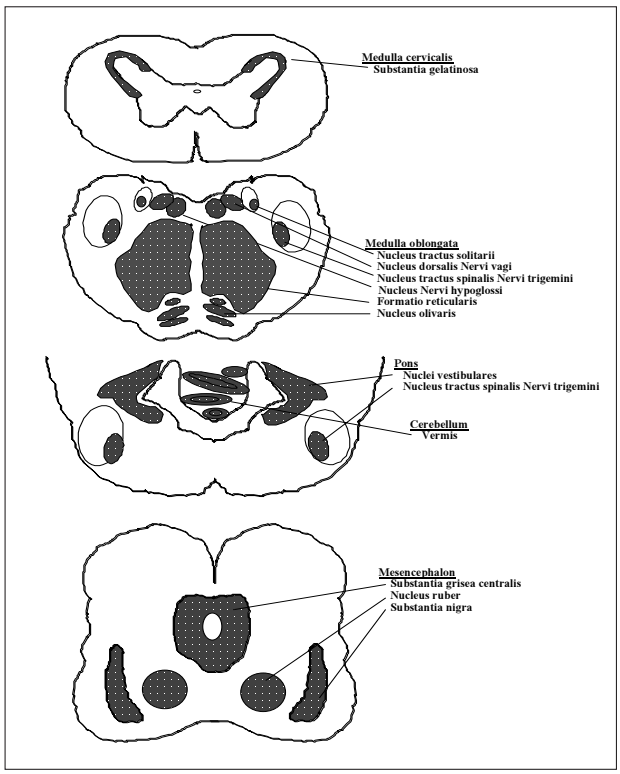


*Figure 1: Brain areas, selected for histological and immunohistological evaluation.*

was evaluated (0 = no change, 1 = unclear, 2 = slight, 3 = medium and 3 = severe spongiform change) based on a series of standard photographs. All areas were graded by the same person. In order to minimize effects of subjective grading the data was compared with the ones obtained by two experienced pathologists and with a set of reference photographs. Bab and nonbab cases were compared by the $Chi^2$Test ($P = 0.01$) analyzing the repartition of the cases onto the different grades. For each brain area a lesion score for spongiform change (vacuolation of the neuropil) and a score for vacuolation of the perikarya were calculated as follows. For each brain area the proportion of cases of every grade was multiplied with the corresponding grade. The lesion score for a brain area was obtained by adding these wheighted grades. No other pathological features than vacuolation were taken into account. The calculated lesion scores were plotted against the area code number (Tab. 2, Fig. 6) in order to produce a comparable, representative lesion profile of the BSE cases of Zurich between 1991 and 2001 and the bab subpopulation (Hawkins et al., 1996; Simmons et al., 1996).

## PrP immunohistochemistry

Paraffin embedded sections mounted on positively charged slides (SuperFrost, Menzel-Gläser, Germany, distributor: Medite, Switzerland) were dried over night at 37°C and deparaffinated. They were treated with proteinase K (Sigma, proteinase sigma Type-XXIV, P6446, distributor: Fluka, Buchs , Switzerland) during 15 minutes at 37°C and then autoclaved at 1 bar at 121°C during 30 minutes. The endogenous peroxidase is followingly blocked by 3% $H_2O_2$ (S2023, Dako, Zug, Switzerland) during 5 minutes and by normal swine serum (1:20, X0901, Dako, Zug, Switzerland) during 20 minutes both at room temperature. The slides were incubated with the primary antibody (polyclonal Rabbit-anti-PrP C15S, kindly provided by A. Zurbriggen, BSE Reference Center, Berne, Switzerland) in a dilution of 1:800 during 1 hour at 41°C or overnight at 37°C and then linked with the secondary antibody coupled with horse radish peroxidase (HRP) (Detection Kit, DakoChemMate, K5003, Dako, Zug, Switzerland). Finally they were visualized by AEC (3-amino-9-ethyl carbazole, DakoChemMate, K5003, Dako, Zug, Switzerland) during 15 minutes and counterstained with hemalaun during 10 seconds (Graber et al., 1995). Quantitative grading was impaired by staining variations between the cases. Therefore only a qualitative grading system was taken into consideration. The selected nuclei were graded from 0 to 2 (0 = no PrP accumulation, 1 = unspecific or questionable and 2 = specific accumulation).

## GFAP immunohistochemistry

After deparaffination the slides were pretreated by microwave cooking in a citric acid buffer (pH6, S2031, Dako, Zug, Switzerland) during 10 minutes at 750 W. Treated with peroxidase blocking solution (DakoChemMate, S 2023, Dako, Zug, Switzerland) the slides were incubated at room temperature during 20 minutes with prediluted GFAP-antibody (H0083, Dako, Zug, Switzerland). The slides were then incubated with the linking antibody (DakoChemMate, K 5003, Dako, Zug, Switzerland) coupled with HRP (DakoChemMate, K 5003, Dako, Zug, Switzerland) each during 10 minutes at room temperature. Finally they were visualized with AEC (DakoChemMate, K 5003, Dako, Zug, Switzerland) during 5 minutes and counterstained with hemalaun during 10 seconds both at room temperature. In 2 or 3 highpower (40x objective) fields in each of the selected nuclei the cells positively stained for GFAP were counted. The arithmetic mean of the 2 respectively 3 values was determined after checking that there was no significant difference between the values of the same location (paired t-test).

## Data analysis / software

Available data of all cases including the results of the histological studies were collected in FileMaker Pro 3.0Dv3. Raw data were processed in Excel 98 and

 

analysed with Statview 4.02. Further we used Claris-Draw™ 1.0Dv3, EndNote 5.0 and Microsoft® Word 98.

## Results

### Negative control animals and differential diagnoses

The 45 cases used as negative controls gave the differential diagnoses as listed in Table 1. 42 animals were clinically BSE-suspect. 32 (76% of the clinically suspect) cases did not show any neurohistological lesions. In seven cases with pathological lesions in the CNS no etiological agent or factor could be found. Among the degenerative conditions two cases were due to a nutrient deficiency and among the inflammatory conditions three cases had a bacterial etiology. No evidence of atypical BSE was found in these suspect animals (Casalone et al., 2004).

### Histology

Spongiform change was symmetrical and was found in the grey matter of the considered nuclei. It appeared as round optically empty spaces with more or less constant diameter of 10 to 20µm. A significant inflammatory response was absent in all cases. In one case there was neuronophagia in the areas examined. In a few cases there was a questionable spongiform change that could be attributed to inappropriate fixation or autolysis. Those cases were taken into account by grade 1. All in this study considered loci in the CNS of BSE-affected cattle showed a certain degree of spongiform change in the neuropil with the only exception of the cerebellar vermis. High proportions of medium to severe spongiform change in positive cases were observed in the Substantia gelatinosa, the Nucleus tractus solitarii and the Nucleus tractus spinalis Nervi trigemini. A majority of cases showed mild to medium grade spongiform change in the Nucleus dorsalis Nervi vagi (Fig. 2a), in the Nucleus trac-

*Figure 2: Cow, 4 years, BSE-positive. Figs. 2a–2c. Nucleus dorsalis Nervi vagi and the Nucleus Nervi hypoglossi in the Medulla oblongata (Obex region) with different stains. The most striking feature is the different reaction pattern of two neighbouring nuclei. Figs. 2d and 2e. A section from the Nucleus ruber in the Mesencephalon is visible.*

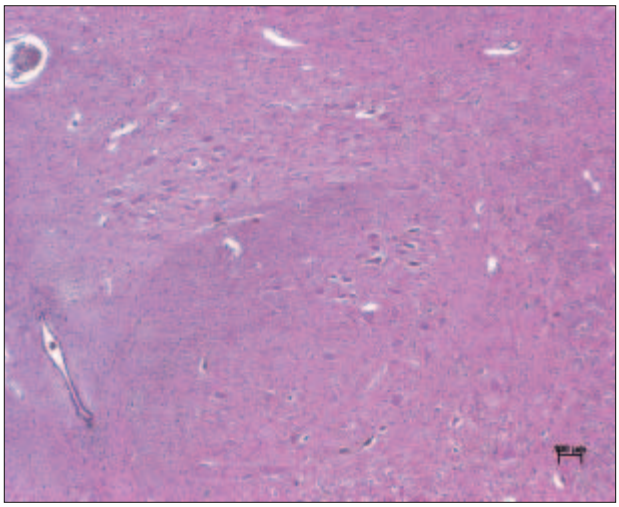

*Figure 2a: Nucleus dorsalis Nervi vagi and Nucleus Nervi hypoglossi in the Medulla oblongata (Obex region). HE. X4.*

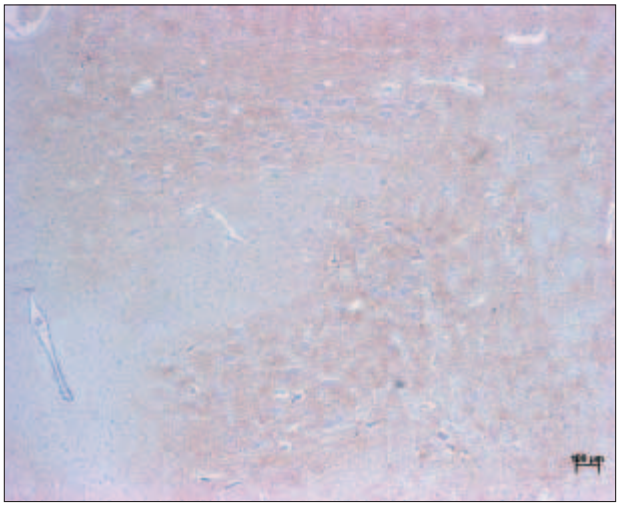

*Figure 2b: Nucleus dorsalis Nervi vagi and Nucleus Nervi hypoglossi in the Medulla oblongata (Obex region). GFAP. IHC. X4.*

*Table 1: Differential diagnoses for clinically suspected BSE found in the 45 negative controls included in the study.*

| Histopathological diagnosis of the CNS | Number of cases | Clinically suspect |
|---|---|---|
| Idiopathic edema | 1 | 1 |
| Degenerative encephalopathy | 1 | 1 |
| Focal neuron degeneration | 1 | 1 |
| Acute CCN | 2 | 2 |
| Tumor (ependymoma) | 1 | 1 |
| Nonpurulent encephalitis | 4 | 4 |
| Actinomycosis | 1 | 1 |
| Listeriosis | 1 | 1 |
| Abscess (bacterial) | 1 | 1 |
| No pathological lesions | 32 | 29 |

 

tus spinalis Nervi trigemini (pons region), in the Substantia grisea centralis and in the Substantia nigra. Grades 1 or 2 of spongiform change were predominant in Nucleus Nervi hypoglossi, the Nuclei vestibulares and the Nuclus ruber (Fig. 2d). BSE-negative cases did not show spongiform change apart from a few cases with grade1.

Vacuolation of the perikarya consisted in clear, large, single or multiple spaces distending the perikarya. Neuronal vacuoles were variable in size. Vacuolation of the perikarya could only be observed in the Nucleus dorsalis Nervi vagi, the Nuclei olivares, the Nuclei vestibulares, the Substantia grisea centralis, the Nucleus ruber and the Substantia nigra. Only in the Nucleus ruber and in the Nuclei vestibulares vacuolation was found in over 90% of the BSE-positive cases.

The heighest mean lesion scores (Tab. 2) for spongiform change were found in the Substantia gelatinosa, the Nucleus tractus solitarii and the Nucleus tractus spinalis Nervi trigemini followed by the Nucleus dorsalis Nervi vagi, the Nucleus olivaris, Nucleus tractus spinalis Nervi trigemini (Pons) and the Substantia grisea. The nuclei in the Medulla oblongata varied remarkably. The lowest scores were in the Vermis and the Nucleus Nervi hypoglossi.

In over 95% of the positive cases the Nuclus ruber and the Nuclei vestibulares showed vacuolation of the perikarya with a mean score in a rank from 0 to 3 of 2.3 respectively of 1.8. 8 out of 19 BSE-negative cases showed vacuolation of the perikarya in the Nucleus rubber. In about 40% of the positive cases also the Nucleus dorsalis Nervi vagi showed vacuolation, whereas it was a rather rare observation in the Nucleus olivaris, the Substantia grisea and the Substantia nigra. The separately regarded and scored bab cases did not differ significantly from the results obtained by all the positive cases together (P= 0.01).

Splitting the positive cases into a group of bab animals (born after feedban, that is the 1st December 1990 in Switzerland) and non-bab animals no differences could be found neither in the repartition of the different grades, split by locations, nor qualitatively by the type of alterations found. Similar results were found regarding the age-dependent histological and immunohistochemical alterations. In most of the cases we did not have reliable data about the onset of clinical signs. That's why no relation between the clinical stage and morphological changes could be established.

The shape of the lesion profile obtained from all the included cases did not vary significantly from the le-

*Table 2: Mean lesion scores for spongiform change and vacuolation in BSE-positive animals (SD = standard deviation, bab = born after ban, area code see Fig. 6)*

| | location, area code | total number of cases (bab cases) | score for vacuolation of the neuropil (bab cases) | ± SD (bab cases) | % ≥ grade 2 (bab cases) | score for vacuolation of the perikarya (bab cases) | % ≥ grade1 (bab cases) |
|---|---|---|---|---|---|---|---|
| Medulla cervicalis | Substantia gelatinosa | 41 (30) | 2.9 (3.0) | ± 0.91 (0.98) | 98 (97) | 0 (0) | 0 (0) |
| Medulla oblongata | N.tr. solitarii, 1 | 128 (41) | 3.5 (3.5) | ±0.71 (0.80) | 99 (100) | 0 (0) | 0 (0) |
| | N.tr.spinalis N. trigemini, 2 | 134 (45) | 3.2 (3.1) | ±0.74 (0.70) | 99 (100) | 0 (0) | 0 (0) |
| | N.dors. N.vagi | 134 (45) | 2.2 (2.5) | ±0.77 (0.84) | 86 (93) | 0.45 (0.51) | 41 (42) |
| | N.N. hypoglossi, 3 | 133 (44) | 1.1 (1.0) | ±0.49 (0.51) | 20 (14) | 0.02 (0) | 2 (0) |
| | N. olivaris | 133 (45) | 2.3 (2.1) | ±0.60 (0.61) | 95 (91) | 0.02 (0) | 2 (0) |
| Pons | N.tr.spinalis N. trigemini | 122 (43) | 2.1 (2.1) | ±0.44 (0.36) | 98 (98) | 0 (0) | 0 (0) |
| | Nn. vestibulares, 4 | 133 (46) | 1.7 (1.9) | ±0.54 (0.49) | 69 (85) | 1.8 (2.0) | 96 (96) |
| Cerebellum | Vermis, 6 | 130 (44) | 0.1 (0.1) | ±0.28 (0.35) | 0 (0) | 0 (0) | 0 (0) |
| Mesen-cephalon | Substantia grisea centralis, 7 | 132 (44) | 2.4 (2.6) | ±0.63 (0.61) | 96 (98) | 0.02 (0.02) | 2 (2) |
| | N. ruber | 74 (18) | 1.6 (1.9) | ±0.64 (0.92) | 60 (72) | 2.3 (1.9) | 97 (72) |
| | Substantia nigra | 124 (39) | 1.9 (2.0) | ±0.51 (0.54) | 86 (85) | 0.1 (0.1) | 10 (10) |

 

sion profile of the bab cases taken alone. The lesion profile did not contain the values for the Nucleus cochlearis (brain area code 5; Hawkins et al., 1996) which were not included in the study.

### PrP immunohistochemistry

In all BSE-positive cases an accumulation of $PrP^{sc}$ was evident as a coarsely granular brownish staining of the perikarya or the neuropil (Fig. 2c, 2e). A quantification of $PrP^{sc}$ staining of the positive cases resulted to be varying rather due to the method than to $PrP^{sc}$ quantity. Within a case however there were clear differences between the nuclei. In the Vermis of the Cerebellum and in the Nucleus Nervi hypoglossi none to unspecific staining was consistently found whereas there was specific staining in all other considered areas.

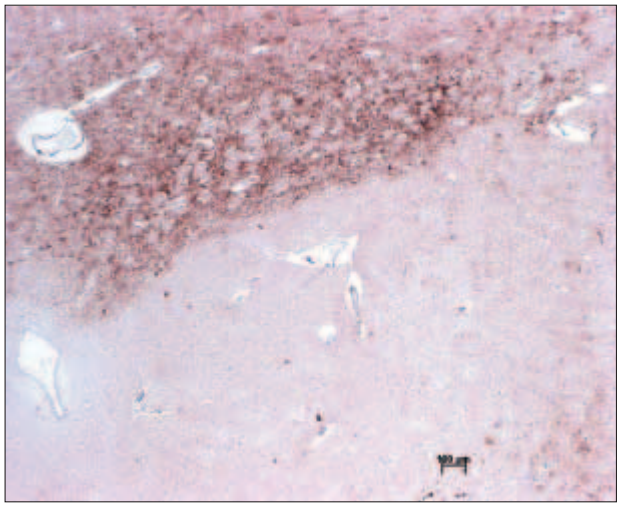

*Figure 2c: Nucleus dorsalis Nervi vagi and Nucleus Nervi hypoglossi in the Medulla oblongata (Obex region). PrP-IHC. X4.*

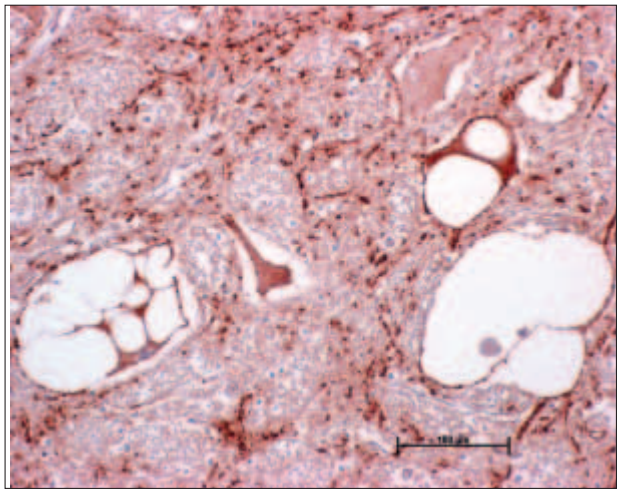

*Figure 2e: Severe $PrP^{sc}$ accumulation in the Nucleus ruber of the Mesencephalon at a higher magnification. $PrP^{sc}$-IHC. X40. Vacuolation of the perikarya alone is not diagnostic in the Nucleus ruber without positive $PrP^{sc}$-IHC.*

### GFAP immunohistochemistry

Positive GFAP staining consisted in a fine granular brownish staining of the cell bodies of astrocytes and their thin processes. The nucleus and a small space surrounding it were usually not stained (Fig. 2b). Comparing all counts of GFAP-positive cells per nucleus between BSE-positive and negative cases there was a significant rise in BSE-positive cases. The mean almost doubled although the variation was considerable (Fig. 3). The ages of animals included in the GFAP and synaptophysin investigation varied between 2.5 and 11 years for BSE-negative cases and between 4 and 8 years for BSE-positive cases. The two groups did

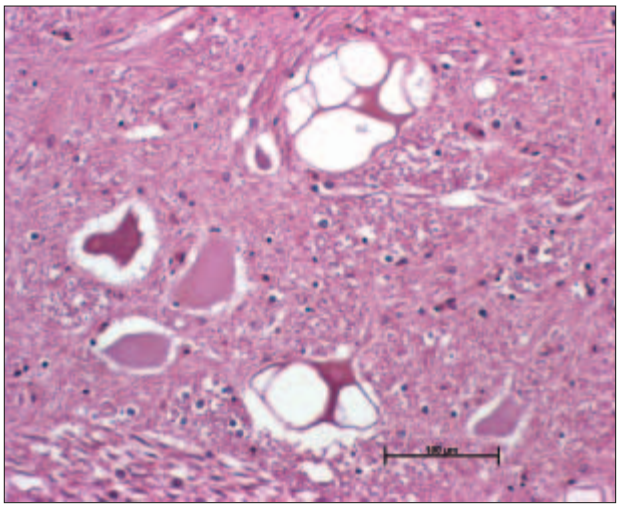

*Figure 2d: Intense vacuolation of the perikarya in the Nucleus ruber of the Mesencephalon. HE. X40.*

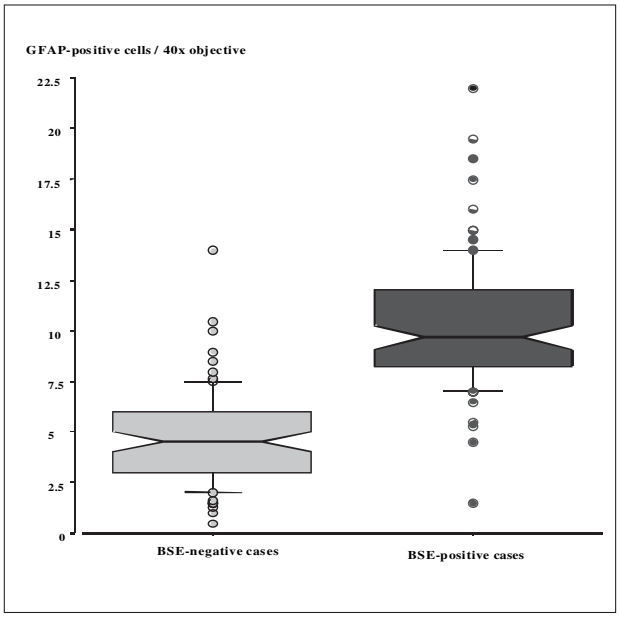


*Figure 3: Distribution of GFAP-positive cell counts in BSE-positive and BSE-negative cases. (Boxplot showing the 10%, 25%, 50%, 75% and the 90% percentiles). The difference was significant ($P < 0.01$ in the U–Test).*

 

not vary significantly. The mean ages were 5.5 years for the BSE-negative cases and 5.0 years for the BSE-positive cases.

The differences per location split into BSE-positive and BSE-negative cases showed to be highly significant for the Nucleus dorsalis Nervi vagi, the Formatio reticularis, the Nucleus tractus spinalis Nervi trigemini and the Nuclei vestibulares. Except for the Nucleus Nervi hypoglossi all locations had significantly lower number of GFAP-positive cells in BSE-negative cases (Fig. 4).

The most considerable relative rise in the number of GFAP-positive cells was observed in the Nucleus dorsalis Nervi vagi, the Formatio reticularis and the Nuclei vestibulares where the numbers rose more than 2.5 fold comparing with mean value of the negative cases.

### Statistical test for effects

Testing the different grades of spongiform change, for differences in GFAP cell counts (Bonferroni/Dunn, significance level 5 %) we found that grades 2, 3 and 4 differed significantly from grade 0 in the Nucleus dorsalis Nervi vagi. Similar results were found in the Nucleus tractus spinalis Nervi trigemini and in the Nuclei vestibulares which showed an increasing number of GFAP-positive cells with increasing grade of spongiform lesions. The differences in the Nucleus Nervi hypoglossi and in the Vermis were not significant (Fig. 5).

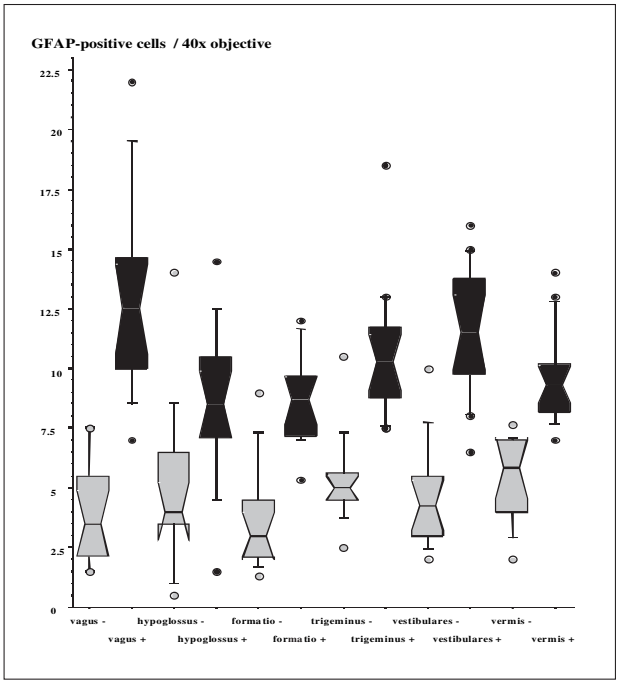


*Figure 4: Distribution of GFAP-positive cell counts per area (Boxplot showing the 10%, 25%, 50%, 75% and the 90% percentiles). All areas showed a significant difference between the positive and the negative cases with the only exception of the N.N.hypoglossi (P< 0.01 in the U–Test). BSE-negative = "-" = grey, BSE-positive = "+" = black.*

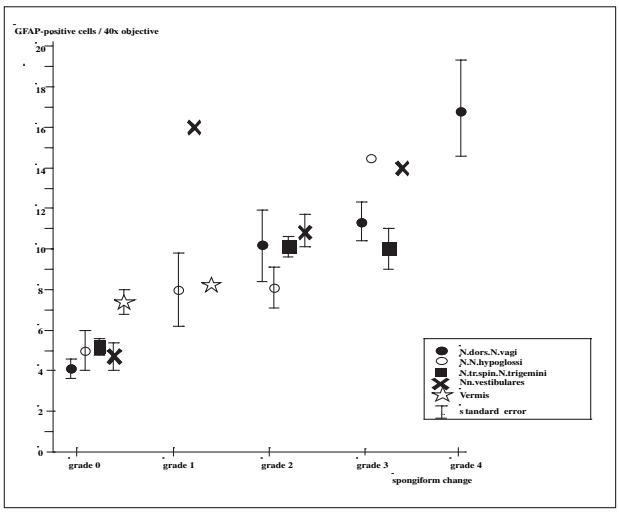


*Figure 5: Mean counts of GFAP-positive cells per grade of spongiform change and per brain area with standard error. Missing standard error bars indicate that only one case was found in the considered grade and brain area.*

PrP accumulation gave significant differences between negative and unspecific staining on one hand and specific staining on the other hand in all examined areas except the Nucleus Nervi hypoglossi and Vermis. Specific staining could not be observed in the Vermis. In the Nucleus Nervi hypoglossi specific staining was not accompanied by a significant rise in the number of GFAP-positive cells. In the GFAP study the Nuclei vestibulares were the only areas showing vacuolation of the perikarya. The difference between grades 1 to 3 and 0 was highly significant. The grades 1, 2 and 3 did not differ.

## Discussion

Spongiform change of the neuropil was the most consistent finding in the included brain areas. Vacuolation of the perikarya was most frequently found in the Nn. vestibulares and, as a nonspecific finding in the Nucleus ruber. These findings are remarkably consistent and contrast with the variability of distribution patterns in sheep Scrapie (Wells et al., 1991).

In Scrapie the most obvious alteration is a vacuolation of the cytoplasm of neurons mainly found in Medulla, Pons, Diencephalon and Thalamus. This feature is accompanied by other signs of neuron degeneration like chromatolysis and pyknosis. As to the distribution of Scrapie lesion profiles there is an important variability between the different strains which has not been observed in BSE of cattle (Groschup and Kuczius, 2001).

In goats challenged orally with BSE, one case was described with only slight vacuolation in the midbrain and thalamus and another had more extensive lesions, especially in the thalamus. Little vacuolation was iden-

 

tified in cortical areas. In sheep challenged orally with BSE, vacuolation throughout the brainstem was found, including the raphe, dorsal vagus, olivary and facial nuclei, thalamus and hypothalamus, but there was no evidence of cortical lesions (Foster et al., 2001).
Multiple-route parenteral inoculation in pigs resulted in severe vacuolar change of the molecular and granule-cell layers of the Cerebellum. In the caudal brainstem vacuolar changes were least severe. Occasional neuronal vacuoles were seen within neurons of the dorsal nucleus of the vagus nerve. In four pigs the entire cerebral cortex showed severe neuropil vacuolation which is not commonly found in cattle (Ryder et al., 2000). In mule deer *(Odocoileus hemionus)* and elk *(Cervus elaphus nelsoni)* with CWD (chronic wasting disease) vacuolation of perikarya could be found in Purkinje cells (Williams and Young, 1993). This feature was not found in BSE of cattle.
The lesion profiles established on the 135 BSE positive cattle and on its subpopulation of Bab cases were very similar to the English findings (Hawkins et al., 1996; Simmons et al., 1996) with the restriction that the brain areas included in our study consisted only out of 6 areas in contrast to the 16 mentioned in the English study (Fig. 6). A diagram of the topography and relative severity of vacuolation sorted by brain region established on the mean scores for each of the brain areas showed similar results (Wells et al., 1991; Jeffrey et al., 1992; Jeffrey and Halliday, 1994; Wells and Wilesmith, 1995). Assuming that the sites scored are representative for different intensities of vacuolar pathology in the lesion profile of BSE the hypothesis that the English and the Swiss BSE epidemic including the bab cases is sustained by a single strain of agent is given some support but it cannot be conclusive. The phenotypic uniformity of BSE in cattle can partly be explained by the genotypic invariability of the PrP gene in cattle in contrast to the variability of the PrP genes in humans and cattle (Goldmann et al., 1994; Hunter and Goldmann, 1996).
Using immunohistochemistry for $PrP^{sc}$, all cases that had previously been diagnosed in our institute as BSE by routine histology since 1991 were confirmed. The most often mentioned differential diagnoses for clinically BSE-suspect animals are all diseases leading to behavioral changes and/or ataxia. Essentially these are metabolic disorders like hypomagnesemia, nervous acetonemia and peripartal hypocalcemic paresis, bacterial infections like listeriosis, viral infections like rabies and pseudorabies (Aujeszky), intoxications with lead or botulinustoxin and illnesses of the backbone and the spinal cord (Wilesmith et al., 1992; Braun et al., 1997; Braun et al., 1999). In 32 animals of the 42 (76%) clinically suspect cows, which were BSE-negative, no pathologic alterations could be diagnosed in the brain. A far bigger study in England (Simmons et al., 1996) gave a comparable proportion of suspect but BSE-negative cases with no diagnosis in the CNS. None of the BSE cases in Zürich showed signs of atypical spongiform change neither in histology nor in immunohistochemistry. Up to now atypical BSE was not found in other countries. Silent BSE cases were not included in this study.
$PrP^{sc}$ expression was mainly confined to the grey matter including the different nuclei. A semiquantitative grading system going further than no, unspecific and specific immunohistochemical reaction could not be held upright. Nevertheless, there were considerable differences between $PrP^{sc}$ accumulation within the same brain level. All the positive cases had a far stronger immunohistochemical $PrP^{sc}$ reaction in the N.dors.N.vagi than in the N.N.hypoglossi which lie side by side in the Medulla oblongata. This may give some emphasis to the hypothetical vagal spread of $PrP^{sc}$ from the gut into the central nervous system (CNS) (Beekes et al., 1998). Different authors high-

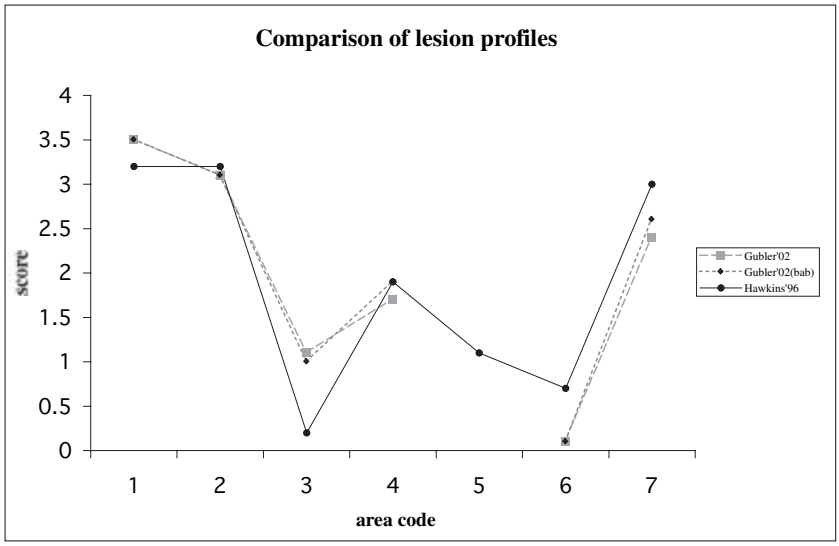


*Figure 6: Brain lesion profile comparison: Gray dots/lines and lines representing results from Swiss cattle /bab and non-bab; black dots/lines representing results published from experimentally (orally) infected cattle in UK (Hawkins et al., 1996). Area code see Table 2.*

 

lighted the importance of prions appearing in the peripheral nervous system (PNS) prior to the onset of replication in the brain and stated that $PrP^{sc}$ expression in the PNS is required for neuroinvasion (Beekes et al., 1996; Race et al., 2000). Scrapie infectivity and $PrP^{sc}$ were found in the PNS of a Scrapie-affected sheep (Groschup et al., 1996). By a double immunolabelling strategy proximity of noradrenergic endings with $PrP^{sc}$-accumulating cells was demonstrated (Bencsik et al., 2001). The lymphoreticular system (LRS) may not be an essential mediator for neuroinvasion because in immunodeficient mice prions make their way into the CNS, probably through the PNS (Lasmezas et al., 1996). Nevertheless the disappearance of mature follicular dendritic cells (FDC) which abolished splenic prion accumulation retarded neuroinvasion following intraperitoneal Scrapie infection (Weissmann et al., 2001). These findings give rise to a similar question. Which is the role of FDC in the spleen and of an increased number of astrocytes in the CNS in the pathogenesis of prion disease?

The often mentioned astrocytosis or astrocytic gliosis in BSE was substantiated by quantification of the GFAP-positive cells. Also in sheep Scrapie a significant increase of astrocytes was detected, but astrocytosis was not usually related to the severity of the characteristic vacuolar lesions (Georgsson et al., 1993). Double-label immunohistochemistry for proliferating cell nuclear antigen (PCNA) and GFAP confirmed that the astrocytosis in Scrapie-infected animals is, at least in part, owing to actual replication of astrocytes and not only owing to the overexpression of GFAP (Ye et al., 1998b). Astrocytosis correlated fairly well in our results with $PrP^{sc}$ accumulation, vacuolation of the perikarya and spongiform change, which was in contrast to other studies (Fatzer et al., 1996). Astrocytosis is also found in many other pathological alterations of the CNS and it is somehow like an unspecific response to various neurologic insults. This feature is well conserved across a variety of different species. It is, therefore, of limited diagnostic value for TSE's (Eng and Ghirnikar, 1994; Hewicker-Trautwein et al., 2001). Nevertheless there is a pronounced astrocytosis in all TSE's and the question arises which then is its role in the pathogenesis. GFAP itself does not seem to be crucial in the pathogenesis as mice devoid of GFAP develop normally and are susceptible to Scrapie prions (Gomi et al., 1995). Oxidative stress has been shown to be important in several neurodegenerative disorders. In Scrapie-infected mouse brains peroxynitrite mediated neuronal degeneration was found (Guentchev et al., 2000). Concerning the role of the astrocytosis there are different theories. Some point out a indirect toxic effect (Raeber et al., 1997), others think that $PrP^{sc}$ is on the one hand toxic for neurons and on the other trophic for astrocytes (Ye et al., 1998a). Interactions between astrocytes and oligodendroglia are also suggested, but its significance remains unclear (Liberski et al., 1997).

Another remarkable feature of our study is an astrocytic response whose intensity is not the same in all areas. The mean rise of GFAP-positive cells per brain area is not the same for all areas. But which is the cause for this differential response which is furthermore in good relation with other pathological features. This observation cannot give an answer whether the astrocytic gliosis happens prior to nerve cell degeneration or whether it is just reactive. Anyway, there may be an involvement of the astrocytes and the microglia in the pathogenesis of prion disease (Giese et al., 1998).

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**Prorils lésionnels et gliose dans le tronc cérébral de 135 vaches suisses souffrant d'encéphalopathie spongiforme bovine (ESB)**

Les profils lésionnels sont importants pour la comparaison entre les diverses encéphalopathies spongiformes animales et humaines et les souches de prions concernées. On a utilisé pour cette étude lésionnelle 135 cas positifs et 45 cas négatifs diagnostiqués sur une période de 10 ans. Sur chaque cas, 13 zones des noyaux cérébraux ont été examinées du

**Profilo delle lesioni e gliosi nel tronco cerebrale di 135 mucche svizzere affette da encefalopatia bovina spongiforme (EBS)**

I profili delle lesioni sono di importanza capitale per paragonare le encefalopatie spongiformi negli animali e nell'uomo e del ceppo di prioni coinvolti. Per il nostro studio retrospettivo sono stati esaminati 135 casi con diagnosi positiva alla EBS e 45 con diagnosi negativa, diagnosticati su una durata di 10 anni. In ciascun caso sono state analizzate, sotto il

 

point de vue histologique et immunologique dans 4 plans de coupe différents du tronc cérébral ($PrP^{sc}$, GFAP) et un profil lésionnel a été établi. Nos constatations ont été comparées à celles provenant d'Angleterre. Les cas suisses et anglais ne se différencient pas quant au type et à la qualité des lésions dans les principales aires cérébrales. En outre, les animaux nés avant ou après l'interdiction d'affouragement présentent les mêmes lésions avec les mêmes profils lésionnels. Cette constatation soutien l'hypothèse que l'épidémie suisse a été causée par la même souche stable d'agent de l'ESB que celle qui a causé l'épidémie en Angleterre. Dans cette étude, une bonne corrélation entre l'accumulation de $PrP^{sc}$ et les modifications spongiformes étaient constatables particulièrement dans les zones qui présentaient les plus fortes altérations morphologiques. Dans les cas d'ESB, l'astrocytose a été quantifiée. Une augmentation significative des cellules positives à la GFAP a pu être démontrée malgré une grande variabilité entre les cas et les zones examinées lorsque l'on comparait les zones du tronc cérébral d'animaux positifs à l'ESB avec celles d'animaux négatifs. L'astrocytose est bien corrélées avec la vacuolisation du neuropil et des péricaryes ainsi qu'avec l'accumulation de $PrP^{sc}$ chez les animaux positifs.

profilo istologico e immunologico ($PrP^{sc}$, GFAP), 13 diverse zone del tronco cerebrale su 4 diversi piani/sezioni del tronco cerebrale. È stato quindi costituito un profilo delle lesioni. I nostri risuitati sono stati paragonati a quelli inglesi. I casi svizzeri e inglesi non si differenziano in riferimento ai tipi e alla qualità delle lesioni nelle zone cerebrali rilevanti. Inoltre gli animali, sia che erano nati prima o dopo la proibizione del mangime, mostravano le stesse modifiche con lo stesso profilo di lesioni. Ciô sostiene l'ipotesi che l'epidemia svizzera sia stata provocata dall' agente patogeno della EBS con Io stesso ceppo stabile che ha provocato l'epidemia in Inghilterra. ln questa analisi era ben visibile la buona correlazione tra accumulo di $PrP^{sc}$ e variazioni spongiformi, in particolare nelle zone centrali del cervello, le quali sono state colpite maggiormente sotto l'aspetto morfologico. Nei casi di EBS è stata quantificata la astrocitosi. Un significativo aumento di GFAP nelle cellule positive veniva evidenziato, malgrado la grande variabilità tra i casi e le zone centrali esaminate, se fossero state comparate le zone centrali del tronco cerebrale in manzi con EBS positiva e con EBS negativa. L'astrocitosi si correla molto bene con la vacuolizzazione dei neuropils e dei pericari come anche con l'accumulo di $PrP^{sc}$ negli animali positivi alla EBS.

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