Breed predisposition for BSE: Epidemiological evidence in Bavarian cattle

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Summary

We present epidemiological data from Bavaria that indicates that animals of the Brown Swiss (BS) cattle breed might be more susceptible to BSE than animals from other breeds, both in terms of disease prevalence and length of the incubation period. BS animals were disproportionately represented among the BSE cases (BS represented about 9% of the susceptible population but 27% of actual cases). BS were slaughtered at a higher age (5.8 years vs. 5.0 years for other breeds), and there is a higher prevalence of feeding proprietary feeds to BS calves than calves from other breeds. There was no difference in the recorded feeding practice of BSE-positive animals from BS or other breeds. These results would lead to expect a higher prevalence of BSE in the BS population, with BS BSE animals being of equal age or older than BSE animals from other breeds. In contrast, median age at BSE detection was significantly lower in BS animals than in other breeds (61.4 vs. 68.8 months). There was no difference in the identification categories of BSE between BS animals and animals of other breeds that could explain this difference in age. BS cattle are reported to have more octapeptid repeats in the prion protein gene than other breeds, which could account for shorter incubation periods and higher susceptibility. These observations suggest that BS animals and their tissues should be used in further studies into genetic determinants of BSE susceptibility in cattle.

Keywords: BSE, Brown Swiss, predisposition, Bavaria

Rasse-Prädisposition für BSE: Epidemiologische Hinweise bei Bayerischen Rindern

Wir stellen epidemiologische Daten aus Bayern vor, die darauf hinweisen, dass Rinder der Rasse Braunvieh (BV) empfänglicher für BSE sind als Rinder anderer Rassen, sowohl hinsichtlich einer höheren Prävalenz als auch einer kürzeren Inkubationszeit. BV war unter den BSE-Fällen überproportional vertreten (ca. 9% der empfänglichen Population, aber 27% der tatsächlichen Fälle). BV hatte ein höheres Schlachtalter (5.8 Jahre vs. 5.0 Jahre für andere Rassen) und BV Kälber erhielten häufiger Zukaufs-Futtermittel als Kälber anderer Rassen. Es gab aber keinen Unterschied bei der berichteten Fütterungspraxis von BSE-positivem BV und Tieren anderer Rassen. Diese Ergebnisse lassen eine höhere BSE-Prävalenz bei BV vermuten, wobei BSE-positives BV älter oder gleich alt wie BSE-positive Tiere anderer Rassen sein sollte. Im Gegensatz dazu war das mediane Alter von BV bei BSE-Festellung signifikant niedriger als bei anderen Rassen (61.4 vs. 68.8 Monate). BV und Tiere anderer Rassen unterschieden sich nicht hinsichtlich der Identifikations-Kategorie, die diesen Altersunterschied hätte erklären können. BV hat laut Literatur mehr Octapeptid-Repeats im Prion-Protein-Gen als andere Rassen, was eine kürzere Inkubationszeit und eine erhöhte Empfänglichkeit erklären könnte. Diese Beobachtungen legen nahe, dass BV und ihr Gewebe für weitere Studien zu genetischen Determinanten von BSE bei Rindern herangezogen werden sollte.

Schlüsselwörter: BSE, Braunvieh, Prädisposition, Bayern

Introduction

In several species, a genetic predisposition for a spongiform encephalopathy has been demonstrated, such as in certain sheep breeds for scrapie (Tranulis, 2002) or in certain humans for CJD (Raymond et al., 1997). In contrast, there have been no reports on a breed predisposition among cattle breeds for BSE and experiments did not reveal any difference between the investigated breeds with respect to BSE susceptibility. During the search for genetic determinants or correlates of BSE susceptibility in cattle, it has been obser-

ved that most animals have six and some five octapeptide repeats (OR) within the repetitive octapeptide region of the prion protein gene (Prnp) (Goldmann et al., 1991; McKenzie et al., 1992; Brown et al., 1993; Hunter et al., 1994; Neibergs et al., 1994; Premzl et al., 2000; Leone et al., 2002; Walawski and Czarnik 2003; Sander et al., 2004). An increased number of OR in the Prnp has been associated with inherited prion diseases in humans (Krasemann et al., 1995; Cochran et al., 1996). In mice, extra OR insert mutation in the Prnp resulted in a reduction of incubation time of BSE and led to an earlier detection of BSE by immunohistochemical and Western blot analyses (Castilla et al., 2004). Therefore, it could be suspected that cattle with more OR are affected more often by BSE or have shorter incubation periods. However, studies so far have not found a difference in the OR frequency between affected and non-affected cattle (Hunter et al., 1994; Neibergs et al., 1994; Sander et al., 2004).

Until now, seven OR have only been reported for the two related cattle breeds Brown Swiss (BS; Schläpfer et al., 1999) and Bruna Alpina (Leone et al., 2002), both dairy breeds primarily used for milk production. In theory, a certain proportion of Prnp 7/7, might make BS cattle either more susceptible to BSE or prone to shorter BSE incubation times. On the one hand, BS cattle have not been included in studies that compared the OR frequency of BSE-affected and non-affected cattle and on the other hand, BS cattle are traditionally kept in significant proportions in the southern part of Germany, particularly in Bavaria. The predominant cattle breed in Bavaria is German Fleckvieh, but the second most frequent breed is BS, representing about 9% of the total Bavarian cattle population (Bayerisches Staatsministerium für Landwirtschaft und Forsten, 2002). Therefore, the epidemiological data on the Bavarian BSE cases were used to compare BS BSE-cases with cases of other breeds.

Materials and Methods

Several different data sets were used to investigate potential indications for a breed disposition for BSE in Bavarian cattle. Data on the breed composition of cattle leaving the Bavarian cattle population and data on the age at slaughter of different cattle breeds in Bavaria were available from the German «HI-Tier» database («Herkunftssicherungs- und Informationssystem für Tiere», a central database for information on origin and holding place of each individual piece of cattle in Germany) provided by the Bavarian State Ministry for Agriculture and Forestry. Data on the screening category in which a BSE case was detected (regular testing or animals >24 months of age or

testing of clinically diseased or dead animals as part of the TSE [transmissible spongiform encephalopathy] monitoring program) and the age of the BSE-cases were available from the Federal Ministry of Consumer Protection, Nutrition and Agriculture. Data on feeding practices were available from a questionnaire investigation that collected information on characteristics and management practices of Bavarian farms that had not had a case of BSE (Clauss et al., ★2006★). Data on the feeding practices of BSE farms were available from the national database on BSE-animals from the Friedrich-Löffler-Institut, Wusterhausen. Data handling and data analysis were conducted in MS Access and SPSS v.12 (Microsoft, 2003; SPSS Inc., 2003) and included Chi-squared tests and Mann-Whitney U-tests (Bortz, 1999).

Results

Within the time period from November 2000 to May 2004 more than 2 Mio cattle over 24 months of age left the farms in Bavaria due to being slaughtered or due to death for other reasons. Of these animals 8.4% were BS. If breed had no influence on susceptibility to BSE, one would expect an equivalent distribution of all 121 BSE cases in Bavaria. However, BS were affected significantly more often (33 cases, or 27% of all cases) than expected by the breed distribution of the risk population ($P < 0.00001$). According to data from the German HI-Tier database, in Bavaria BS cattle are slaughtered at an average age of 5.8 years (± 0.35) but other breeds at an earlier mean age of 5.0 years (± 0.33; $P < 0.0001$, Fig. 1; all data for animals >24 months of age to exclude meat production animals).

Regarding the answers to a questionnaire on feeding practices sent out to non-BSE farms as part of the Bavarian BSE Risk Assessment Study, a higher proportion of BS calves (67.6%) received proprietary feeds than calves of other breeds (51.8%; Tab. 1). While the use of milk replacers did not differ between
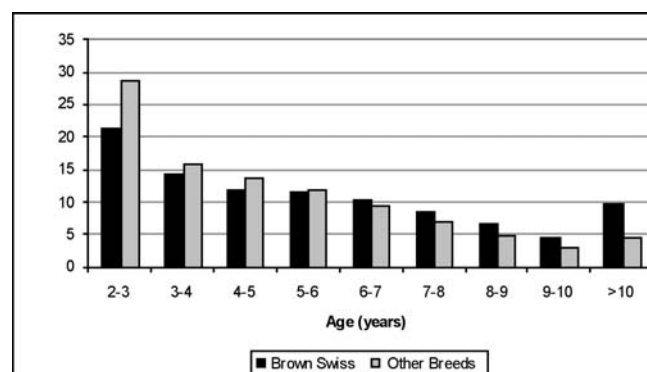

Figure 1: Comparison of slaughter age of cattle over 24 months in Brown Swiss and in other breeds in Bavaria.

Table 1: Feeding practices in Brown Swiss calves and calves of other breeds in Bavaria (data from Bavarian non-BSE farms).

	Brown Swiss (n=469)	Other breeds (n=4423)	Test
Use of milk replacer	183 (39.0%)	1656 (37.4%)	$\chi^2 = 0.46$; P = 0.50
Use of commercial concentrates	270 (57.6%)	1496 (33.8%)	$\chi^2 = 103.7$; P < 0.0001
Combinations			
Milk replaces only	47 (10.0%)	796 (18.0%)	$\chi^{2\star}$=1.21; P = 0.271
Commercial concentrates only	134 (28.6%)	637 (14.4%)	$\chi^{2\star} = 42.5$; P < 0.0001
Milk replaces and commercial concentrates	136 (29.0%)	859 (19.4%)	$\chi^{2\star} = 78.1$; P < 0.0001
Neither nor	152 (32.4%)	2131 <(48.2%)	

⋆ compared to the «neither nor» category.

Table 2: Feeding practices of Bavarian BSE cases in Brown Swiss calves and calves of other breeds. Note that data on questions are not always available from each BSE case.

	Brown Swiss	Other breeds	Test
Use of milk replacer	27 of 31 (87.1%)	55 of 64 (85.9%)	P = 0.877
Use of commercial concentrates	24 of 28 (85.7%)	61 of 70 (87.1%)	P = 0.851
Combinations	of 28	of 63	
Milk replaces only	2 (7.1%)	4 (6.3%)	P = 0.819⋆
Commercial concentrates only	2 (7.1%)	6 (9.5%)	P = 0.569⋆
Milk replaces and commercial concentrates	22 (78.7%)	50 (79.4%)	P = 0.661⋆
Neither nor	2 (7.1%)	3 (4.8%)	

⋆ compared to the «neither nor» category.

the breeds, significantly more farms keeping BS cattle fed proprietary concentrates to their calves than farms keeping other breeds. In contrast, when the feeding records of the Bavarian BSE-positive animals were compared (Tab. 2), no difference between BS and other breeds was observed. BSE-positive BS animals were significantly younger at detection than positive animals of other breeds (Fig. 2). The 33 BSE-positive BS animals had a median age of 61.2 months as compared to the median age of 68.2 months of the 88 animals from other breeds (P = 0.043). Additionally, these BS animals also had a lower median age than all 197 BSE-positive animals from the rest of Germany (70.8 months; P = 0.035).

In Germany, all cattle slaughtered more than 24 months after birth are tested for BSE. Furthermore, all animals that display clinical symptoms indicative of BSE are tested as part of the so-called TSE-monitoring, as well as those that die of unknown causes or that are slaughtered because of disease or that are cohort animals of a BSE case. Thus, five groups can be differentiated: normally slaughtered, BSE-suspected, dead, slaughtered for disease, and cohort animals. The group from which each BSE case originated is recorded by the Federal Ministry of Consumer Protection, Nutrition and Agriculture (Tab. 3). There was no significant difference in the cause of death between the BSE cases in BS cattle and in other breeds (P = 0.337).

Discussion

The results indicate a fundamental discrepancy. On the one hand, the proportion of BS animals from all cattle aged over 24 months that were slaughtered or died agrees with the general proportion of BS in the
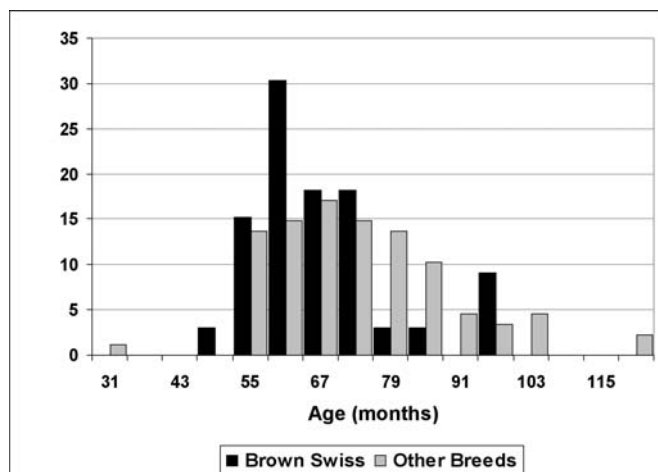
Figure 2: Comparison of the age at BSE identification in Brown Swiss cattle and cattle of other breeds in Bavaria.

Table 3: Cause of death of Bavarian BSE cases in Brown Swiss cattle and other breeds.

Cause of death	Brown Swiss (n=33)	Other breeds (n=88)
BSE-Symptoms	5 (15.2%)	9 (10.2%)
Other diseases	7 (21.2%)	22 (25.0%)
Died	10 (30.3%)	21 (23.9%)
Cohort animals	1 (3.0%)	1 (1.1%)
Normal slaughter	10 (30.3%)	35 (39.8%)

Bavarian cattle population; however, BS animals were disproportionately often affected with BSE. This finding in itself does not necessarily imply breed susceptibility but could be the result of other predisposing factors: 1) BS cattle could be slaughtered at a later age than other cattle breeds; this would allow infected animals more time to develop detectable levels of BSE and would result in a higher proportion of BSE-positive animals at testing. The fact that BS were actually slaughtered at a higher age supports this suspicion. If this consideration was of influence, then one would expect BSE-positive BS animals to have a higher average age than BSE-positive animals of other breeds. 2) BS cattle could be fed differently from other cattle breeds. If a higher proportion of BS cattle had received proprietary feedstuffs as calves, an increased BSE prevalence among BS cattle would be understandable. As proprietary feedstuffs are considered to be the most important vector for BSE (Wilesmith et al., 1992) because of an inclusion of or a contamination with animal products (Clauss and Kienzle, 2003), the difference documented in calf feeding practices between the breeds could explain the higher prevalence of BSE in the BS population, given that cattle are usually infected with BSE early in life (Anderson et al., 1996). In contrast, BSE-positive animals of BS and other breeds were exposed to a similar sequence of frequency of potential vectors – milk replacer and proprietary concentrate feeds. This leads to the prediction that while BS animals should generally be more affected by BSE due to the difference in the overall feeding practice, BSE-

positive animals of BS and other breeds should be of similar age, as they were all fed in a similar way.

These considerations would make a higher BSE prevalence in BS animals understandable if BSE-positive BS animals were of similar or older age than BSE-positive animals of other breeds. However, in contrast to this expectation, BSE-positive BS animals were younger than BSE-positive animals of other breeds. Yet, the lower age of the BS animals in comparison to BSE-animals of other breeds could be explained if a higher proportion of BS-BSE animals had been identified in the TSE-monitoring program. However, no differences in the identification rate between BS and other breeds in the TSE-monitoring program were evident. Thus, a breed predisposition, manifesting itself either in a higher susceptibility or shorter incubation times, remains as the only possible explanation. To our knowledge, this is the first report of a breed predisposition for BSE in cattle. In this respect, comparative data from Switzerland, where there is also a high proportion of BS animals in the cattle population, would be revealing. Whether this predisposition is caused by an increased proportion of 7 OR animals in the BS population or specific DNA polymorphisms, which were observed more frequently within the genomic region of the Prnp gene than in other (German) breeds, remains to be investigated. Our results suggest that BS animals and their tissues should be used in further research to identify genetic markers of BSE susceptibility. In particular, tissue from BSE-positive and control BS animals should be analysed for the number of OR present and the DNA polymorphisms.

Prédisposition raciale pour l'ESB: indices épidémiologiques chez les bovins de Bavière

On présente des données épidémiologiques provenant de Bavière qui donnent à penser que les bovins de la race brune sont plus sensibles à l'ESB que ceux d'autres races, que ce soit en terme de prévalence ou de temps d'incubation plus court. La race brune était représentée de façon disproportionnée dans les cas d'ESB (environ 9% de la population réceptive mais 27% des cas effectifs). Les animaux de la race brune présentaient un âge d'abattage plus élevé (5,8 ans contre 5 ans pour les autres races) et leurs veaux recevaient plus souvent des aliments achetés que ceux d'autres races. Il n'y avait toutefois pas de différences quant aux méthodes d'alimentation rapportées entre les animaux de la race brune positif face à l'ESB et ceux d'autres races. Ces résultats laissent supposer une prévalence plus élevée de l'ESB chez la race brune, les animaux de la race brune positifs à l'ESB devant être du même âge ou plus âgés que ceux d'autres races également positifs. A l'opposé de cela, l'âge moyen des animaux de la race brune atteints d'ESB était significativement plus faible que celui des autres races (61,4 contre 68,8 mois). Les animaux de la race brune et ceux des autres races ne se différenciaient pas quant à leur catégorie d'identification, ce qui aurait pu expliquer cette différence d'âge. Selon la littérature, la race brune présente plus de repeats d'octapetides dans les gênes de la protéine à prions que les autres races, ce qui pourrait expliquer un temps d'incubation plus cours et une plus grande sensibilité. Ces observations amènent à penser que les animaux de la race brune et leurs tissus devraient être utilisés pour d'autres études relatives aux déterminants génétiques de l'ESB.

Predisposizione di razze per la BSE: indicazioni epidemiologiche per i manzi bavaresi

Presentiamo dati epidemiologici dalla Bavaria che mostrano che manzi della razza di bovini bruna sono più sensibili alla ricezione della BSE che manzi di altre razze, tanto riguardo l'alta prevalenza quanto il tempo di incubazione inferiore. Bovini di razza bruna sono molto più rappresentati proporzionalmente (ca. il 9% della popolazione ricevente, ma il 27% dei casi effettivi). Questa razza aveva un'alta età di macellazione (5.8 anni contro 5.0 anni per altre razze) e i vitelli di razza bruna ricevevano più spesso mangime comprato che vitelli di altre razze. Non vi si è notata nessuna differenza nella prassi nutrizionale tra bovini di razza bruna BSE positivi e animali di altre razze. Questi risultati lasciano presupporre un'alta prevalenza di BSE nella razza bruna, anche se bovini di razza bruna con BSE positivo dovrebbero essere più vecchi o di pari età di animali di altre razze con BSE positivo. Al contrario l'età media alla constatazione di BSE nella razza bruna era significativamente inferiore che per le altre razze (61.4 contro. 68.8 mesi). Animali di razza bruna e animali di altre razze si differenziano non per quanto riguarda l'identificazione della categoria che avrebbe potuto spiegare questa differenza di età. I bovini di razza bruna stando alla letteratura scientifica hanno più octapeptide repeats nel gene del prione della proteina che altre razze cosa che potrebbe chiarire il breve periodo di incubazione e l'alta ricettività. Queste osservazioni dovrebbero portare a considerare più da vicino i bovini di razza bruna e i loro tessuti per altri studi sui determinanti genetici della BSE nei manzi.

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