Autoantibodies to Brain Components and Antibodies to 
Acinetobacter calcoaceticus Are Present in Bovine 
Spongiform Encephalopathy

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Bovine spongiform encephalopathy (BSE) is a recently discovered neurological disorder of cattle which was first reported in the United Kingdom after 1985, following a change in the preparation of “meat and bone meal” (MBM) feeds used especially during the winter months (1). The disorder has attracted public concern lest it be transmitted to humans following consumption of meat or other animal products (20). It has been suggested that BSE is caused by either abnormal prions (PrPSc) (11, 12) or exposure to organophosphates (13) and to these three common microorganisms (18). Since BSE was thought to be caused by consumption of MBM winter feeds, it was believed that the mucosal immunoglobulin A (IgA) isotype was more likely to show any possible differences in the titer of autoantibodies to brain components. Molecular modelling suggested three possible microbes which showed cross-reactivity, and these were tested by using a total Ig (IgG + IgA + IgM) assay in an endeavor to detect any immunological signal.

MATERIALS AND METHODS

Sera from animals with or without BSE. Sera from 29 animals (mean age, 74.4 months; range, 44 to 122 months) which were found at postmortem to satisfy the criteria of having BSE and 18 animals which did not have the disorder were supplied by the Central Veterinary Laboratory (CVL) (New Haw, Addlestone, Surrey, England), an executive agency of the Ministry of Agriculture, Fisheries and Food. The 18 animals which did not have BSE had been referred to CVL because of abnormal behavior involving ataxia and suggesting a neurological disease. Postmortem examinations were carried out to exclude BSE. The BSE and control sera (CVL) were obtained from animals raised on farms in different parts of England, each having its own water supply and belonging to separate herds. The majority of the BSE-positive animals came from dairy Friesian herds. Specifically, there was no genetic or breeder link between the various animals that had developed BSE or the controls.

Sera from animals from an organic farm. In addition, sera were obtained from an additional 58 healthy animals to act as extra controls: 30 serum samples from animals aged less than 30 months (8 Friesians and 21 Hereford-Friesian and 1

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TABLE 1. Comparison of similar sequences in bovine neurofilaments compared with A. calcoaceticus molecular sequences

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEALEK</td>
<td>Neurofilament (326–331)</td>
</tr>
<tr>
<td>REALEK</td>
<td>Mercuric reductase (24–29)</td>
</tr>
<tr>
<td>LKKVHEE</td>
<td>Neurofilament (222–228)</td>
</tr>
<tr>
<td>IEKVEE</td>
<td>RNA polymerase sigma-S4 factor (54–60)</td>
</tr>
<tr>
<td>EALEKOL</td>
<td>Neurofilament (327–333)</td>
</tr>
<tr>
<td>EALEYGL</td>
<td>Lysyl tRNA synthetase (471–477)</td>
</tr>
<tr>
<td>ELEDKON</td>
<td>Neurofilament (335–341)</td>
</tr>
<tr>
<td>ALEDKSN</td>
<td>Protocatechuate 3,4-dioxygenase (212–218)</td>
</tr>
<tr>
<td>EALEQKL</td>
<td>Neurofilament (327–333)</td>
</tr>
<tr>
<td>EAYAQKL</td>
<td>âβ-Carboxy-lys-muconate cyclomerase (218–224)</td>
</tr>
<tr>
<td>KKIVHEE</td>
<td>Neurofilament (223–228)</td>
</tr>
<tr>
<td>KKVKKKE</td>
<td>Regulatory protein (13–18)</td>
</tr>
<tr>
<td>EIRDL</td>
<td>Neurofilament (141–146)</td>
</tr>
<tr>
<td>EIRDLE</td>
<td>Secretion protein (279–284)</td>
</tr>
<tr>
<td>EQIEIRDL</td>
<td>Neurofilament (139–146)</td>
</tr>
<tr>
<td>EQIVDAR</td>
<td>Acyl coenzyme A dehydrogenase (174–181)</td>
</tr>
</tbody>
</table>

Note: Sequences retrieved from the Protein Information Resource database release 44. Identical amino acids are shown in boldface.

RESULTS

Measurement of autoantibodies to brain components. Elevated levels of IgA autoantibodies to bovine neurofilaments (Fig. 1a) and bovine myelin (Fig. 1b) were found in the 29 animals with BSE (respective mean ODs ± SEs, 0.451 ± 0.029 and 0.260 ± 0.019) when compared to 18 animals free of BSE (0.149 ± 0.009; P < 0.001) or in animals less than 30 months of age (0.149 ± 0.007; P < 0.001) (0.078 ± 0.005; P < 0.001), and 28 organically raised cows greater than 30 months of age (0.157 ± 0.006; P < 0.001) (0.078 ± 0.005; P < 0.001). Absorption of BSE sera with sonicated A. calcoaceticus reduced autoantibodies to bovine myelin and neurofilaments almost to the levels found in control sera (Table 2), although some activity to neurofilaments remained.

Measurement of antibacterial antibodies. Antibodies to A. calcoaceticus of total Ig (IgG + IgA + IgM) were significantly elevated in the sera from animals with BSE (0.99 ± 0.05) (Fig. 2a) compared to CVL controls (0.65 ± 0.06; P < 0.001) and organic farming controls, either in animals greater than 30 months of age (0.57 ± 0.03; P < 0.001) or in animals less than 30 months of age (0.53 ± 0.02; P < 0.001). There was no significant difference between the CVL controls and the organic farming controls aged more than 30 months, but there was a small, statistically significant difference when compared with the sera from animals aged less than 30 months (P < 0.05). However, there was no significant difference in the level of anti-A. calcoaceticus antibodies between organic farming animals aged more than 30 months when these animals were compared to those aged less than 30 months. There was no significant difference between the BSE sera and the three control groups in the levels of either anti-A. tumefaciens (Fig. 2b) or anti-E. coli antibodies (Fig. 2c).

Measurement of serial dilutions. ELISA estimations of three BSE serum samples which had high, medium, and low respective reactivities to the respective antigens bovine neurofilaments, bovine myelin, and A. calcoaceticus.

DISCUSSION

Elevated levels of autoantibodies to bovine neurofilaments and myelin, as well as elevated levels of specific antibodies to A. calcoaceticus, have been shown to be present in BSE-affected cattle when compared to three different groups of controls, whilst no such elevations have been seen against either E. coli.
coli or *A. tumefaciens*. This is clearly a specific observation, since the other two species of microorganisms tested did not show such elevations in their antibody levels. The agent responsible for the production of these specific autoantibodies is unclear, but it would seem that BSE cattle have been exposed to *A. calcoaceticus*. Whether this implies a link to the neurological features of the disease remains to be determined. This interesting observation requires confirmation with a larger sample of sera from animals with BSE selected from different parts of the United Kingdom and with the analysis carried out with different species of *Acinetobacter*. Furthermore, such sera should be tested against other bacteria commonly present in the bowel flora, as well as against peptides derived from the cross-reacting sequences resembling bovine neurofilaments, myelin, and other brain tissues.

*A. calcoaceticus* is a species of saprophytic and aerobic gram-negative bacteria that is widely distributed in soil and water supplies, but can also be cultured from skin, mucous membranes, and body secretions from both animals and humans. It is relevant to note that *A. tumefaciens* antibodies are not elevated in animals with BSE. This microbe does not have glutamic acid in the cross-reacting epitope when compared to either *Acinetobacter* or bovine myelin (4), and furthermore, it is a plant pathogen of small trees and shrubs, which makes it unlikely that grass-eating animals like cows would have been exposed to it.

One clear result from these studies is that in at least one TSE disease, namely BSE, specific immune responses predominantly involving IgA, suggesting antigenic exposure across a mucosal surface such as the gut, can be demonstrated against a microbe that is found readily in the environment of cattle and which also happens to possess molecular sequences resembling bovine neurofilaments and myelin. Determinations of whether this microbe was introduced into the food chain of cattle following changes in the preparation of winter feeds or has any pathological significance in the development of BSE await further studies.

Autoantibodies to neuronal components have previously been reported in TSEs, especially in patients with kuru and Creutzfeldt-Jakob disease (15) and in animals with natural scrapie (2). The pathological significance of these autoantibodies remains unclear, but there are three human autoimmune diseases in which molecular mimicry occurs between bacterial antigens and self tissues: rheumatic fever (*Streptococcus pyog-
Rheumatic fever is the classic model of an autoimmune disease caused by an infection. A bacterial infection of the tonsils by *S. pyogenes* evokes the formation of antibodies which bind to heart tissue, resulting in acute rheumatic fever, because there is molecular mimicry or similarity between cardiac tissues and streptococcal antigens. Furthermore, antistreptococcal an-

gens) (9), rheumatoid arthritis (*Proteus mirabilis*) (18, 21), and ankylosing spondylitis (*Klebsiella*) (3, 6).

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FIG. 2. Total antibody titers (mean ± SE) of high-, medium-, and low-reactivity BSE sera against bovine neurofilaments (a), bovine myelin (b), and *A. calcoaceticus* (c).

FIG. 3. Serial doubling dilutions (mean ± SE) of high-, medium-, and low-reactivity BSE sera against bovine neurofilaments (a), bovine myelin (b), and *A. calcoaceticus* (c).
tibodies can also bind to the basal ganglia of the brain, thereby evoking abnormal gait movements, and this is known as rheumatic fever. Chorea or Sydenham’s chorea (8). Injection of antistreptococcal antibodies into rabbits will produce abnormal neurological features of disordered gait and postmortem elution from the rabbit basal ganglia will lead to recovery of an antibody with specificity for streptococcal antigens.

A similar neurological disorder could occur in cattle with BSE following the production of anti- A. calcoaceticus antibodies, since this microbe possesses antigens resembling brain tissue. Another possibility is that these anti- A. calcoaceticus antibodies appeared following damage to brain tissues by prions, a situation that frequently occurs in patients with burns who develop antiskin antibodies or following a myocardial infarction, when antinecrotic autoantibodies can be detected. A third possibility is that direct infection of brain tissues could occur, similar to the recent observation that Chlamydia microbes can be isolated from the cerebrospinal fluid of patients with multiple sclerosis (16). Further studies are required to determine whether anti- A. calcoaceticus antibodies exhibit cytotoxic responses against neurons, involving complement activation and NK cells, and to assess the possible relationships between normal (PrPc) and abnormal (PrPSc) prions, A. calcoaceticus, and brain autoantibodies in BSE. The mechanism responsible for these serological observations remains unclear, but at least these results confirm and extend the observations of Gajdusek’s group that autoantibodies to brain components are present in TSEs.

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REFERENCES


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