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Experimental Bovine Spongiform Encephalopathy: Detection of PrP^{Sc} in the Small Intestine Relative to Exposure Dose and Age

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Summary

European regulations for the control of bovine spongiform encephalopathy (BSE) decree destruction of the intestines from slaughtered cattle, therefore producers have been obliged to import beef casings from countries with a negligible BSE risk. This study applies immunohistochemical and biochemical approaches to investigate the occurrence and distribution of disease-associated prion protein (PrP^{Sc}) in the duodenum, jejunum and ileum of cattle orally exposed to a 1 g or 100 g dose of a titrated BSE brainstem homogenate. Samples were derived from animals at various times post exposure. Lymphoid follicles were counted and the frequency of affected follicles recorded. No PrP^{Sc} was detected in the duodenum or jejunum of animals exposed to a 1 g dose or in the duodenum of animals receiving a 100 g dose. PrP^{Sc} was detected in the lymphoid tissue of the ileum of 1/98 (1.0%) animals receiving the 1 g dose and in the jejunum and ileum of 8/58 (13.8%) and 45/99 (45.5%), respectively, of animals receiving the 100 g dose. The frequency of PrP^{Sc}- positive follicles was less than 1.5% per case and biochemical tests appeared less sensitive than immunohistochemistry. The probability of detecting lymphoid follicles in the ileum declined with age and for the 100 g exposure the proportion of positive follicles increased, while the proportion of positive animals decreased with age. Detection of PrP^{Sc} in intestinal neural tissue was rare. The results suggest that the jejunum and duodenum of BSE-infected cattle contain considerably less BSE infectivity than the ileum, irrespective of exposure dose. In animals receiving the low exposure dose, as in most natural cases of BSE, the rarity of PrP^{Sc} detection compared with high-dose exposure, suggests a very low BSE risk from food products containing the jejunum and duodenum of cattle slaughtered for human consumption.

Keywords: bovine spongiform encephalopathy; immunohistochemistry; PrP^{Sc}; small intestine

Introduction

Bovine spongiform encephalopathy (BSE) is a zoonotic transmissible spongiform encephalopathy (TSE) or prion disease of domestic cattle. It was initially diagnosed in the UK in 1986 (Wells *et al.*, 1987) and subsequently occurred throughout Europe (Ducrot *et al.*, 2008), in Japan (Kimura *et al.*, 2002) and in North America (Richt *et al.*, 2007). The occurrence of a variant phenotype of the human TSE, Creutzfeldt–Jakob Disease (CJD) (vCJD), 10 years after the recognition of BSE is considered to have resulted from the consumption of BSE-contaminated beef products (Will *et al.*, 1996, 1998; Cousens *et al.*, 1997).

Regulations for the protection of human and animal food chains within the European Union (EU)

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from contamination with the agents of domesticated ruminant TSEs (BSE and scrapie of sheep and goats) require the removal at slaughter of certain tissues from cattle, sheep and goats. These tissues, the specified risk materials (SRM), include, in cattle of all ages, the intestines from the duodenum to the rectum (European Commission Scientific Steering Committee, 1997; 2000; European Commission, 2001). The position of the World Organization for Animal Health (OIE) differs in that it recommends that in countries classified as having a controlled or undetermined BSE risk, the ileum, but not the rest of the intestine, must be discarded at slaughter (OIE, 2010).

The major use of small intestines from pigs, sheep and cattle is for the production of edible natural casings for sausages and a large variety of casings with specific qualities and calibres are required. From cattle, the duodenum and jejunum, but not the ileum, are used for casing production (Wijnker *et al.*, 2008). Unlike sheep and pig casings, which, after processing, consist essentially only of submucosa, beef casings are comprised of submucosa, muscularis propria and serosa. While beef casings are consequently thicker and practically unpalatable, consumption cannot be excluded and therefore the SRM regulations have, since their inception, prevented the production of beef casings in Europe. Thus, beef casings for use in the EU are allowed only as imports from countries with a negligible BSE risk (European Commission, 2001).

Studies of infectivity and/or the disease specific form of the prion protein (PrP^{Sc}) in intestinal tissues of BSE-infected animals have been limited, but have indicated a restricted degree of lymphoid tissue involvement relative to the widespread and high incidence of involvement of such tissues in scrapie (Wells *et al.*, 1994, 1996, 1998, 2005; Terry *et al.*, 2003; Espinosa *et al.*, 2007; Hoffmann *et al.*, 2007; Arnold et al., 2009). After experimental exposure of calves to 100 g of BSE-affected brain tissue, but not in natural cases, PrP^{Sc} has been demonstrated in the Peyer's patches (PPs) of the ileum (Terry *et al.*, 2003; Hoffmann *et al.*, 2007). Involvement of the ileum throughout much of the course of the disease in this experimental model is also borne out by wild type and transgenic (Tg) mouse bioassays and cattle bioassays (Wells *et al.*, 1994, 1996, 1998, 2005; Espinosa *et al.*, 2007; Arnold *et al.*, 2009). Information on naturally-infected cattle is more sparse: infectivity has been confirmed in the ileum of a clinically-affected cow when assayed in Tg mice overexpressing the bovine prion protein gene (Buschmann and Groschup, 2005) and PrP^{Sc} has been detected inconsistently in the myenteric plexus, but not in gut-associated lymphoid tissue (GALT) of the ileum, in small numbers of natural cases of BSE (Iwata *et al.*, 2006; Terry *et al.*, 2003). Regions of the small intestine other than the ileum have rarely been examined for PrP^{Sc}, but in these studies the results have largely been negative in natural (Iwata *et al.*, 2006) and experimental (Wells *et al.*, 1998; Terry *et al.*, 2003; Hoffmann *et al.*, 2007) BSE. The exception is a single case of BSE in Japan (Kimura and Haritani, 2008) in which equivocal immunohistochemical reactivity was reported in Schwann cells of the myenteric plexus of the duodenum, jejunum and ileum.

The aim of the present study was to investigate the occurrence and topography of the accumulation of PrP^{Sc} in the duodenum, jejunum and ileum of experimental cattle that were exposed orally (receiving either 1 g or 100 g of titrated BSE-infected brainstem) and killed sequentially. Data on animals infected with the low oral dose (1 g) are considered to be particularly informative of the responses anticipated after natural exposure to the BSE agent (Arnold *et al.*, 2007), where single oral exposures of the order of 0.1–1.0 g are consistent with the range of mean incubation period range (5–5.5 years) calculated from studies of the BSE epidemic in the UK (Wells *et al.*, 2007).

Materials and Methods

Experimental Design

Tissues for this study were derived from a previously reported experimental, oral exposure, sequential kill study of BSE pathogenesis (Arnold *et al.*, 2007). In that study, 300 calves born in 1998 were sourced from farms in Great Britain (GB) with no prior history of BSE. Two-hundred calves were dosed at 4–6 months of age; 100 calves received 1 g and 100 calves received 100 g of a pool of titrated BSE brainstem homogenate ($10^{3.1}$ RIII mouse intracerebral + intraperitoneal ID_{50}/g), derived from 254 cases of BSE sourced in GB in 1996 and 1997. The remaining 100 calves served as age-matched, undosed controls. Clinical monitoring of cattle throughout the study was conducted to detect the onset of disease. Six exposed and three control cattle were selected randomly and killed at 3-monthly intervals after exposure, increasing to 6-monthly intervals after the first year post exposure in the case of the 1 g dose group. For the present study, the duodenum, jejunum and ileum were sampled from cattle at selected sequential timed-cull points (Table 1). The approach was based on knowledge gained from previous studies (Terry *et al.*, 2003; Wells *et al.*, 2005; Espinosa *et al.*, 2007;