PRESENCE OF CENTRAL NERVOUS SYSTEM (CNS) TISSUE IN ADVANCED MEAT RECOVERY (AMR) PRODUCTS

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INTRODUCTION

The consumption of Central Nervous System (CNS) tissue in beef from cattle with Bovine Spongiform Encephalopathy (BSE) is the commonly accepted cause of new variant Creutzfeldt Jakob Disease (nvCJD), the human equivalent of BSE in cattle. Chemical tests have identified the presence of the BSE agent in the brain, spinal cord, retina and nervous tissue located near the backbone and bone marrow of cattle experimentally infected with this agent by the oral route. There is strong epidemiological and laboratory evidence for a causal association between nvCJD and BSE. The absence of confirmed cases of nvCJD in other geographic areas free of BSE supports a causal association.

On March 3, 2000, the European Commission published the following proposal; "In a renewed effort to set out harmonized rules to ensure that tissues likely to be infected with BSE are kept out of the animal and human food chain, the European Commission has endorsed a proposal for a decision to regulate the use of specific risk materials (SRM) presenting a BSE risk. Removal of SRM is the single biggest contribution that can be made to reducing the risk to humans from BSE, and thereby reducing the possibility of human infection by variant Creutzfeldt Jakob Disease (vCJD). All member states would be obliged to remove the skull (including brains and eyes), the tonsils and the spinal cord of cattle, sheep and goats. The proposal also prohibits the use of certain slaughtering techniques which entail a risk of contamination of animal blood by the release of BSE infected brain tissue into the blood stream".

The presence of brain or spinal cord as an inadvertent contaminant of meat may result from the stunning of livestock or the preparation of advanced meat recovery (AMR) products from the vertebral column. In light of current consumer concern about bovine spongiform encephalitis (BSE), a disease believed to be transmitted by consumption of Central Nervous System (CNS) tissue, a reliable analytical test for CNS tissue in meat products is essential to ensure consumer confidence and allay consumer fears of BSE in meat products. The continued effort to eliminate CNS tissue from the food supply, even in the absence of any known BSE cases in the USA, is a prudent approach to protecting the markets and consumers of American beef.

A method to detect the presence of CNS tissue in meat products has been developed by a group of researchers. This method uses an enzyme-linked immunosorbent assay (ELISA) to detect an antigen called glial fibrillary acidic protein (GFAP) which is generally restricted to cells in the CNS. This chemical analysis provides a method to detect small amounts of CNS tissue in meat products which is simple, cost-effective and efficient. The assay is capable of detecting one nanogram (10^{-9}g) of the specific antigen and can be used on product samples that have been stored at 4°C for up to 8 days.

OBJECTIVES

The purpose of this beef checkoff funded research was to continue validation of the CNS detection method and increase the sensitivity through the use of fluorescent labels. The project included a survey of ground beef patties destined for restaurants for the presence of CNS tissue and an evaluation of methods to remove spinal cord from beef neck bones prior to processing in advanced meat recovery equipment. In addition, a survey of products from several different AMR production facilities to determine the incidence and sources of CNS tissue in AMR products was conducted.

PROJECT OUTLINE

Advanced meat recovery (AMR) products from six beef processing plants around the United States were used for this study, as well as modified (spinal cord channel removed) AMR samples. Samples included 50 separate samples from the machinery and recovery systems from the plants obtained over a one hour period. Modified AMR samples were obtained from the start of the production shift from clean machinery, and again later in the production shift. Both samples were originally 30 lbs, and were then analyzed as 50 sub-samples for GFAP. Ground beef patty samples were obtained from seven major suppliers of large fast food chains from diverse geographic locations throughout the U.S. Ten different ground beef patties were obtained per plant, which were then sampled raw and analyzed for GFAP.

RESULTS

As the initial data for this project was being collected, it was clear that the major problem with contamination with CNS tissue occured during the AMR processing of beef neck bones. Well over 50% of the samples of AMR tissue from beef neck bones had detectable to high levels (>5 ng/mg) of CNS tissue as determined by the GFAP/ELISA method. Neck bones are processed through AMR equipment in many plants. This process yields considerable weight of lean meat from a beef carcass with minimal hand labor. All plants in this study were processing beef chuck neck bones with tandom piston pressure recovery systems followed by drum type mechanical desinewers.

Table 1 presents the GFAP levels in AMR product produced from beef chuck vertebrae collected at 6 fed cattle plants. AMR product was re-sampled from plant A from chuck bones which were intact (AA) or had been modified (AA-Modified AMR) by eliminating the cervical vertebrae and sawing the chine and associated spinal canal from the first 5 thoracic vertebrae. It is clear that removal of the spinal canal and remaining spinal cord remnants greatly reduced the level of GFAP in the resulting AMR product from 0.61 ng/mg (Plant AA) to 0.015 ng/mg (Modified AMR-AA).

The GFAP levels in ground beef patties from 70 commercial production lots at 7 different plants located throughout the USA are also shown in Table 1. All but one value was less than 0.1 ng/mg.

Spinal cord contains the most GFAP antigen on a fresh weight basis, followed by brain tissue. As peripheral nerves (e.g. sciatic nerve) contain approximately 0.50% of the GFAP present in spinal cord, a great deal of peripheral nerve (>1%) would have to be present to invoke a false positive in meat products. Cervical ganglia, bone meal, AMR and ground beef contain very low levels of detectable GFAP. All ground beef and many AMR samples contain <1 ng/mg wet weight, equivalent to 0.05% of spinal cord GFAP.

Less than 1.0 ng GFAP/mg tissue was found on most beef sub-primals, advanced meat recovery (AMR) product and in all ground beef patties (Tables 1&2). This represents only 0.05% of the GFAP found in spinal cord, so the meat products sampled in this study contained only trace amounts of neural tissue contamination.

The presence of normal sausage ingredients or heating the product to 180°F for 60 minutes did not affect the detection of GFAP in meat products spiked with spinal cord (Tables 3&4). The GFAP ELISA test was found to be effective on cooked meat and sausage, but not on canned meat and sausage.

TABLE 1

GFAP levels in advanced meat recovery (AMR) product prepared from unmodified and modified beef chuck vertebrae collected at 7 fed cattle slaughter plants and in ground beef from 7 plants.*

Plant		GFAP ng/mg tissue
		Mean
AMR		
	A	1.308**
	B	2.461
	D	0.433
	F	0.238
	J	0.067
	K	0.692
	AA	0.610
Modified AMR		
	AA	0.015
Ground Beef		
	A	0.015***
	B	0.029
	C	0.025
	D	0.025
	E	0.049
	F	0.121
	G	0.000
Mean, All Patties		0.037

**Glial fibrillary acidic protein (ng/mg tissue).*
***Least square means of plant site (n=50 samples).*
****Least square means of plant site (n=10 samples).*

TABLE 2

GFAP levels on cut surfaces of beef sub-primals, in bone saw dust and heart blood clots.

Carcass Site/Sample	Mean
MEDIAL NECK	0.541*
VENTRAL NECK	0.390
RIBEYE	0.140
MEDIAL RIB	0.495
MEDIAL LUMBAR	0.227
DORSAL TOP BUTT	0.478
MEDIAL TOP BUTT	0.306
SAGITTAL INSIDE ROUND	0.264
SAGITTAL OUTSIDE ROUND	1.464
MEDIAL CHUCK	1.482
MEDIAL BRISKET	0.349
SAGITTAL CLOD	0.135
BONE SAW DUST	1.349
HEART BLOOD CLOT	0.718

**GFAP ng/mg tissue, average of 30 samples (3 from each of 10 plants).*

TABLE 3

GFAP levels detected in raw or cooked ground chuck and sausage with 0 - 1% added spinal cord.

	0		0.2		0.4		0.6		0.8		1.0	
Spices*	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
Temp °F												
Raw	0.60**	0.02	4.8	3.95	12.10	8.75	10.65	9.90	21.78	20.30	25.85	19.80
140	0.08	0.03	6.03	3.43	10.25	9.70	22.88	8.43	29.80	25.85	31.25	17.13
150	0.00	0.00	2.85	2.68	5.23	9.28	7.43	13.10	16.95	16.13	11.95	21.60
160	0.14	0.07	1.98	4.50	5.00	6.10	11.13	12.63	19.05	20.23	17.50	22.25
170	0.10	0.05	1.13	2.50	6.85	4.30	8.68	15.78	19.80	15.23	22.00	14.83
180	0.04	0.00	4.28	5.80	12.55	6.40	17.38	22.83	14.73	12.63	23.75	18.63
Canned***	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.80	0.00	0.00	0.00	0.00

**(-) = ground chuck with no additives*
***GFAP ng/mg tissue average of two replicates*
(+) = ground chuck with added water, cure and other sausage indgredients
**** Product heated at 240°F for 100 minutes*

TABLE 4

GFAP levels detected in raw or cooked advanced meat recovery (AMR) products and AMR sausage with 0 - 1% added spinal cord.

	0		0.2		0.4		0.6		0.8		1.0	
Spices*	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
Temp °F												
Raw	0.38**	0.30	7.78	3.28	11.30	5.33	17.38	6.80	19.05	11.05	32.25	17.30
140	1.28	0.59	13.28	8.25	20.70	8.38	27.13	20.50	37.15	19.08	38.90	19.28
150	1.82	0.48	3.88	1.83	12.28	5.08	14.90	14.25	30.75	22.43	22.28	15.10
160	0.86	0.52	4.48	4.65	8.03	5.40	18.88	7.73	22.28	11.10	34.25	21.98
170	1.47	1.09	6.60	4.15	5.88	16.33	11.85	11.70	30.33	21.23	34.50	24.48
180	1.19	0.97	7.90	2.43	11.60	7.10	19.68	13.55	28.93	15.85	22.75	15.65
Canned***	0.38	0.00	0.60	1.70	1.55	0.15	2.40	0.75	2.90	0.00	3.80	2.35

**(-) = ground AMR product with no additives*
*** GFAP ng/mg tissue, average of two replicates*
(+) = ground AMR product with added water, cure and other sausage ingredients
**** Product heated at 240°F for 100 minutes*

The safety of beef products must be a continual focal point for the entire industry so that consumer confidence is maintained and improved. The ability to detect CNS tissue in beef products will enable the U.S. beef industry to assure consumers that there is little risk of contracting nvCJD from eating beef products that are monitored for the presence of CNS tissue.

POSSIBLE APPLICATIONS

The implementation of Good Manufacturing Practices for spinal cord removal in all plants will play an important role in minimizing the presence of spinal cord in AMR products. Analytical methods can be used to detect CNS tissue in AMR products and to verify that spinal cord removal procedures have been properly implemented.

ADDITIONAL RESEARCH NEEDS

The further development of this test to assure accuracy, ease of analysis and rapid testing would be helpful to the beef industry.

PUBLICATIONS

Schmidt, G.R., R.S. Yemm, K.D. Childs, J.P. O'Callaghan and K.L. Hossner. 2001. The presence of central nervous system tissue on beef carcasses and in comminuted beef. Journal of Food Protection 64: 2047-2052.

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