Poster exhibition parallel session 5: Assessing and managing risk

PE5.01 Study on presence /absence of central nervous tissues in meat products 12.00

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In this study using commercially available Ridascreen® Risk material ELISA kit, evaluate the efficiency of removing BSE specific risk material from meat products sold in Obihiro Japan. Further more, efficiency of using GFAP as a marker protein for detection of central nervous tissues (CNS) in ageing meat were evaluated using ELISA kit and western blotting techniques. Total of 84 meat product samples were tested. All the tested samples absorbance values were less than the minimum detection limit of the kit. To evaluate the effect of ageing porcine meat obtained from the day of the slaughter were mixed with 0.5% (W/W) porcine spinal cord (SC) tissues and allowed to age at 4ºC. Absorption values obtained from SC contaminated meat after ageing for 0, 4 and 7 days shows significant reduction (P>0.05) of GFAP with the ageing time. However, after 7 days of ageing clear detection of GFAP was possible with Ridascreen ELISA kit. Thus, it concluded, mixing of CNS tissues with meat products was found to be completely absence or less than the minimum detection level of Ridascreen® risk material kit in Obihiro, Japan.

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Terms- BSE, CNS tissue, GFAP, Meat products

I. INTRODUCTION

Variant Creutzfeldt-Jakob disease (vCJD) in humans is most likely transmitted by consumption of infectious tissues from animals with Bovine Spongiform Encephalopathy (BSE) (Hill et al., 1997; Will et al., 1996). Tissues of central nervous system (CNS) such as brain and spinal cord (SC) which are classified as specified risk material (SRM) are regarded to be main source of infection and carry up to 95% of the total infectivity (SSC,1999). As a consequence, the presence of CNS tissue in beef and beef products is banned in many countries such as USA, Canada, Japan and the countries of EU. In Japan, current practices in SRM elimination whether guarantees the total food safety or not is still a debating question among the consumers. Thus, qualitative study to determine the presence of CNS tissue in meat and meat products in various food outlets of Japan may lead to enhance the consumer confidence in food safety. In our previous study (Barana et al., 2009) conducted in late 2006 reported that total absence of CNS material or presence was less than the minimum detection level (0.1% CNS) of the Ridascreen® Risk material kit in Obihiro Japan. Ridascreen® Risk material kit (R-biopharm, Germany) is a commercially available test kit, which uses the sandwich enzyme immunoassay for the quantitative analysis of CNS in meat products, ensues by the determination of glial fibrillary acidic protein (GFAP) as a marker for CNS. Since BSE is a very sensitive issue concerning food safety aspects, continuous surveillance on implementing strict controlling regulation to avoid the invasion of SRM material in to human food chain is necessary. In the present study we analyzed samples of commercial meat products sold through out Obihiro area in Hokkaido Japan during December 2008 using Ridascreen® Risk material kit. In the case of evaluating ageing meat which is potentially contaminated with CNS tissue may have the potential of reducing the presence of GFAP due to the proteolytic activity during the ageing thus, experimentally contaminated meat particles with SC tissues were evaluated for the presence of marker protein GFAP using the Ridascreen® Risk material kit and the western blot method after ageing.

II. MATERIALS AND METHODS

Meat products analysis for CNS material

A total of 84 meat product samples bought from different supermarkets throughout the Obihiro, Japan during the first week of December were simultaneously tested for the presence of CNS tissue using Ridascreen® Risk material kit. Information related to the source of the each meat sample was obtained using the Japans beef traceability program. The processed (sausages, salami) and raw (ground meat/ meat ball) meat products, which are consumed commonly in Japan, were included in this study. Extraction and test steps were performed as described by the Ridascreen® Risk material kit producer (Ridascreen® risk material test, Art No.: 6701) and absorbance was obtained at 450nm using microplate reader.

Meat samples contaminated by SC tissues

As seen in the table 1, absorbance values obtained at different concentrations of bovine and porcine SC tissues using Ridascreen® risk material kit were not significantly different ($P<0.05$). Therefore, in the current study, artificially contaminated porcine SC tissues were used instead of bovine tissues to avoid the practical difficulties in using bovine materials. Fresh non-cured left pork thighs were obtained on the day of slaughter and the semi-tendineous muscle was removed from thigh for the study. Meat was ground and portioned in to units of 50 g and pre-determined amounts of freeze dried SC tissues of porcine were individually added to the meat portions and homogenized to yield a final concentrations of 0.5% (w/w) SC tissues in meat. Then experimentally contaminated meat was divided in to three groups and two of them were vacuum packed and stored in a refrigerator at 4ºC for ageing for 4 days and 7 days, respectively. The other group of meat sample was analyzed by Ridascreen® Risk material 10/5 kit ELISA test and western blotting without ageing. After ageing for 4 and 7 days, refrigerated two meat samples were analyzed using the same techniques.

SDS-PAGE and western blotting

SDS-PAGE was performed on 15 % Tris-glycine slab gel with a 6% stacking gel (Laemmli, 1970) for the prepared protein samples. The gel was run at 20 mA, until the dye track reached the end of the gels and stained with 0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid. Proteins were transferred from the slab gel to a nitrocellulose membrane by a buffer-transfer method (Towbin et al., 1970; Negishi et al., 1996). Membrane was incubated in phosphate-buffered saline (PBS, pH 7.4) containing 10% (w/v) skim milk overnight at room temperature, and washed three times in PBS for 5 min at room temperature. Using vector stain ABC kit membranes were stained for GFAP.

First, membranes were incubated with mouse GFAP monoclonal antiserum (Clone name: GA5) 40 min at 37°C, and washed three times in PBS. Then they were again incubated with biotinylated anti-mouse secondary antibody for 30 min at 37°C, and followed by three washes in PBS for 5 min each.

Finally, membranes were incubated with avidin/biotinylated horseradish peroxidase and followed by detection with 3, 3′-diaminobenzidene (DAB) as a chromogen after washed 3 times with PBS for 5 min each. Each analysis was done at least in duplicate.

Statistics

Data are presented as the mean and standard deviation for absorbance values obtained in ELISA test. The significance of differences among the absorbance values were analyzed using Duncan`s multiple range test (SAS Institute, USA). Differences were considered significant at $p<0.05$.

III. RESULTS AND DISCUSSION

According to the R-biopharm Ridascreen® Risk material kit instruction manual the lowest detection limit is $\leq$ 0.2% of CNS tissue. However, the manufactures have stated that any absorption value, which is 2-fold higher than absorbance of standard 1 (0%), provided with the kit, can be valued as positive results (Ridascreen® Risk material, article no. R6701, 2005). According to that we determined our positive cutoff value and all the absorbance values of analyzed meat samples were less than that cut off value. This was in line with our previous findings (Barana et al., 2009) for samples obtained from the same area in Japan. Further in previous

study we determined the minimum detection limit for both brain and SC tissues in minced meat using the same kit (Barana et al., 2009) and reported that kit detected both brain and SC tissues at 0.1%, below its claimed sensitivity level, for brain and SC combined.

Meat supplies to the Obihiro city mainly come from Hokkaido region. Hokkaido is the nation's number one beef-producing region and which is home to the most of the identified cases of BSE in Japan. Thus, continuous surveillance in this area may greatly contribute to the consumer confidence in safety of the meat. Yeşilbağ & Kalkan (2005) as well as Kale et al., (2007) showed that mixing of CNS tissues to the meat products still happens in different parts of Turkey.

This was further accompanied by previous studies conducted in Germany a few years ago (Lucker et al., 2001; Agazzi et al., 2002), while no previous literature was available on mixing of CNS tissues to meat products in Japan. Absorbance values at the 450nm obtained from Ridascreen® Risk material kit 10/5 kit ELISA test were significantly ($p>0.05$) lower from 0 days of ageing to 7 days of ageing (Figure1). Thus, it indicates the reduction of GFAP in ground meat. This was confirmed by the western blotting results (Figure 2). This reduction in the presence of GFAP in the meat can attribute mainly to the proteolytic systems in muscle tissues, which are active in the meat during the post-mortem ageing (Sentandreu et al., 2002). Therefore, not only the GFAP also the other marker proteins, such as NSE (Lucker et al., 1999), and proteolipid protein (Sandmeier et al., 2006) which are using as a CNS tissue marker may vulnerable to the reduction during ageing of meat. However, even after the 7 days of ageing ELISA kit was able to detect the clear evidence of presence of GFAP in experimentally contaminated meat even though the contaminated concentration of CNS material is very low (0.5%).

Most of the beef offered for sale as retail cuts at supermarkets is aged from 5 to 7 days. Therefore, the existing effect of ageing on the protein marker GFAP not completely diminished the effect of using this kit as a tool to determine the presence of CNS material in meat products during this study.

IV. CONCLUSION

According to the results from present study, mixing of CNS tissues with meat/meat products was found to be completely absence or less than the minimum detection level of Ridascreen® Risk material kit in Obihiro, Japan. Evaluation the effect of ageing of meat on the CNS marker protein GFAP revealed the risk of possible under estimation of the presence of CNS on the meat products when analyzed after the period of ageing. Therefore, further studies on finding a suitable non –protein marker for CNS material need to be enhanced.

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REFERENCES

[1] Hill, A.F., Desbruslais, M., Joiner, S., Sidle, K. C. L., Gowland, I., Collinge, J., Doey, L. J., & Lantos, P. (1997). The same prion strain causes vCJD, and BSE. Nature, 389, 448-450.

[2] Will, R.G., Ironside, J.W., Zeidler, M., Cousens, S.N., Estibeiro, K., Alperovitch, A., Poser, S., Pocchiari, M., & Hofman, A. (1996). A new variant of creutzfeldt-Jakob disease in the UK. Lancet, 347, 921-925.

[3] SSC (Scientific steering committee), European Commission. (1999) Opinion on the human exposure risk (HER) via food with respect to BSE. Available : http://ec.europa.eu/food/fs/sc/ssc/out67_en.html

[4] Jayawardana, B.C., Shimada, K., Fukushima, M., & Sekikawa, M. (2009). Study on presence/absence of central nervous tissues as BSE specified risk material in processed and raw meat products in Japan. Food Control, 20 (3), 187-190.

[5] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

[6] Towbin, H., Staehelin, T., & Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Science USA, 76, 4350-4354.

[7] Negishi, H., Yamamoto, E., & Kuwata, T., (1996). The origin of the 30kDa component appearing during post-mortem ageing of bovine muscle. Meat Science, 42, 289-303.

[8] Ridascreen® risk material, article no. R6701. (2005). R-biopharm, Germany.

[9] Ye şilbağ, K., & Kalkan, A. (2005). Detection of central nervous system tissues as BSE specified risk material in meat products in Turkey. Food Control, 16 (1), 1-13.

[10] Kale, M., Kursan O., & Pehlivanoglu, F. (2007). Detection of central nervous system tissues as bovine spongiform encephalopathy specified risk material in processed and raw meat products in Turkey. Journal of Food Safety, 27, 56-65.

[11] Lucker, E., Horlacher, S., & Eigenbrodt E. (2001). Brain in human nutrition and variant Creutzfeldt-Jakob disease risk (vCJD): Detection of brain in retail liver sausages using cholesterol and neuron specific enolase (NSE) as markers. British Journal of Nutrition, 86, 115-119.

[12] Agazzi, M. E., Barrero-Moreno, J. M., Lucker, E., Von Holst, C., & Anklam, E. (2002). Performance comparison of two analytical methods for the detection of tissues of the central nervous system in sausages: Results of the inter-laboratory study. European Food Research and Technology, 215, 334-339.

[13] Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. (2002). Trends in Food Science & Technology, 13, 400-421.

[14] Lucker, E., Eigenbrodt, E., Wenisch, S., Failing, K., Leiser, R., & Bullet, M. (1999). Development of an integrated procedure for the detection of central nervous tissue in meat products using cholesterol and neuron specific enolase as markers. Journal of Food Protection, 63, 258-263.

[15] Sandmeier, B., Bauerlein, R., Villmann, C., Düthorn, T., Gareis, M., Becker, C. M., & Pischetsrieder, M. (2007). Detection of central nervous system tissue in meat and meat products with a newly developed immunoassay selective for myelin proteolipid protein. Food chemistry,105, 871-878.

Table 1: The summery of mean absorption values obtained by Ridascreen® risk material kit at various percentages of bovine and porcine spinal cord tissues (SC). No significance difference ($p<0.05$) between mean absorption values at same tissue concentration of SC.

Tissue %	SC Tissue	
	Bovine	Porcine
0	0.111 (0.028)	0.128 (0.014)
0.01	0.222 (0.033)	0.190 (0.009)
0.025	0.466 (0.090)	0.402 (0.065)
0.05	0.731 (0.063)	0.625 (0.064)
0.1	0.1423 (0.063)	0.1281 (0.116)
0.2	2.690 (0.488)	2.138 (0.105)

[a]Bovine (n=4) and porcine (n=6)Mean absorption value

[b]Standard deviation values of means in parenthesis

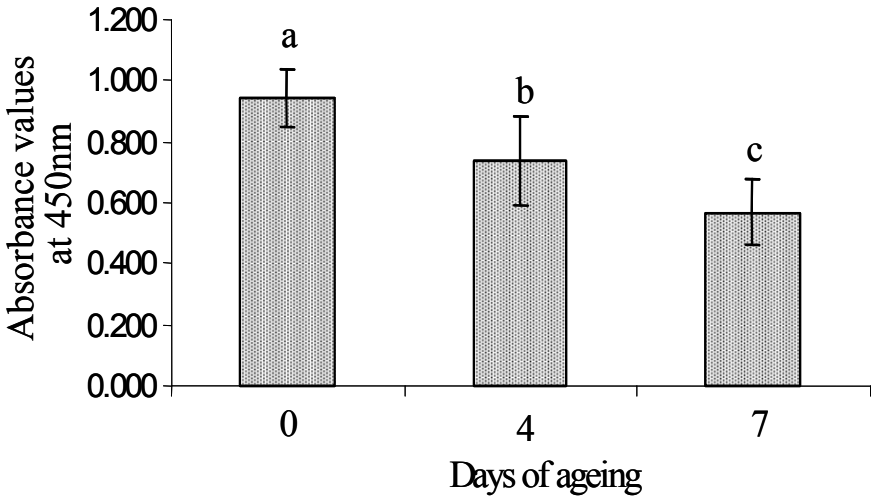

Figure 1: Absorption values obtained from meat contaminated with SC tissues before ageing or after ageing 4 or 7 days at 4ºC. Values are expressed as mean ±standard deviation for n=3. Different letters denote significantly different values ($p<0.05$).

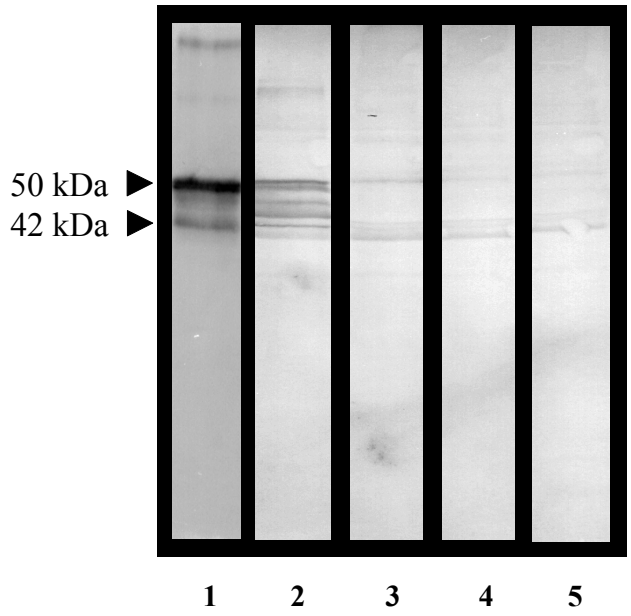

Figure 2: Western blot analysis of GFAP protein. Lane 1: pure GFAP, Lane 2:porcine spinal cord tissue, Lanes 3 to 5 : Meat contaminated with 0.5% spinal cord tissues without ageing (0 day), 4 days of ageing and 7 days of ageing respectively.