The Presence of Central Nervous System Tissues in Meat Products Sold in Istanbul

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SUMMARY

Bovine Spongiform Encephalopathy (BSE) is a transmissible, neurodegenerative and fatal disease of cattle that affects the central nervous system (CNS) tissues. Contamination of edible meat with specific risk materials (SRMs) may occur during stunning, slaughtering, carcass splitting and meat production processes. ELISA is an available test for the detection of CNS tissues in heated and no heated food. The aim of this study is to determine the presence of some risky tissues and organs in meat and meat products which are consumed frequently in Turkey. As a result, 3 out of 75 minced meat samples (4%), 9 out of 75 (12%) meat ball samples, 4 out of 50 (8%) fermented sausage samples, 5 out of 50 (10%) salami samples and 7 out of 50 (14 %) sausage samples were positive. In conclusion, meat and meat products are intentionally or accidentally, contaminated with CNS tissues in any step of slaughtering (stunning, carcass splitting etc.) and meat production. So, risk contamination sources such as equipments, personnel, surfaces, tools and meat production process must be scrutinized. Necessary precautions have to be taken and complied with hygiene practices.

Keywords: BSE, ELISA, Meat, Meat Products, Specific Risk Material

RÉSUMÉ

L’encéphalite spongiforme bovine (ESB) est une maladie du bétail transmissible, neuro-dégénérative et fatale, qui affecte les tissus du système nerveux central. La contamination de la viande par un matériel à risque spécifique (SRM) peut apparaître à l’étourdissement, à l’abattage, à la découpe des carcasses et puis pendant le processus de transformation des viandes. L’ELISA est un test utilisé pour la détection des tissus nerveux dans l’alimentation crue et cuite. Le but de cette étude est de déterminer la présence de tissus et organes à risque dans la viande et dans les produits dérivés qui sont fréquemment consommés en Turquie. Il en résulte que 3 échantillons sur 75 de viandes hachées (4 %), 9 sur 75 (12 %) échantillons de boulettes de viande, 4 sur 50 (8 %) échantillons de saucisses fermentées, 5 sur 50 (10 %) échantillons de salami et 7 sur 50 (14 %) échantillons de saucisses ont été détectés positifs. En conclusion, la viande et ses produits dérivés sont intentionnellement ou accidentellement contaminées par des tissus nerveux au cours de l’abatage (stunning, découpe, etc...) et de la transformation des viandes. Ainsi, les sources de contamination comme les équipements, le personnel, les surfaces et les outils ainsi que le processus de production de viande doivent être contrôlés. Les précautions nécessaires doivent être prises et mises en application dans le cadre des bonnes pratiques d’hygiène.

Mots-clés : ESB, ELISA, Viande, Produits à base de viande, Système nerveux central

Introduction

Bovine Spongiform Encephalopathy (BSE) which belongs to the family of diseases known as transmissible spongiform encephalopathy (TSE), is a transmissible, neurodegenerative and fatal disease of cattle, that affects the central nervous system (CNS) tissues [10, 13, 23, 26]. The disease has a long incubation period of 4 to 5 years, but can be effective and fatal for cattle within 1 month to 1 year [11, 26].

BSE is caused by an irregular prion protein (PrP) known as PrPSc, which is derived from its normal cellular precursor (PrPC). This formation makes the PrPSc, resistant to proteolysis and as a result this protein accumulates in the CNS and in some organs of lymphoreticular system [13, 15, 19]. This prion has resistance to high temperatures, formaldehyde treatments, UV irradiation and enzyme degradation [12, 27]. Contrarily to this autoclaving with alkaline solutions such as 1M NaOH may be effective in inactivation of the agent [24, 25]. On the other hand, a dose of 25,000 Gray (2.5 mrad) of X-Rays, can partially inactivate the agent [12].

The cattle feedstuffs of meat and bone meal, which was contaminated with a transmissible spongiform encephalopathy agent, rendering the carcasses of livestock including sheep and feed the ruminants with these rendering products for a protein-rich nutritional supplement and insufficient heating process in rendering systems are the main causes of this disease [6, 8, 17]. Therefore, in July 1988 the “Feed Ban” was presented to prevent the use of ruminant-derived protein in ruminant feedstuffs [17, 22].

The various experimental studies showed that, the oral application of infected brain tissues to different animal species such as cattle, sheep, goat, cat, pig and rat induced the infection [11]. Similar to this, the disease could be transmitted to humans by the consumption of meat and meat products from BSE infected cattle and CNS contaminated food. Especially the tissues of CNS such as brain and spinal cord which are named as Specified Risk Materials (SRMs) are regarded to be the main source of BSE. [6, 20, 28]

In order to prevent the contamination of CNS into human food chain, the use of CNS tissues in food production had been banned by the member states of EU. According to legis-
lation (Commission Decision 2000/418/EC); (i) the skull including the brains and eyes, the tonsils, the spinal cord and the ileum of bovine animals aged over 12 months, (ii) the skull including the brains and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months or that have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages are designated as SRMs. In addition to these following tissues are also designated as SRMs in the United Kingdom, Northern Ireland and Portugal; (i) the entire head excluding the tongue, including the brains, eyes, trigeminal ganglia and tonsils, the thymus, the spleen, the intestines from the duodenum to the rectum and spinal cord of bovine animals aged over six months, (ii) the vertebral column, including dorsal root ganglia of bovine animals aged over 30 months [2, 4, 7]. The presence of CNS in any kind of meat products has been strictly banned in our country [5].

Currently, there are no ante mortem tests available for field use to detect the disease [10]. However, diagnostic tests such as ELISA, Western Blot and Immunocytochemistry are present for the detection of BSE, but are performed post mortem. Especially, ELISA is an available test for the detection of CNS tissues in heated and no heated food. This test is based on the interaction of specific antibodies with the Glial Fibrillary Acidic Protein (GFAP) which mainly exists in astrocyte cells of the CNS tissues [9, 21, 25].

This study was planned to determine the presence of some risky tissues and organs (CNS) in meat and meat products which are consumed frequently in Turkey.

Materials and Methods

Test Material: In this study, a total of 300 meat product samples (75 minced meat, 75 meatballs, 50 fermented sausage, 50 salami and 50 sausage) were obtained from different markets in Istanbul and used as test material.

Extraction Procedure: Extractions were performed as described by r-Biopharm Ridascreen Risk Material manual. 2 g of homogenized sample was mixed with 10 ml of extraction solution, and heated at 100 °C for 15 minutes. After the chilling process, centrifuged at 3500 g for 10 minutes at 10 °C. Upper fat layer was removed completely and diluted with the sample dilution buffer. The sample extracts were kept at -20 °C, until ELISA test is performed [6].

ELISA Test Procedure: 50 µl of extracted samples and standards (0 %, 0.1 %, 0.2 % ve 0.4 %) were transferred separately to micro wells which are coated with GFAP antibodies. After that, 50 µl of enzyme conjugate was added and incubated for 30 minutes at room temperature (20-25 °C). After the washing process (for 3 times with washing buffer solution), 100 µl of substrate/chromogen solution was added and incubated at dark for 30 minutes at room temperature. Finally, 100 µl of stop solution was added to each well and the absorbance values were measured at 450 nm against an air blank [6].

Recovery Test: The sensitivity and the validity of the method was tested with brain contaminated samples with different concentrations. First of all it was detected that, the minced meat, meatball, fermented sausage, salami and sausage samples did not maintain any CNS tissues. Then, these samples were contaminated with different concentrations (0.2 %, 0.5 %, 1.0 % and 2.0 %) of brain tissues. The extraction procedure which was explained above was performed and repeated for 3 times.

Results

The sensitivity and the validity of the method were tested with brain tissue contaminated samples with different concentrations (0.2 %, 0.5 %, 1.0 % and 2.0 %) were found to be positive.

As it can be seen from Table 1, 3 out of 75 minced meat samples (4 %), 9 out of 75 (12 %) meat ball samples, 4 out of 50 (8 %) fermented sausage samples, 5 out of 50 (10 %) salami samples and 7 out of 50 (14 %) sausage samples were positive. CNS tissue positive samples were shown in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>The Number of Samples</th>
<th>The Number of Positive Samples</th>
<th>The Percentage of Positive Samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minced meat</td>
<td>75</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Meat ball</td>
<td>75</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Fermented sausage</td>
<td>50</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Salami</td>
<td>50</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Sausage</td>
<td>50</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>28</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Table I. Numbers of Prepared Meat and Meat Products Containing CNS Tissues.

Discussion

Nowadays, there are no antemortem tests available for detection of BSE agent, but there are ongoing studies about the subject [10]. The analysis of meat and meat products regarding the contamination of CNS tissues has a great significance in protection of consumer’s health.

In this study, the ELISA technique was used to detect the presence of CNS tissues in meat and meat products sold in Istanbul. The validity of the method was confirmed by a recovery test, in our study. According to Ridascreen test kit manual, the absorbance values which are equal to 0.2 % or higher values of standard solutions, evaluated as positive. Positive results were achieved at the concentration of 0.2 % in recovery test samples which were experimentally contaminated with bovine brain at different concentrations of 0.2 %, 0.5 %, 1.0 % and 2.0 %. These results indicated the sensitivity of the method (n = 3). The method had been previously validated by Liebig University Institute for Veterinary Food Science with Western Blot [9].
ELISA is one of the available tests for the detection of CNS tissues in heated and no heated food. The principle of this test is based on the detection of GFAP as a marker for to determine the CNS tissues. SCHMIDT et al. [21] stated that GFAP was a specific marker for CNS tissues. HAJMEER et al. [14] compared two test kits, including the Ridascreen Test kit which was used in this study and reported that Ridascreen test kit was 10 times more sensitive, easier and faster to run than the other kit. This test kit was preferred in our study because of its advantages.

In our study, 28 out of 300 examined samples (9.3%) were found to be positive. These results point out that, the meat and meat products are still contaminated with CNS tissues by accidentally or designedly. In Turkey, there is no other study about this subject except YEŞİLBAĞ and KALKAN’s [28] research. In their study, they examined 179 meat product samples (49 salami, 34 sausage, 31 fermented sausage, 22 hamburger meatball, 43 meatball/minced meat samples) about the presence of CNS tissues. The researchers detected 18 out of 179 examined samples as positive. These results are similar with ours.

We found the highest positive percentage in sausage samples (14%) and in meatball samples (12%). YEŞİLBAĞ and KALKAN [28] found the highest positive percentage in salami samples (30.61%). Beside this, in our study, 4 out of 50 examined fermented sausage samples (8%) were found positive. Contrarily, none of the fermented sausage samples were found to be positive by YEŞİLBAĞ and KALKAN. In spite of these differences, there was similarity in the total number of positive samples in both performed studies.

Contamination of edible meat with CNS tissues may occur during stunning, slaughtering, carcass splitting and meat preparation and fabrication processes. Stunning with a captive bolt gun may cause the transportation of brain tissues via the blood stream to the edible part of carcass. Also, current slaughter methods may allow the brain and spinal cord tissues to contaminate the carcass. In addition to this, while the splitting downs the carcass from vertebral column with a saw, the carcass can be contaminated with spinal cord tissue and enter the food chain. On the other hand, CNS tissues can be mixed to meat products by producers designedly [114, 14, 16, 18].

According to Turkey’s Ministry of Agriculture statements, it has not been come across to BSE and scrapie disease in cattle and sheep. Similarly, up until now there is no any report about vCJD in humans in our country. BSE disease has been taken to “Notifiable Disease” scope. For precautions, on 25.05.1990 the importation of live animal, animal products and animal feed from the related countries has been restricted. Also, the presence of CNS tissues in any kind of meat products has been strictly banned in Turkey.

As a conclusion of our study, meat and meat products can be contaminated with CNS tissues in any step of slaughtering (stunning, carcass splitting etc.) and meat production. To dispose of the SRMs (the skull including the brain, eyes, the tonsils, the spinal cord, the ileum etc.) as inedible products, dispose of the SRMs (the skull including the brain, eyes, the tonsils, the spinal cord, the ileum etc.) as inedible products, to remove the head without contamination of the carcass or other meat products with SRMs and to remove the spinal cord and its entirety are some important precautions in preventing the contamination of SRMs to carcass during slaughtering. In addition to these, risk contamination sources such as equipments, personnel, surfaces, tools and meat production process must be scrutinized. In order to protect the public health, mixing of CNS tissues to meat and meat products have to be strictly prohibited.

Acknowledgement

This work was supported by The Research Fund of Istanbul University. Project Number: 68/15052003.

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