

---

# **Scientific Research on Natural Sausage Casings**

Second Edition



International Scientific Working Group

Editor: Dr. Joris J. Wijnker – Scientific Advisor ISWG

Copyright © International Scientific Working Group 2014  
All rights reserved

Cover design and artwork: Multimedia Centre, Faculty of Veterinary Medicine, Utrecht University, The Netherlands  
Printed by: Markade L.L.C., Alexandria, Egypt

## PREFACE

---

In 2009 the first edition of this book on scientific research on natural sausage casings was launched. It was an instant success, as it provided the highlights of 40 years of research on natural casings in a practical and accessible way and because its recipients were not only limited to the natural casing industry but also included competent authorities and researchers.

Also, it gave credit to those who took the initiative for scientific research on natural casings as a common practice to provide solid arguments on the quality and safety of the product. This initiative has already led to the inclusion of specific facts on natural casings in certain standards such as the OIE's Terrestrial Animal Health Code, HACCP manual, competent authorities' scientific opinions and (inter-)national legislation.

This has encouraged the natural casing industry to continue its commitment to this work where already more than 2 million US dollars have been committed and spent on these scientific projects.

The book's basis lays in the natural casing industry, where practical questions are raised on various casing-related topics that are subsequently discussed by the ISWG, the International Scientific Working Group. This is a dedicated committee of INSCA, the International Natural Sausage Casing Association. The committee members are delegates of the continental and national associations covering the entire world.

The ISWG was originally established as the INSCA Research and Development Committee in 1989 and became a joint ENSCA / INSCA R&D program in 1993. In 2000, the Joint INSCA/ENSCA/NANCA Scientific Working Group (SWG) was formed, followed in 2006 by the formation of the International Scientific Working Group (ISWG) when the Japanese (JSCIA) and Chinese (CNSCA) associations joined the SWG. Past chairmen of these working groups since 1989 are **Mr. Eddie Tonks**, **Mr. Mounir Shehfe**, **Mr. Lex van Hessen** and **Mr. Philippe Leymonie** who is currently serving his second term, this time as chairman of the ISWG.

As time progressed, different issues were raised and subsequent research projects launched. With more information becoming available each year, a first compilation of all finalised projects was prepared by **Dr. Gisela Panzer** in 2002. The 2009 edition was prepared by **Dr. Joris J. Wijnker** and subsequent editions are logical follow-ups to this original compilation where the key message is still the same: to provide a scientific update on natural casings and give practical guidance on its application.

This second edition provides you with the latest information, describing each project in a brief summary and overview of its conclusions and recommendations. In order to list all studies logically and comprehensively, a subdivision has been made into five major categories, based on comparable subjects:

**Chapter 2**, Public Health, is on microbiological studies and possible presence of unwanted residues, focusing on the safety of natural casings;

**Chapter 3**, Animal Health, describes the studies on the inactivation of certain viruses, such as foot-and-mouth disease and classical swine fever, which have been done in order to prevent the spread of these contagious animal diseases via natural casings;

**Chapter 4**, Technological Developments, focuses on the different studies done on the usability and preservation of natural casings;

**Chapter 5**, BSE/ TSE research, presents an overview of the different reports and papers published and how these were evaluated in EFSA opinions.

**Chapter 6**, Special Projects, describes the projects that have had a major impact on the natural casing industry.

Included in the **Annexes** is the protocol for the treatment of natural casings with phosphate supplemented salt, the inventory part of the EU – CRAFT – Project, describing the histology and microbiology of hog and sheep casings and the SWG statement on the definition of *ileum*. Finally in **References**, all reports and scientific papers are listed alphabetically to which references have been made in the different chapters. These reports and papers are made available as full-text documents on the INSCA website.

In repeating the final paragraph of the 2009 edition, its intended message and commitment is again underlined:

*“This book, besides summarizing past projects, will clearly show what still needs to be done and where we should go from here.*

*At the same time, this group of industry delegates forming the ISWG is convinced that there is much more that needs to be studied and clarified and proactive work is required in order to meet the ever-changing requirements. With the moral and financial support of all the associations and the companies in this industry this work can be done, striving to ensure the future of our industry”.*

Hong Kong May 2014,

Hans Martin Kersting – INSCA Chairman

Philippe Leymonie – ISWG Chairman

Joris Wijnker – ISWG Scientific Advisor

# CONTENTS

---

<b>1</b>	<b>General introduction</b>	
1.1	History	8
1.2	Production of natural sausage casings	11
1.3	Microbial contamination of casings	14
<b>2</b>	<b>Public health</b>	
2.1	Microbiological investigation of natural casings	20
2.2	Study on emerging pathogens in natural sausage casings	21
2.3	A survey of dry-salted natural casings for the presence of <i>Salmonella</i> spp., <i>Listeria monocytogenes</i> and sulphite-reducing <i>Clostridium</i> spores	22
2.4	Antimicrobial properties of salt (NaCl) used for the preservation of natural sausage casings	24
2.5	Reduction of <i>Clostridium sporogenes</i> spore outgrowth in natural sausage casings using nisin	26
2.6	Bacterial spores on natural casings and means of control or elimination	27
2.7	Establishing safe thresholds for spore formers in the casings industry	28
2.8	Shelf life of natural sausage casings	29
2.9	Nutritional values of natural sausage casings	32
2.10	Substances and residues in natural sausage casings	34
2.11	A simulation model for the prediction of tissue:plasma partition coefficients for drug residues in natural casings	36
<b>3</b>	<b>Animal health</b>	
3.1	Presence of foot-and-mouth disease virus in organs of infected sheep	38
3.2	Removal of foot-and-mouth disease virus infectivity in salted natural sausage casings by minor adaptation of standardized industrial procedures	39
3.3	Inactivation of classical swine fever virus in porcine casing preserved in salt	40
3.4	Virus inactivation by salt (NaCl) and phosphate supplemented salt in a 3D collagen matrix model for natural sausage casings	41
3.5	Inactivation of foot-and-mouth disease virus in various bovine tissues used for the production of natural sausage casings	42
3.6	Scientific Opinion on animal health risk mitigation treatments as regards imports of animal casings	43
3.7	Implementation – OIE Terrestrial Animal Health Code	44
<b>4</b>	<b>Technological developments</b>	
4.1	Effect of initial mild curing, with additives, of hog and sheep sausage casings on their microbial quality and mechanical properties after storage at difference temperatures	46
4.2	Effect of different curing treatments on the usability of beef, hog and sheep casing	47
4.3	Residues of curing agents in natural sausage casings previously subjected to anti-viral treatments - Hog and sheep casings treated with either lactic acid, citric acid or orthophosphates	48
4.4	Effect of trisodium phosphate on slip and textural properties of hog and sheep natural sausage casings	49

# CONTENTS

---

4.5	Biochemical and microbiological changes in natural hog casings treated with ozone	50
4.6	Phosphate analysis of natural sausage casings preserved in brines with phosphate additives as inactivating agent – Method validation	51
4.7	Implementation	52
<b>5</b>	<b>BSE / TSE research</b>	
5.1	Natural sausage casings and the BSE / TSE risk	58
5.2	Assessment of the risk of exposure to the BSE agent through the use of natural sausage casings	60
	Note on weights of intestine in small ruminants and expected risk reduction	61
	Risk assessment of the use of sheep natural casings and legs of lamb	62
5.3	Comparative histological studies of mechanically versus manually processed sheep intestines used to make natural sausage casings	63
5.4	Histology of bovine natural casings	64
5.5	Quantitative histological analysis of bovine small intestines before and after processing into natural sausage casings	65
5.6	Opinion on quantitative histological studies and the re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings	66
5.7	TSE risk assessment for use of bovine casings	67
5.8	Scientific Opinion on BSE risk in bovine intestines	68
5.9	Experimental bovine spongiform encephalopathy: detection of PrP <sup>Sc</sup> in the small intestine relative to exposure dose and age	69
5.10	Scientific Opinion on a review of the BSE-related risk in bovine intestines	70
5.11	Scientific Opinion on BSE risk in bovine intestines and mesentery	71
<b>6</b>	<b>Special projects</b>	
6.1	Improved treatment of natural sausage casings for quality improvement in automated stuffing processes (EU – CRAFT – Project (BRC 2.CT 94.1495))	74
6.2	HACCP manual for processing natural sausage casings	76
6.3	The Road Map: Consumer safety of natural sausage casings	77
6.4	The Road Map 2: Safety and quality of natural casings	81
6.5	Aspects of quality assurance in processing natural sausage casings	84
	<b>Annexes</b>	
	I. Phosphate supplemented salt treatment of natural sausage casings	89
	II. Inventory part - Histology and microbiology of hog and sheep casings (EU – CRAFT – Project (BRC 2.CT 94.1495))	99
	III. SWG statement on the definition of ileum	110
	<b>References</b>	113

## *CHAPTER 1*

---

### **GENERAL INTRODUCTION**

---

## History

Natural sausage casings ("casings") are traditional products that have been used in the production of meat specialities for centuries, and have remained virtually unchanged in function and appearance. A large variety of sausage is produced world-wide using the processed intestines of pigs, sheep, goats and cattle (and sometimes horses).

It is often assumed that sausages were invented by the Sumerians in the region that is Iraq today, around 4000 BC. Reference to a cooked meat product stuffed in a goat stomach like a sausage was known in Babylon and described as a recipe in the world's oldest cooking book 3750 years ago (Yale Babylonian collection, New Haven Connecticut, USA).

The Chinese sausage *Làcháng*, which consists of goat and lamb meat, was first mentioned in 589 BC. The Greek poet Homer mentioned a kind of blood sausage in his *Odyssey* (book 20, poem 25); Epicharmus (ca. 550 BC - ca. 460 BC) wrote a comedy entitled *The Sausage*. Numerous books report that sausages were already popular among the ancient Greeks and Romans.

During the reign of the Roman emperor Nero, sausages were associated with the Lupercalia Festival. The early Catholic Church outlawed the Lupercalia Festival and declared the consumption of sausages to be a sin. For this reason, the Roman emperor Constantine banned the consumption of sausages. Early in the 10th century, the Byzantine emperor Leo VI forbade the production of blood sausages following cases of food poisoning, known in Germany as sausage poisoning.

Interestingly, the word sausage is derived from old French word *saussiche*, which could be found in a dialect spoken between 1000 and 1300 AD in a geographic region spanning the north of France and parts of Belgium and Switzerland. *Saussiche* comes from the Latin word *salsus*, meaning salted and creating a clear link to the long-known preservation method of casings using salt.

The art of producing sausages using animal intestines survived the fall of the Roman Empire and continued through the Middle Ages. With the development of cities throughout Europe, the butcher profession re-emerged, garnering great respect and even power. At the beginning of the 12th century, during the time of German Kaiser Heinrich V, butchers were recognized as eminent citizens.

Advances in meat processing were widely observed and sausage making was practiced throughout the Old World. As early as the 12<sup>th</sup> century, slaughterhouses in England separated more perishable materials (tripe, intestine) from carcass meat, and the French and Germans had set inspection requirements for meat products in the 13<sup>th</sup> century.

By the late medieval period, sheep were perhaps the most important domesticated animals; both individual farmers and monasteries owned huge flocks. However, the supply of casings could not meet the steadily growing demand of the sausage makers. Because of this, salt preserved casings became an important trade commodity across south and central Europe.

Intestines were praised as delicacies in medieval Europe. The German poet Kunig von dem Otenwalt in his song "Von der Küewe" (Küewe = cow) regarded large intestines as popular food and Steinmar (around 1200) spoke in his



“Schmauslied” (Feast poem) about intestines (“Dermel”) as pleasing and luxurious products. Later, around 1300 Johannes Hadlaub, a lyric poet, described that the German people highly valued meat products, including mesentery, intestines viscera and sausages. In his painting “The Butcher Shop”, the Dutch painter Pieter Aertszen (1507 - 1573) depicted the details of common sausages amongst other meat specialities of that time such as ring sausages, link sausages, and double links of small sausages.



*The Butcher Shop (Pieter Aertszen)*

In 1662, under the pseudonym Marcus Knackwurst, a book was written describing a number of famous sausage formulations with the emphasis on the use of natural sausage casings.

The late 18<sup>th</sup> and first half of the 19<sup>th</sup> century were the years of the industrial revolution. Improvements in meat preservation and processing methods resulted not only in dehydrated foods and dried meats, but also in new sausage production processes, again making use of animal casings as natural envelop for meat preparations.

The public health and hygienic problems have for long been regarded as critical points in the practical application of casings. As a result the casings imported into Germany from foreign countries have been subjected to veterinary inspection since the introduction of the Meat Inspection Act (“Fleischbeschauengesetz”) on June 3<sup>rd</sup> 1900. Problems related to the quality of casings, such as intestinal parasites and tuberculous knots as well as other sanitary defects, were discussed in the literature of that time (i.e. Von Ostertag, 1905). Gröning (1905) described the results of veterinary-sanitary examination of imported casings. In his studies, published in 1910 and 1920, he discussed the issue of hygiene and quality of

imported casings (hog fat ends), including microbial red discolorations of salted products, commonly known as "*Red Dog*". The problems related to the cleaning of casings concerned meat hygienists of that time. Schilling (1901) found in hog and beef casings several grams of faecal residue, consisting partly of straw fragments, corn and animal hairs. Improvements in cleaning efficiency were permanently required.

During the first half of the 20<sup>th</sup> century the processing techniques of intestines into finished sausage casings were gradually improving. Knowledge of casing production was growing and new technology emerged. Von Ostertag (1905) described the methods of quality control of casings. New methods and machines were developed for the de-sliming and cleaning of casings (Heiss-Straubig, 1902; Nägele's cleaning machine – Patents Stohrer, 1919 and 1927).

Regulations, strictly applied to the growing import of casings, had an active and positive influence on the hygiene and technology of sausage preparation, not only in Europe but also around the world. The import of casings in Europe started to be more and more rigorously and successfully controlled, not only because of microbial risks but also because of the possible use of inedible salt and other inedible preservatives and additives.

Development of meat processing machines was an important stimulus for an accelerated growth of sausage production in this period. As a result the need for casings increased which encouraged both further improvement of processing and preserving methods of casings and paved the way for the invention of new alternative types of casings from natural and man-made materials (Savic and Savic, 2002).

This change from a traditional style of sausage production to a uniform industrial approach did not make natural casings obsolete. Dr. Gisela Panzer wrote in 1977 that sausages stuffed in natural casings are, due to their non-uniform appearance, clearly distinguishable from mass-produced products and are therefore acceptable as a higher quality product. Prof. Sakata (1998) stated that natural casings achieve marked consumer preferences over artificial casings due to their better bite-resistance (The -"knack"- in knackwurst).

A quick search on the internet shows that sausages have not lost their appeal to the modern consumer. A country well-known for its sausage tradition is Germany, where the average consumer buys some 30 kilos of sausage per year – representing half of his annual meat consumption. This high consumption is associated with a range of more than 1500 different kinds of sausage, produced mostly locally and with regional variations regarding composition, smoking and spicing techniques (Wijnker, 2006).

Despite the appreciation of sausages as meat products, the attention of the scientific community in this product remained limited. Apart from a handful of studies, most investigations focussed on meat (products) addressing hygienic issues and the control of contagious animal diseases. However, since the 1970s natural casings have been studied more closely and these investigations are presented in more detail in the next chapters.

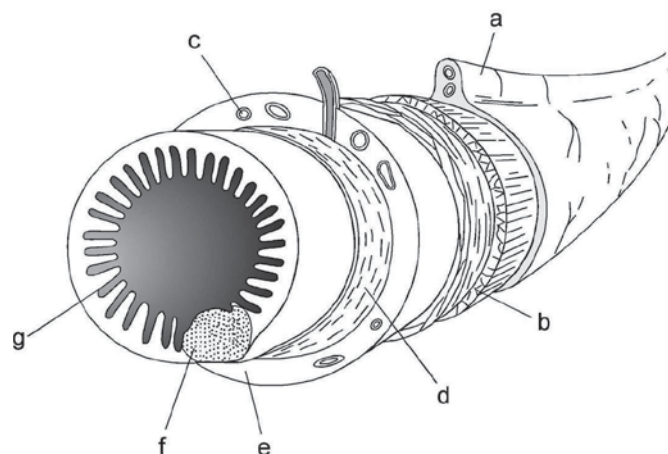
## Production of natural sausage casings

### Anatomy

From pigs the entire intestinal tract is used for the production of casings, specifically the small intestines (duodenum, jejunum, ileum), bung (caecum), large intestines (colon ascendens & transversum), after end (colon descendens) and fat end (rectum). From sheep, only the small intestines are used, particularly the duodenum and jejunum and sometimes also the ileum. The intestinal tract of cattle is also used entirely with the exception of the ileum. Its shape differs too much from the jejunum to produce the classic beef rounds and is therefore removed prior to the cleaning process and destroyed. Beef casings are produced from the weasand (oesophagus), small intestines (duodenum, jejunum) which are processed into beef rounds, bung (caecum), large intestines (colon) which are processed into beef middles, and bladders (Ockerman and Hansen, 2000).

Although there is quite a large variety in shapes and sizes of the intestinal tract between the different species used for the casing production, their basic anatomy and function are remarkably similar. The intestinal wall is composed of four basic layers (Figure 1).

Figure 1: Schematic diagram of sheep small intestine showing mesentery and serosa (a), inner and outer muscle layers (b), submucosal blood vessels (c), muscularis mucosae (d), submucosa (e), lymphoid nodule (Peyer's patch) (f), and tunica mucosa (villus and crypt layers) (g). The tunica mucosa, the muscularis, the serosa and Peyer's patches are removed during processing, so the natural casing consists of only the submucosa (e).



The tunica serosa is the outermost layer covering the intestinal tract. The tunica muscularis consists of two layers of smooth muscle, with an inner layer in a circular and an outer layer in a longitudinal orientation. The tunica submucosa, lying beneath the tunica muscularis, has a microstructure characterized by a network of collagen fibres (type II), elastin and blood vessels of different sizes. For hog and sheep casings, this submucosa is the remaining layer of the intestine after processing and forms the natural sausage casing (Figure 2).

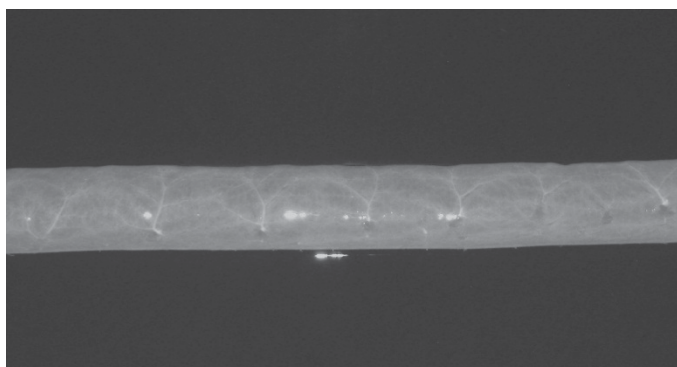


Figure 2: Fully processed sheep casing



The tunica mucosa is the innermost layer of the intestinal tract and lines the lumen. Embedded in the mucosa lies lymphatic tissue which occurs irregularly along the length of the small intestine as isolated lymphoid nodules (lymphonoduli solitarii), but tend to be most prominent in the ileum. These aggregated lymphoid nodules (lymphonoduli aggregati) are known as Peyer's Patches and are anatomically located on the convex side of the intestine opposite to the mesenteric attachment.

Taking a sheep casing as example, figure 3 shows the full thickness of the uncleaned small intestine and figure 4 shows the tunica submucosa as remaining tissue layer after the cleaning process is finished. A cleaned sheep casing is on average 0.11 mm thick, whereas a cleaned hog casing, also comprising only of the submucosa, is 0.32 mm (Bartenschlager-Blässing 1979; Koolmees and Houben 1997).

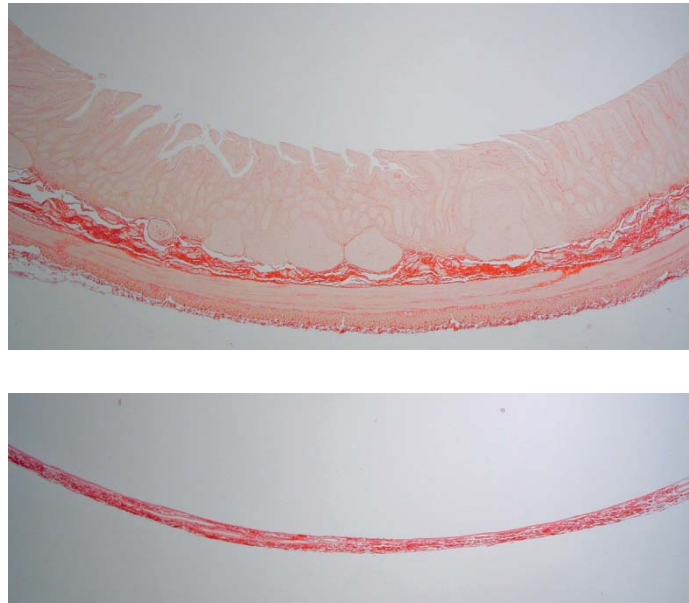


Figure 3 & 4: Sheep casing, before and after cleaning (HE colouring (red), magnification X 25)

A study done by Nishiumi and Sakata (1999) on the histological and biochemical properties of casings found that each casing was composed of numerous sheet-like layers of collagen fibres in a criss-cross arrangement. Differences in gross organisation of the collagen fibres was not observed, but the outer layers of Chinese sheep casings contained more stretched collagen fibres and was packed more densely than Australian sheep casings. These casings had relative fine collagen fibres of loosely packed fibrils. Casings contained approximately 2 % elastin accumulated in blood vessels, without any differences in morphology, localisation and density between samples of different species and origin. Only Japanese hog casings seem to be an exception with lower elastin content. According to Nishiumi and Sakata, the mechanical properties are determined by the size, arrangement of collagen fibres and heat-solubility of collagen present in casings. Differences in the heat-solubility of collagen in casings of various origins may therefore influence usability and palatability of these casings and subsequent studies to tenderize casings will be discussed below.

## Processing

The subsequent cleaning process of small intestines can vary between species and geographical location of the cleaning operation. Clear differences exist in how the intestines are pulled from the viscera, being either with a knife, by hand or by machine (Smits and Keizer, 2003). Contrary to hog casings, sheep casings are fermented prior to the cleaning process. After the manure is stripped from the intestines, sheep casings are placed overnight in a cooled storage during which the mucosa will degrade for easier removal. In general for hog and sheep casings, the manure is stripped out of the small intestines, the mucosa is crushed and removed in various steps and the outer layers, being the tunica

muscularis and tunica serosa, are scraped off, leaving the tunica submucosa (Fisher and Schweflinghaus, 1988; Ockerman and Hansen, 2000).

The porcine large intestines are mostly processed into hog chitterlings and fat ends by hand. All layers remain identifiable, whereas it depends on the operation if the mucosa is (partially) removed (Schweigmann and Seeger, 1988; Ockerman and Hansen, 2000).

In contrast to the extensive processing of hog and sheep small intestines into casings, beef casings retain all original layers after cleaning (Botka-Petrak et al., 2001). A pilot study on the histology of beef casings (Koolmees, 1998) indicated that although most of the tunica mucosa is removed from the small intestines, the tunica muscularis and serosa can be clearly identified and that Peyer's Patches also remain present.

In order to determine whether a difference existed in cleaning efficacy between manual and mechanical processing techniques, a comparative histological study was done by Koolmees et al. (2004) using sheep casings. Results showed that no significant differences existed between both techniques and that no lymphatic tissue (Peyer's patches) remained after cleaning.

### **Mechanical and biochemical properties of casings**

An important issue in the quality of casings is their usability for the stuffing process of a great variety of sausages and the consumers' preferences regarding texture and tenderness. Although these technological aspects are beyond the scope of this thesis, a brief overview is warranted to indicate certain developments.

A study (den Reijer, 1996) done on the toughness of casings in the production of smoked sausages, indicated that many factors not directly related to the casings themselves have a significant influence on the toughness perception. Higher fat content of the sausage meat batter and improved smoking procedures enhance the overall quality.

Various additives, used during the initial curing process of the casings (Bakker et al., 1999) prior to storage in salt, were tested for their influence on the mechanical properties of casings. Combinations of citric acid /  $\text{Na}_3\text{-citrate}$ , lactic acid /  $\text{Na-lactate}$  or phosphates ( $\text{Na}_3\text{PO}_4$  /  $\text{Na}_2\text{HPO}_4$ ) and  $\text{NaCl}$  improved the mechanical properties, with prevalence for phosphates on the final usability of the casings (Verkleij and Keizer, 2003).

Japanese studies aiming to tenderize hog casings evaluated the effect of lactic acid and pepsin (Sakata et al., 1998) or high pressure treatment in combination with lactic, acetic or citric acid (Nishiumi et al., 2005), whereas Reichert (1996) studied the effect of a proteolytic fruit enzyme solution on casings after stuffing. A recent study (Nakae et al., 2008) on the influence of trisodium phosphate (TSP) gave a clearer indication than Bakker et al. (1999) that phosphates can reduce the maximum force and breaking strain of casings.

An overall conclusion could be that phosphates are preferred over organic acids and other substances, although a comprehensive study would be required to make a full assessment of the different treatments and their effects on various quality parameters of casings. Such an attempt was made by Schwanz and Schnäckel (2007a; 2007b) who developed an elaborate set-up to test various additives and their influence on the quality of casings. Interestingly, their results also showed that soaking desalinated casings in plain water (45 °C) for around 30 minutes prior to stuffing was sufficient to create an acceptable bite.

## 1.3

### Microbial contamination of casings

#### Bacteria

As casings are cleaned in batches at water temperatures around 40 °C, microbiological cross-contamination and bacterial outgrowth is evident. Koolmees and Houben (1997) illustrated the bacterial load of casings at various cleaning steps, underlining the necessity to take appropriate actions to prevent quality loss and possible food safety risks.

	Hog casings (counts cfu <sup>b</sup> / g)	Sheep casings (counts cfu / g)
Total aerobic count	10 <sup>5.8</sup>	10 <sup>6.5</sup>
Enterobacteriaceae	10 <sup>4.4</sup>	10 <sup>4.3</sup>
<i>S. aureus</i>	10 <sup>3.4</sup>	10 <sup>4.3</sup>
Salt tolerant org.	10 <sup>3.5</sup>	10 <sup>3.4</sup>
Lactic acid bacteria	10 <sup>4.7</sup>	10 <sup>5.5</sup>
Bacterial spores <sup>a</sup>	<10 <sup>2</sup>	<10 <sup>3</sup>

a) if detected

b) colony forming units

Table 1: Bacteriological results of freshly cleaned hog and sheep casings

Riha and Solberg (1970) investigated the microflora of salted sheep casings used for the production of fresh pork sausages. Various bacterial contaminants were identified including *Bacillus*, *Pseudomonas*, *Clostridium*, *Micrococcus*, *Proteus* and *Lactobacillus*. Casings were stored in either dry-salt or saturated brine and sampled within 4 weeks after production. Aerobic total plate counts were comparable between the different samples and corresponded to the counts found immediately after cleaning (Table 1). However, Riha and Solberg (1970) did not mention the exact period between casing production and sampling. They acknowledged the wide variety of spore-forming bacteria present and concluded that the potential shelf-life reduction of fresh sausage contributed by the casing material is minimal, provided the sausage is processed properly and stored at 4 °C.

In 1974 the study by Gabis and Silliker was published on *Salmonella* in beef, sheep and hog casings. Results indicated a complete removal of *Salmonella* (10<sup>6.2</sup> cfu / g) in dry-salted beef and sheep casings after 7 days (stored at 6 °C) or after 21 days in hog casings stored in saturated brine (stored at 6 °C). A study conducted by Bartenschlager-Blässing (1979) reported the absence of high bacterial counts in sheep and hog casings and a survey done by Houben (2005) on the presence of *Salmonella*, *Listeria* and *Clostridium* spores in dry-salted casings indicated that only spores were present after prolonged storage. In addition, Schweigmann and Seeger (1988) showed that casings produced from large intestines are preserved in less salt than small intestine casings and can therefore contain higher microbial counts.

Bockemühl (2000) studied the presence and survivability of certain pathogens using fresh and salted beef, sheep and hog casings. *Listeria monocytogenes* was confirmed in beef casings and *L. monocytogenes*, *Salmonella* and *Campylobacter* were confirmed in fresh hog casings after 30 days of storage in dry-salt at 15 °C. These bacteria were no longer present except for *Salmonella* and *L.*

*monocytogenes* in hog casings. However, information on salt concentrations or actual bacterial counts was not given in this report, preventing any clear conclusions.

A comprehensive study by Wirth (1990, 1994) reported not only the microbial aspects of casings after prolonged storage but evaluated also whether or not the casings were still usable for further processing. Storage at 22 °C provided lower bacterial counts than 4°C, although the quality of casings was better preserved at lower temperatures. Wirth concluded that most likely the influence of light and higher oxygen concentrations were detrimental to the quality loss, a situation specific for this experiment and in contrast to the general storage of casings in closed casks.

All these results indicated that the high bacterial counts found during and directly after the cleaning procedure could be countered by correct curing and subsequent storage in dry-salt or brine. However, the specific concentration-dependent influence of salt and brine on various bacteria has not been clearly determined and remain to be studied.

### **Prions**

A recently emerging sanitary problem is the possible presence of infective prions in tissues of slaughter animals, particularly in tissues derived from cattle. Normal prion protein (PrP<sup>C</sup>) is a glycoprotein expressed by neurons and other cells and functions as a copper dependent antioxidant (Brown et al., 1999). The transition of healthy, routinely synthesized PrP<sup>C</sup> to infectious prion proteins (PrP<sup>Sc</sup>), based on the prion dimer theory of Prusiner, follows the merging of a normal (healthy) and an infectious prion molecule to form a PrP<sup>C</sup>-PrP<sup>Sc</sup> heterodimer, in which the normal PrP<sup>C</sup> molecule is restructured into PrP<sup>Sc</sup> (Prusiner, 1998).

All known forms of Transmissible Spongiform Encephalopathies (TSEs) are characterized by the extracellular aggregation of Infectious prion proteins that can cause neurodegenerative disease within the CNS by forming plaques known as amyloids, which disrupt the normal tissue structure (Doherr, 2007). This disruption is characterized by "holes" in the tissue with resultant spongy architecture due to the vacuole formation in the neurons. These progressive lesions cause impairment of brain function (memory, behaviour, movement) and most often result in death of the patient. Known prion diseases are Bovine Spongiform Encephalopathy (BSE) and Variant Creutzfeld-Jakob disease (vCJD) in humans, which has been associated with the exposure to BSE prions via animal products.

As a result of the initial epidemiological assessment in the 1980ies and in consideration of the possible link between BSE and vCJD, restrictive measures were put in place. This resulted by the end of 1997 in the listing of all cattle tissue types and organs that could be contaminated with BSE as Specified Risk Material (SRM), including brain, spinal cord, vertebral column and the entire intestinal tract from duodenum to rectum of cattle and the ileum of sheep.

The plausible route of infection via contaminated feed suggested that the intestinal tract of cattle could play a major role in the uptake of the BSE agent. From the first studies done at the European reference laboratory for BSE in the UK it became apparent that only the distal ileum, containing higher quantities of lymphatic tissue than the jejunum or duodenum, was found to be infective (Wells, 1994). Subsequent studies did not find any other part of the intestines to be positive for BSE prions (Terry, 2003; Buschmann and Groschup, 2005; Hoffmann et al., 2007).

To determine the actual BSE infectivity risk in cattle intestines more studies are required to generate sufficient data for statistical analysis. However, the options to perform such studies are extremely limited and a distinction needs to be made between uncleaned cattle intestines and processed beef casings regarding the possible presence of BSE-related infectivity. A different approach would be to determine the contribution of beef casings in a human exposure assessment. This route has been used for various beef products and has led to several amendments of the SRM list.

## **Viruses**

As casings are sourced, processed and subsequently shipped to sausage producers worldwide, they have been identified as possible carriers of infectious animal diseases, such as food-and-mouth disease (FMD) and classical swine fever (CSF). Overviews given by Blackwell (1984) and Farez and Morley (1997) provide limited information on casings and when reviewing the original studies, casings were never the intended subject of the study.

McKercher et al. (1978; 1980) mentioned that residual infective FMD virus remains in untreated processed natural casings for as long as 250 days. Unfortunately the authors provide neither reference to the original studies on the natural casings involved, nor information on processing and storage conditions of these casings (temperature, pH, salting).

Panina et al. (1989, 1992) showed that lactic acid formation in fermented sausages led to complete loss of FMD virus infectivity and that CSF virus could survive for at least 75 days in cured sausages. However, the casings used for the production of these sausages did not originate from infected animals.

Only two studies were found which actually investigated CSFV infectivity in processed casings (Helwig and Keast, 1966; McKercher et al., 1980). However, information from these studies was either incomplete or based on incomparable processing methods to allow for an accurate risk assessment.

Böhm and Krebs (1974) were the first to report on different FMD virus titres in specific tissues of experimentally infected sheep including intestines and freshly cleaned casings. They also confirmed the efficacy of a 5-minute treatment with 0.5% citric acid on infected sheep casings to inactivate the FMD virus. Although this was also mentioned by McKercher et al. in 1978, no other studies on FMD virus inactivation in natural casings are known.

As a result from the absence of specific data for FMD and CSF virus survival in processed casings, risk assessments for the international trade in casings have been extrapolated from other products, with either insufficient risk reduction or resulting in unnecessary trade restrictions. Therefore it will be of great relevance to clarify this situation and to determine the potential threat of casings in the dissemination of these contagious animal diseases.

## **Cross-contamination of casings via salt**

Halophilic bacteria are introduced to casings not via the original contamination of the uncleaned animal intestines or cleaning process but via the salt used as preservation agent. The salt used for preservation must meet the "Food Grade" requirements included in the Codex Alimentarius (Codex Stan 150-1985) but can be of different origin and produced under various conditions. Solar evaporation techniques are used, salt is mined or pumped as brine from underground salt layers and processed yielding evaporated salt of the highest purity. Although the presence of halophilic bacteria has little relevance in respect to food safety, they



play an important role in product quality due to odour development, discoloration and proteolysis of the casing due to bacterial outgrowth.

Known halophilic bacteria to cause "red dog" (Rust, 1988) on the surface of casings are *Halobacterium salinarum* and *H. cutirubrum* and require a salt concentration of more than 150 g NaCl per litre water. These bacteria are Gram negative facultative aerobic, non-spore forming rods or cocci, depending on growth conditions (Labots, 1967; DasSarma 2001). They produce a red-orange carotenoid pigment for self-protection against the high levels of ultraviolet radiation in their normal habitat (salt lakes, solar evaporation ponds) and can also produce buoyant gas vesicles. Its purpose is to enable these bacteria, whose primary metabolism is aerobic, to float to the more oxygenated surface areas (DasSarma 2001).

Studies done by Labots and Krol (1964) indicated that increased growth occurs at salt concentrations higher than 20% (wt/wt), temperatures higher than 20 °C and neutral pH. Removal of halophilic bacteria was suggested by flushing casings in potable water (> 30 minutes) and storage at temperatures around 10 °C to prevent outgrowth of any remaining bacteria.

Wirth (1990, 1994) reported on the microbial contamination of casing samples from various origins after prolonged storage (3, 6 & 12 months) at 4 or 22 °C. Results showed only a clear reduction in halophilic bacteria after prolonged storage for 12 months at either storage temperature. Results also showed that the origin of the casings and therefore the origin of the salt played a major role in the original contamination with halophilic bacteria.

Bakker et al. (1999) used freshly cleaned casings in their experiments to determine the effects of initial curing with additives on microbial quality and mechanical properties. Salt of unknown origin was used and halophilic bacteria and presence of "red dog" were confirmed in slush cured and dry cured sheep casings stored at different temperatures for 3 and 6 months. It was shown that, whether curing additives (citrate, lactate or phosphate) were used or not, spoilage occurred by halophilic bacteria with the distinctive red discoloration and smell occurring after 3 months storage at 20-40 °C and after 6 months also at 10 °C in slush cured sheep casings.

These results confirm the original findings by Wirth (1990, 1994) that halophilic bacteria can remain present after prolonged storage. However, they also contradict the results found by Labots and Krol (1964) that a lower storage temperature prevents further outgrow. Most likely a combination of original contamination, storage time and temperature determine whether halophilic remain present. As a result the focus should lie on prevention instead of elimination. Adequate control of salt quality and prevention of cross-contamination of contaminated batches should further reduce the loss of quality due to halophilic bacteria.

### **Recent developments in the preservation of casings**

Several studies have been done recently on the usability of gamma irradiation as preservation method for casings (Trigo and Fraqueza 1998; Byun et al., 2001; Jo et al., 2002), culminating in the study by Chawla et al. (2006) on the inclusion of gamma irradiation into the principle of Hurdle Technology.

Trigo and Fraqueza (1998) used fresh, locally produced, hog casings and dried beef casings. Analysis of the bacterial population prior to irradiation yielded high counts (i.e. total aerobic count  $7.54 \log_{10}$  cfu / g casing), which could only be fully eliminated in the fresh hog casings after exposure to 10 kGy of gamma radiation.

The studies by Byun et al. and Jo et al. (2001; 2002, one experimental set-up, two separate articles), used freshly salted and semi-dried hog and sheep (lamb) casings. Casings were exposed to gamma radiation either as salted product or after washing in de-ionized distilled water, used for sausage production (Bratella Weiss Wurst) and subsequently a bacterial and sensory evaluation was done. A combination of washing and exposure to 5 kGy was sufficient to eliminate all Enterococci and coliform bacteria, although other aerobic bacteria remained present. Sausages produced using irradiated casings had an increased tenderness at both 3 and 5 kGy but there were no significant differences found in the sensory analysis in comparison to non-irradiated sausages (flavour, colour, texture).

The final study by Chawla et al. (2006) used fresh, locally produced, sheep (lamb) casings, salted with NaCl (10% w/w) to reduce water activity from 0.95 to 0.80, which were irradiated at 5 and 10 kGy and stored at ambient temperatures. Microbiological analysis was done up to 90 days post irradiation and sensory analyses of sausages produced with these casings were done at day 0 and day 30 post irradiation. Only the highest dose of 10 kGy succeeded in eliminating the total aerobic count ( $>10^6$  cfu / g casing) and spores of sulphite reducing *Clostridia* ( $10^3$  cfu / g casing). The sensory evaluation (colour, odour, texture) revealed no significant differences at either interval or radiation dose. Contrary to Byun et al. (2001), no textural changes indicating an increased tenderness were observed.

Based on these studies a combination of reduced water activity and radiation processing can improve the safety of natural casings without affecting their functional properties. However, in general casings are stored for a prolonged time in either dry salt or saturated brine prior to sausage production. For instance, a study done by Gabis and Silliker (1974) showed that after 3 weeks of storage in dry salt at 6 °C, no *Salmonella* could be found. Secondly, Houben (2005) found only sulphite reducing *Clostridia* spores in dry-salted hog and sheep casings. These studies may question the necessity to use gamma irradiation as another hurdle whereas preservation in salt after a certain storage period is sufficient to remove microbial contamination of casings, with bacterial spores as the known exception.

Ozonated water was used in a study by Benli et al. (2008) to determine its efficacy on the preservation of hog casings, taking into account its effects on the biomechanical properties after treatment. Ozone is a strong oxidant and ozonated water has been reported to effectively kill spoilage micro-organisms, environmental and faecal contaminants and food-borne pathogens in low ozone demand media. However, a high protein environment negatively affects the stability of dissolved ozone. Results indicated that prolonged exposure of casings to ozone, necessary to obtain a relevant reduction of resident micro-organisms, would lead to a substantial weakening of the casing. Subsequently the use of this technique for the preservation of casings was rejected.

### **Remark**

The text from the General Introduction was included in the PhD thesis of Dr. Joris Wijnker, which he obtained on January 8<sup>th</sup> 2009 at Utrecht University in The Netherlands. A great achievement by itself, but more importantly, a clear step forward for the natural casing industry as it underlines the validity and necessity to provide solid scientific information for the casing industry.

## *CHAPTER 2*

---

### **PUBLIC HEALTH**

## 2.1

### Microbiological investigation of natural casings

Author F. Wirth<sup>1</sup>

#### Report

Investigation No. 60-81, 1990

Investigation No. 34-55, 1994

#### Summary

In 1990 the German Zentralverband Naturdarm (ZVN) commissioned a study on the microbial aspects of casings after prolonged storage at 4 and 22 °C. In addition, the usability of these casings for further processing was also evaluated. Results showed that storage at 22 °C provided lower bacterial counts than 4 °C, although the quality of casings was better preserved after storage at lower temperatures.

It was concluded that most likely the influence of light and higher oxygen concentrations were detrimental to the quality loss, a situation specific for this experiment and in contrast to the general storage in closed casks.

All these results indicated that the high bacterial counts found during and directly after the cleaning procedure could be countered by correct curing and subsequent storage in dry-salt or brine.

#### Implementation

The results from these studies formed the basis for the microbiological recommendations made by ENSCA in November 1996.

	Fully acceptable	Maximum value	Reference
Total aerobic count	$<1.0 \times 10^5$	$5.0 \times 10^6$	ISO 4833
<i>Enterobacteriaceae</i>	$<1.0 \times 10^2$	$1.0 \times 10^4$	ISO 21528-2
<i>Staphylococcus aureus</i>	$<1.0 \times 10^2$	$1.0 \times 10^3$	ISO 6888-1
Sulphite reducing <i>Clostridium</i> - spores	$<1.0 \times 10^2$	$1.0 \times 10^3$	ISO 15213

Table 1: Recommended microbiological values (colony forming units per gram)

These microbiological values were included in the ENSCA community Guide to Good Practice for Hygiene and the application of the HACCP principles in the production of natural sausage casings and the INSCA HACCP Guide.

---

<sup>1</sup>Federal Office for Meat Research, Institute for Technology, Kulmbach, Germany

## 2.2

---

### Study on emerging pathogens in natural sausage casings

**Author** J. Bockemühl<sup>2</sup>

#### **Report**

ENSCA report May 9<sup>th</sup>, 2000

#### **Summary**

The objectives of this study were to investigate the incidence of *Salmonella*, *Listeria*, *Shiga toxin-producing Escherichia coli* (STEC) and *Campylobacter* in freshly harvested natural sausage casings and to examine the effect of traditional salting of the casings on the elimination of the mentioned organisms after 30 days of salt treatment at 15 °C.

In total 330 casing samples were tested, including beef rounds (30), middles (30) and bung (30), sheep casings (60) and hog casings (60), chitterlings (60) and fat ends (60).

Results showed a moderate contamination rate of 5 to 10% for *Salmonella*, *Listeria* and *Campylobacter* in fresh beef and sheep casings which were fully eliminated after storage for 30 days.

In hog casings, *Salmonella* was found in 53% of the fresh samples, *Listeria* in 7% and *Campylobacter* in 16%. After storage for 30 days in salt at 15 °C, *Salmonella* was found in 4% and *Listeria* in 2%. *Campylobacter* was no longer present.

STEC was not found in any sample tested in this study.

#### **Recommendation**

Based on the outcome of this study it was decided to do a large scale survey on the presence of *Salmonella*, *Listeria* and *Clostridium* spores on casing samples with various origins after prolonged storage in salt.

---

<sup>2</sup>Abteilung Bakteriologie, Hygiene Institut Hamburg, Postfach 261551, 20505 Hamburg, Germany

## 2.3

---

### **A survey of dry-salted natural casings for the presence of *Salmonella* spp., *Listeria monocytogenes* and sulphite-reducing *Clostridium* spores**

**Author** J.H. Houben<sup>3</sup>

#### **Journal**

Food Microbiology, 22 (2005) 221-225

#### **Summary**

A monitoring study was performed for the presence of *Salmonella* spp., *Listeria monocytogenes* and sulphite-reducing *Clostridium* spores in dry-salted natural hog and sheep casings. These casings were sourced from New Zealand (sheep casings), United Kingdom (sheep casings), China (hog and sheep casings) and The Netherlands (hog casings).

Two hundred and fourteen consignments were examined for *Salmonella* spp. and *L. monocytogenes*, and 138 for the *Clostridium* spores.

- None of the 214 sampled consignments (25 g per sample investigated) were found to contain any *Salmonella* spp., or *L. monocytogenes*.
- Fourteen percent of the 21 New Zealand sheep casing samples were found to contain *Clostridium* spores, with colony forming units (cfu) per gram counts ranging from 3 to 45.
- Five percent of the 19 UK sheep casing samples were found to contain *Clostridium* spores, with cfu per gram counts ranging from 5 to 18.
- Sixty percent of the 35 Chinese hog casing samples were found to contain *Clostridium* spores, with cfu per gram counts ranging from 3 to 2500. Three of these samples had counts over 100 cfu per gram and only 1 higher than 1000 cfu per gram.
- Fifty-nine percent of the 22 Chinese sheep casing samples were found to contain *Clostridium* spores, with cfu per gram counts ranging from 10 to 1180. Seven of these samples had counts over 100 cfu per gram and 2 higher than 1000 cfu per gram.
- Ten percent of the 41 Dutch hog casing samples were found to contain *Clostridium* spores, with cfu per gram counts ranging from 3 to 10.

The relevance of the presence of sulphite-reducing *Clostridium* spores for the manufacture of various meat products is discussed. Regular preservation and processing techniques of these meat products can generally prevent any spoilage due to the outgrowth of the *Clostridium* spores. However, this will not fully eliminate the risk of having *Clostridium* spores present on the casings and efforts must be made to further reduce any presence of spores.

#### **Recommendation**

This study also included a comparison on the efficacy of two different agars. Results show that Iron Sulphite Agar (ISA) overall showed higher *Clostridia* counts as compared to differential reinforced clostridial agar (DRCA). It is

---

<sup>3</sup>Institute for Risk Assessment Sciences, Division Veterinary Public Health, P.O. Box 80.175 NL-3508 TD, University of Utrecht, The Netherlands

therefore recommended to use ISA as a standard medium for future studies on *Clostridia*.

In addition it is recommended that determination of the *Clostridium* strains present on natural casings is carried out and their properties investigated in relation to the manufacture of meat products, since some of the strains may be potentially pathogenic and/or able to spoil products.

## 2.4

---

### **Antimicrobial properties of salt (NaCl) used for the preservation of natural sausage casings**

**Authors** J.J. Wijnker<sup>4,5</sup>, G. Koop<sup>6</sup>, L.J.A. Lipman<sup>4</sup>

#### **Journal**

Food Microbiology, 23 (2006) 657-662

#### **Summary**

Although salt (NaCl) has been used for thousands of years as preservative of natural sausage casings, the influence of a reduced water activity level ( $a_w$ ) due to a high salt concentration has never been studied.

The object of this study was to examine the antimicrobial properties of salt at different water activity levels (Figure 5 & 6), made visible by the supposedly reduced survival of six relevant food pathogens added to casings preserved in dry salt and several different brine concentrations. The following bacteria were used: *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens* and *Escherichia coli* O157:H7.

Additionally, water activity was measured as a novel parameter with possible predictive qualities for the preservation in dry salt and brines.

These results indicate that the antimicrobial properties of salt used for the preservation of natural casings are sufficient to reduce the original bacterial contamination during the cleaning process (except for *Clostridium* spores) well below acceptable levels at a water activity level of 0.85 or lower during a 30-day storage period.

The results found in this study can be directly applicable to the casing industry and may support the known antimicrobial properties of salt used in the traditional methods for the preservation of natural sausage casings. By measuring the water activity level at which no bacteria could be found or where bacteria were below any critical limit, a novel parameter for salt-preserved natural casings becomes available during routine Quality Control Inspection and monitoring of the preservation process.

#### **Implementation**

Preservation of casings in dry salt or saturated brine ( $a_w \leq 0.85$  /  $\geq 22$  °Baumé) and storage for a period of 30 days have been included as Critical Control Points (CCP's) in the ENSCA community Guide to Good Practice for Hygiene and the application of the HACCP principles in the production of natural sausage casings and the INSCA HACCP Guide.

By adequate implementation of these CCP's, casings can be stored at ambient temperatures for prolonged periods of time without an increased food safety risk.

---

<sup>4</sup>Institute for Risk Assessment Sciences, Division Veterinary Public Health, P.O. Box 80.175 NL-3508 TD, University of Utrecht, The Netherlands

<sup>5</sup>VPH Consultancy, Leusden, The Netherlands

<sup>6</sup>Division of Ruminant Health Care, Faculty of Veterinary Medicine, P.O. Box 80.151 NL-3508 TD, University of Utrecht, The Netherlands



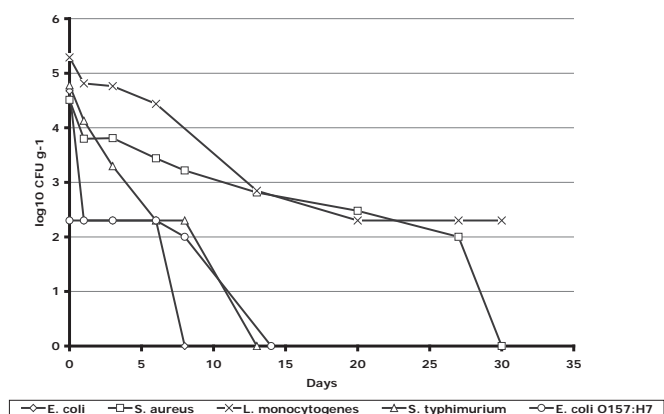


Figure 5: Bacterial reduction over time at a water activity level of 0.85

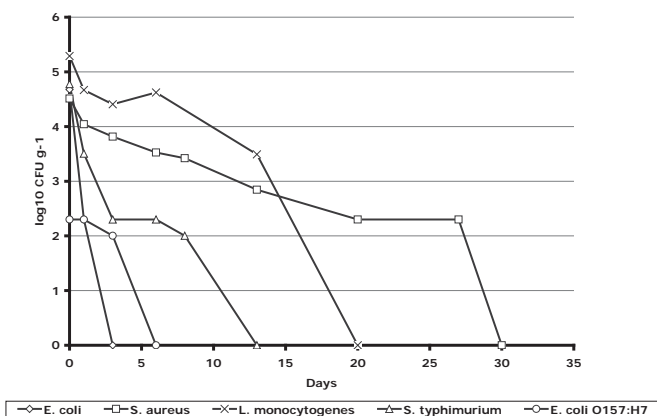


Figure 6: Bacterial reduction over time at a water activity level of 0.75

### **Reduction of *Clostridium sporogenes* spore outgrowth in natural sausage casings using nisin**

**Authors** J.J. Wijnker, E.A.W.S. Weerts<sup>7</sup>, E.J. Breukink<sup>8</sup>, J.H. Houben, L.J.A. Lipman

**Journal**

Food Microbiology, 28 (2011) 974-979

**Summary**

Preservation of natural sausage casings using dry salt or saturated brine is regarded as sufficient to inactivate vegetative pathogenic non-sporeforming bacteria present on the casings. Although the outgrowth of bacterial spores is prevented by salt or saturated brine preservation, these spores will remain present and develop into vegetative cells when conditions are more favourable. To prevent subsequent outgrowth additional preservation measures should be implemented. In the experiments described the use of nisin was evaluated to reduce outgrowth of spores in desalinated casings. The bacteriocin nisin was chosen because of its known efficacy against spore-forming bacteria and their spores in various foodstuffs. *Clostridium* spore suspensions (*C. sporogenes*, ATCC 3584) were used in 2 concentrations to inoculate 3 nisin concentrations (10, 50, 100 µg / mL) in water containing gamma-irradiated casings. Additionally, the binding of nisin to casings, using <sup>14</sup>C-labeled nisin Z and subsequent availability of nisin were evaluated. Results demonstrate that nisin is partly reversibly bound to casings and can reduce the outgrowth of *Clostridium* spores in the model used by approximately 1 log<sub>10</sub> (90%). However, the biological relevance of these results needs to be determined further by conducting industrial trials before any recommendation can be made on the practical implementation of nisin in the preservation of natural sausage casings.

---

<sup>7</sup>Pathology Division, Department of Pathobiology, Faculty of Veterinary Medicine, P.O. Box 80.158 NL-3508 TD, Utrecht University, the Netherlands

<sup>8</sup>Department of Biochemistry of Membranes, Bijvoet Center, Institute of Biomembranes, P.O. Box 80075 NL-3508 TB, Utrecht University, the Netherlands

### **Bacterial spores on natural casings and means of control or elimination**

**Author** B.R. Berends<sup>9</sup>

**Journal**

ISWG report (Project No. 1002) September 2011

**Summary**

This paper provides for an overview of available methods that may control or eliminate spores in and on natural casings, and evaluates which of these methods may be used in the production process without detrimental effects on the technological or organoleptic properties of natural casings.

Part one provides for background knowledge that is needed for considering the reviewed methods of control or elimination.

Part two is the actual overview of scientific publications about methods for control or elimination of spores in natural casings, sausages or meat.

Part three is a concise discussion about which of these (new) techniques could be used by the casing industry for new or optimized ways of controlling or eliminating bacterial spores without compromising the technological or organoleptic properties of natural casings. Recommendations for further research are made also.

The main three conclusions are that:

- 1) Currently, salted natural casings seem to be a relatively safe product, despite the presence of spores of several *Clostridium* species after 30 days storage in salt at ambient temperatures, and that the fundamental question whether the consumer health risks of this product are unacceptable high or not still has to be answered. Without such knowledge the necessity for total destruction of spores has, in fact, not been proven;
- 2) If, despite this, the industry wishes to implement methods that are able to destroy spores at the short term, irradiation is the only of-the-shelf solution available, since all other potential approaches need further research;
- 3) Much can be gained with regard to contamination levels of fresh casings by critical evaluation of current pre harvest and harvest procedures.

The three main recommendations are:

- 1) Global assessment of the prevalence of specific *Clostridium* and/or *Bacillus* species in natural casings is relevant for a better understanding of possible food safety risks and risk factors;
- 2) Construction of a Risk Assessment model will assist in the quantification of any food safety risk connected to natural casings;
- 3) Additional research is required into improving hygiene of the pre- and post-harvest phase, currently used cleaning procedures and the hygienic quality of recirculated process water.

---

<sup>9</sup>Institute for Risk Assessment Sciences, Division Veterinary Public Health, P.O. Box 80.175 NL-3508 TD, Utrecht University, The Netherlands

### Establishing safe thresholds for spore formers in the casings industry

**Authors** W. Alkema<sup>10</sup>, I. Mierau<sup>10</sup>, S. Stringer<sup>11</sup>

#### **Journal**

ISWG report July 2013

#### **Summary**

Establishing correct and safe threshold values for the occurrence of spore forming organisms on natural casings is important for the casings industry. The industry wanted to know whether the current threshold values are valid and scientifically sound or if there is scientific or other data that suggests that these values should be adjusted.

In order to evaluate the validity of the current threshold values, a desk study into the scientific literature and regulatory guidelines related to food and spore forming organisms was performed. In this study several thousands of scientific abstracts were evaluated and a large number of full text papers were analysed in detail.

The literature indicates that *C. perfringens* and *B. cereus* are ubiquitously present but that the prevalence of food poisoning is generally low in sausages. Although there is considerable difference in the guidelines for the levels of *C. perfringens* and *B. cereus* that are acceptable in food samples, the trend is that levels may not exceed  $\sim 10^3$  and  $10^4$ - $10^5$  cfu/g for *C. perfringens* and *B. cereus* respectively in food products.

Since casings constitute 1-2% of the total weight of the sausage,  $10^3$  cfu/g spores in a casing, which is the current lower limit of unacceptable in the casings industry, translates to 10 – 20 cfu/g in the final product, which is well below the current microbiological standards. The current literature therefore does not suggest that the current threshold for *C. perfringens* levels in the casings industry should be adjusted.

#### **Recommendation**

Based on the outcome of this study the following recommendations can be made to change the current approach on microbiological testing:

- 1) Prior to this study it was recommended to test on the presence of sulphite reducing *Clostridium*-spores, using reference method ISO 15213 (see also Chapter 2.1). This can be amended to test specifically on *C. perfringens*, using ISO method 7937. The current recommended values,  $10^2$  cfu/g acceptable and  $10^3$  cfu/g maximum need not be amended.
- 2) It can also be recommended to include standard testing on *B. cereus* using ISO method 7932. Recommend values can be  $10^4$  cfu/g acceptable and  $10^5$  cfu/g maximum.

---

<sup>10</sup>NIZO food research B.V., P.O. Box 20 NL-6710 BA, Ede, The Netherlands

<sup>11</sup>Institute of Food Research, Colney, Norwich NR4 7UA, United Kingdom

## 2.8

### Shelf life of natural sausage casings

**Author** J.J. Wijnker

**Journal**

ISWG report May 2009

#### Introduction

The efficacy of salt as preservative for natural sausage casings has been known for many years and has been described in various studies (Gabis and Silliker, 1974; Wirth, 1990, 1994; Bakker et al., 1999; Houben, 2005; Wijnker et al., 2006). However, the maximum storage period described in these studies has been 12 months, while empirical data indicate that even prolonged storage has no negative effect on the microbiological stability, quality and usability of salted casings.

In order to determine whether a maximum period can be determined for the shelf life of salted casings, randomly chosen samples have been tested over the past years. This report summarizes the outcome of these tests, providing background of the samples included, microbiological results and quality control reports.

All microbiological tests were done by Silliker BV, Ede, The Netherlands and results evaluated according to the 1996 ENSCA microbiological recommendations (see Chapter 2.1) for salted natural casings as incoming product at meat processing establishments (entrance control).

This report is used as a reference document on shelf life in the ENSCA community Guide to Good Practice for Hygiene and the application of the HACCP principles in the production of natural sausage casings and the INSCA HACCP Guide.

#### Test 2005

Background of the samples: samples were taken from the casings that served as negative (untreated) controls in the study by Verkleij et al. (TNO report V5070, 2003).

The study by Verkleij was started in 2002; the casings originated from Van Hessen bv and were at least 1 year old (produced 2001).

	Hog CN origin No. 15	Hog CN origin No. 19	Hog NL origin No. 20	Sheep CN origin No. 25	Sheep NZE origin No. 40
Total aerobic count	<100	100	900	<100	100
<i>Enterobacteriaceae</i>	<10	<10	<10	<10	<10
<i>Staphylococcus aureus</i>	<100	<100	<100	<100	<100
Sulphite reducing	660	0	80	70	90
<i>Clostridium</i> - spores					

Table 2: Results (CFU per gram casing) per casing sample tested of different species and origins

- The microbiological counts are well below the accepted maximum values;
- QC reports show that all tested samples were still within acceptable limits regarding quality and usability;
- Conclusion: a shelf life of 4 years is possible.

### Test 2007

Background of the samples: Based on a company stock aging report the oldest two lots were chosen and sampled.

- Sample 1 was taken from tubed sheep casings, Australian origin, production date 2002;
- Sample 2 was taken from beef casings, Brazilian origin, production date 2002.

	Sheep casings AUS origin	Beef casings BRA origin
Total aerobic count	<100	100
<i>Enterobacteriaceae</i>	<10	<10
<i>Staphylococcus aureus</i>	Not tested	Not tested
Sulphite reducing	20	30
<i>Clostridium</i> - spores		

Table 3: Results (CFU per gram casing) per sample tested

- The microbiological counts are well below the accepted maximum values;
- QC reports show that all tested samples were still within acceptable limits regarding quality and usability;
- Conclusion: a shelf life of 5 years is possible.

### Test 2008

Background of the samples: Based on a company stock aging report the oldest two lots were chosen and sampled.

- Sample 1 was taken from tubed sheep casings, Australian origin, production date 2005;
- Sample 2 was taken from tubed sheep casings, New-Zealand origin, production date 2005.

	Sheep casings AUS origin	Sheep casings NZE origin
Total aerobic count	200	200
<i>Enterobacteriaceae</i>	<10	<10
<i>E.coli</i>	<10	<10
<i>Staphylococcus aureus</i>	<100	<100
Sulphite reducing <i>Clostridium</i> - spores	80	70
<i>Campylobacter</i> spp. thermo tolerant	Absent	Absent
<i>Listeria Monocytogenes</i>	Absent	Absent
<i>Salmonella</i>	Absent	Absent

Table 4: Results (CFU per gram casing) per sample tested

- The microbiological counts are well below the accepted maximum values;
- QC reports show that all tested samples were still within acceptable limits regarding quality and usability;
- Conclusion: a shelf life of 3 years is possible.

### Overall conclusions

All tested casing samples were microbiologically acceptable after storage at temperatures below 20 °C in saturated brine or dry salt for a period up to 5

years. Quality Control reports show that these casings remain usable for their intended use, to serve as edible containers for sausages.

Results are in line with the findings included in the afore mentioned studies and with the recommended microbiological values included in the ENSCA community Guide to Good Practice for Hygiene and the application of the HACCP principles in the production of natural sausage casings and in the INSCA HACCP Guide.

Based on these findings it can be stated that a shelf life for salted natural sausage casings of at least 4 years is possible while remaining suitable for human consumption.

## 2.9

### Nutritional values of natural sausage casings

**Author** J.J. Wijnker

#### Journal

ISWG report April 2012

#### Introduction

Due to the increased demand from consumers and sausage producers to provide accurate information on ingredients, a study was done on the nutritional value of natural sausage casings.

In table 1 an overview is presented of the results for sheep, hog and beef casings. Multiple samples have been included per species result, with a large variety in geographical origin, quality, diameter and production unit.

The analyses have been done by Eurofins<sup>12</sup> and the results can be regarded as indicative for all natural casings.

Some data were previously available in Ockerman and Hansen (2000)<sup>13</sup>, based on the 1995 CRAFT project and are in line with the current findings.

Unit	Energy kcal / 100 g	Energy kJ / 100 g	Carbohydrates %	Protein Kjeldahl %	Protein as nitrogen %	Collagen %	Hydroxyproline %	Collagen/protein (as N) ratio	Moisture %	Fat %	Saturated fat (as % of Fat) %	Ash %	Residual salt as sodium g / 100 g	Residual salt as NaCl g / 100 g
Sheep casings	82	344	2	17	3	16	2	0.19	79	< 0.5	n.a.	26	0.1	0.3
Hog casings	88	370	2	18	3	16	2	0.19	80	1	44	22	0.2	0.4
Hog fat ends	265	1112	2	11	2	3	1	0.67	60	24	59	20	1	3
Beef casings	116	486	< 1.0	19	3	9	1	0.33	75	4	66	24	0.4	1

n.a.: fat content too low for saturated fat analysis

Table 5. Nutritional values of natural sausage casings

For comparison, the nutritional values of three common sausage types are presented. Please note that all data is either as percentile or per 100 g product. Also bear in mind that a sausage consists of 1-2 % natural casing.

Using the amount of salt (as sodium) as an example, natural casings contain 0.1-1.0 g salt (as sodium) per 100 g. Per 100 g of sausage this would be: 1-20 mg of salt (as sodium).

When compared to the amounts of salt (as sodium) present in the three examples, the contribution of the salt in the natural casings is less than 3% of

<sup>12</sup>Eurofins Analytico Food, Heerenveen, The Netherlands ([www.eurofins.nl](http://www.eurofins.nl))

<sup>13</sup>Ockerman, H. W., and C. L. Hansen (ed.). 2000. Sausage Containers, p. 285-323. In: Animal By-Product Processing and Utilization. CRC Press, Boca Raton, FL.

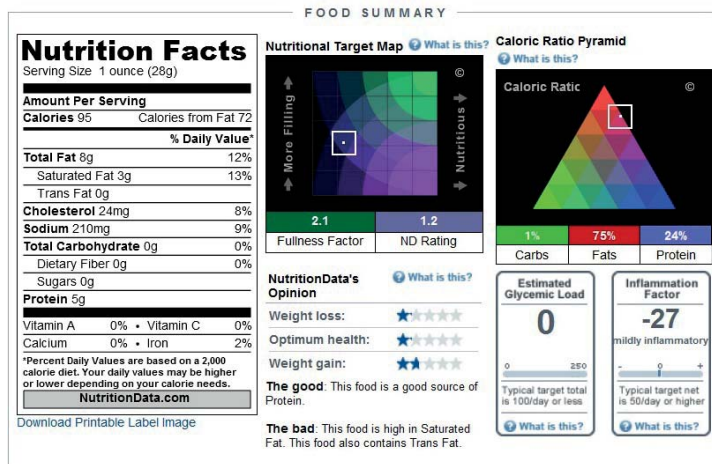


the total amount in the product. In all it can be concluded that the contribution of natural casings to the nutritional value of sausages is extremely limited.

## Pork sausage, fresh, cooked

Serving size: 100 grams

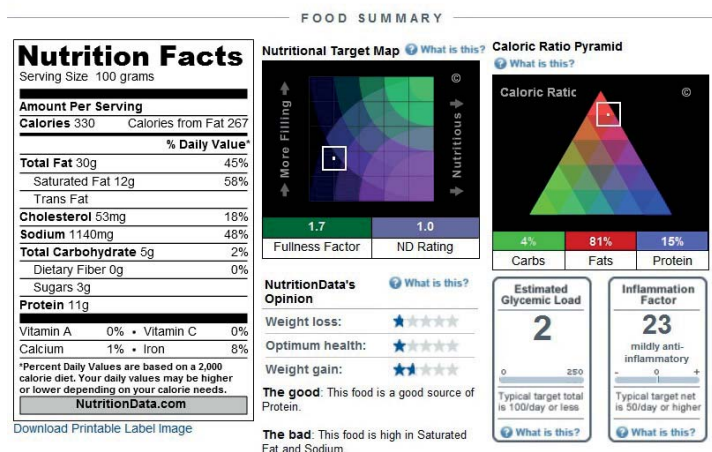
[Add to Tracking](#)  
[Add to Compare](#)  
[Create Recipe](#)  
[Add to My](#)



## Frankfurter, beef [frank, hot dog, wiener]

Serving size: 100 grams

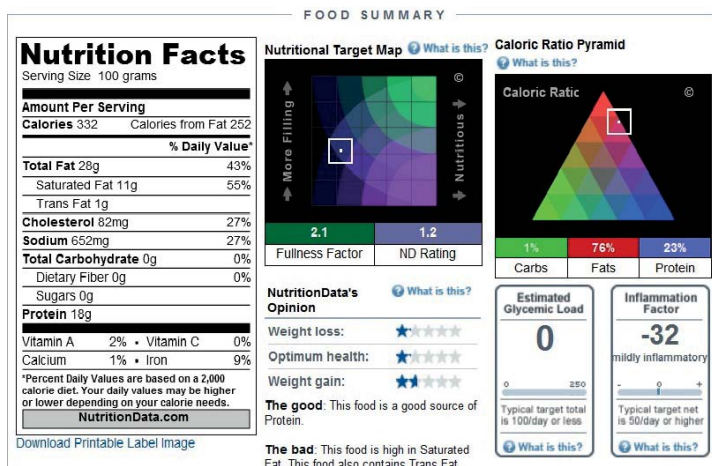
[Add to Tracking](#)  
[Add to Compare](#)  
[Create Recipe](#)  
[Add to My](#)



## Beef sausage, fresh, cooked

Serving size: 100 grams

[Add to Tracking](#)  
[Add to Compare](#)  
[Create Recipe](#)  
[Add to My](#)



### **Substances and residues in natural sausage casings (2000)**

**Authors** B.R. Berends, A.A. Bergwerf<sup>14</sup>, J.H. Houben

### **Report on the analysis of natural casings from third countries (non EU countries) to detect residues of pharmacological substances (2001)**

**Author** H. Schmidt<sup>15</sup>

#### **Summary**

The first desk-top study on this subject showed that there was little scientific interest on possible residues in casings. As a consequence, the literature search evolved into a theoretically based provisional assessment on the likelihood that certain (unwanted) substance might actually pose a problem in (dry-salted) casings. Of all about 50 globally evaluated drugs and contaminants (as principle compounds listed in Directive 96/23/EC) there are compounds in a few categories that in daily practice might be considered as potentially problematic. These "potentially problematic" substances are the organochlorine compounds and certain mycotoxins, primarily the aflatoxins and the ochratoxins.

Furthermore, also based on theoretical grounds, it can be stated that the influence of processing of natural sausage casings, as performed under current Good Commercial Practices, will be such that even in the case a particular animal contains residues or contaminants in levels above a Maximum Residue Limit, this does not automatically mean that the (dry-salted) casings derived from this animal also contain health-threatening levels of the respective compounds, irrespective the maximum amount of casing that a person can consume.

To substantiate the theoretically based statements and assumptions in this report, it was necessary to conduct scientific research into these matters. A surveillance investigation was advised on the current situation of possible contamination of (dry-salted) casings imported from selected regions.

Based on this recommendation a study was done by Prof. Schmidt in 2001 on the presence of residues in casings from various origins outside the EU. The following origins were included:

- Bovine casings from Brazil and Uruguay;
- Sheep casings from Iran, Turkey, China, New Zealand and Australia;
- Hog casings from China, USA and Poland.

---

<sup>14</sup>Institute for Risk Assessment Sciences, Division Veterinary Public Health, P.O. Box 80.175 NL-3508 TD, Utrecht University, The Netherlands

<sup>15</sup>Tiergesundheitsdienst Bayern e.V. Animal Hygiene Department, 85586 Poing, Germany

Analyses were done to detect residues of the following pharmacological substances:

- Antimicrobial substances: sulfonamids, tetracyclines including 4 epimeres and chloramphenicol;
- Hormones and anabolica;
- Stilbenes;
- Anti-parasitica;
- Benzimidazoles.

According to current EU legislation (Council Directive 96/23/EC, annex I; Council Regulation (EEC) No 2377/90, annex IV), most of these products now are banned from use in food producing animals or no Maximum Residue Limit could be determined.

All samples tested in this study showed **no presence** of any residues of the afore-mentioned substances. Although only 23 samples were tested in this study it did show that any risk on the presence of residues in casings was very limited.

However, since 2001 the analytical standard methods have been changed and limits of detection drastically lowered, allowing for the detection of certain residues at ppb (parts per billion) level. Specific Minimum Required Performance Limits (MRPL) have now been put into place for, amongst others, chloramphenicol and nitrofurans (Commission Decision 2002/657/EC).

This increased sensitivity in the monitoring of residues by the European competent authorities has not led to a dramatic change in the number of incidents with unauthorized residues in casings. The casing industry world-wide has placed much emphasize on the importance of this issue, but as the administration of the veterinary medicinal products is done in the living animal and not in the casings itself, any implemented preventive measure taken by the casing industry is limited at best.

However, in order to be able to assess what the actual consumer exposure risk might be of undesirable residues in natural casings, this subject was included in the 2008 report *The Road Map: Consumer safety of natural sausage casings* (Chapter 6.3) and the paper in Chapter 2.11. Results indicate that casings contribute only to a very minor amount to the overall risk of consumers of exposure to undesirable residues in foods.

### **A simulation model for the prediction of tissue:plasma partition coefficients for drug residues in natural casings**

**Authors** A.M. Haritova<sup>16</sup>, J. Fink-Gremmels<sup>17</sup>

#### **Journal**

The Veterinary Journal, 185 (2010) 278-284

#### **Summary**

Tissue residues arise from the exposure of animals to undesirable substances in animal feed materials and drinking water and to the therapeutic or zootechnical use of veterinary medicinal products. In the framework of this study, an advanced toxicokinetic model was developed to predict the likelihood of residue disposition of licensed veterinary products in natural casings used as envelope for a variety of meat products, such as sausages.

The model proved suitable for the calculation of drug concentrations in the muscles of pigs, cattle and sheep, the major species of which intestines are used. On the basis of drug concentrations in muscle tissue, the model allowed a prediction of intestinal concentrations and residues in the intestines that remained equal to or below the concentrations in muscle tissue, the major consumable product of slaughter animals. Subsequently, residues in intestines were found to be below the maximum residue limit value for muscle tissue when drugs were used according to prescribed procedures, including the application of appropriate withdrawal times. Considering the low consumption of natural casings (which represents only about 1–2% of the weight of a normal sausage), it was concluded that the exposure to drug residues from casings is negligible.

---

<sup>16</sup>Department of Veterinary Pharmacology, Veterinary Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Bulgaria

<sup>17</sup>Department of Veterinary Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

## *CHAPTER 3*

---

### **ANIMAL HEALTH**

## 3.1

---

### **Presence of foot-and-mouth disease virus in organs of infected sheep (Nachweis von Maul- und Klauenseuche-Virus in Organen krank geschlachteter Schafe)**

**Authors** H.O. Böhm<sup>18</sup>, H. Krebs<sup>18</sup>

#### **Journal**

Berliner und Münchener Tierärztliche Wochenschrift, 87 (1974) 410–412  
Die Fleischwirtschaft, 6 (1974) 1051-1053

#### **Summary**

Lactic and citric acid in 0.5 and 2.0% concentrations were tested for their effect on sheep's casings experimentally treated with foot-and-mouth disease (FMD) virus. After 0.5% lactic acid or 0.5% citric acid had been allowed to take effect for 5 minutes on the virus-contaminated casings it was no longer possible to detect infectious virus either in baby mice or BHK<sub>21</sub> cell cultures. The increase in the volume of the casings which was noted after they had been treated with acid had no disadvantageous effect on the filling or the scalding process.

#### **Implementation**

The research project was done in 1974 in response to import restrictions on sheep casings from Iran, where an outbreak of FMD was registered. Within one month after the results were presented the import ban was lifted when casings were treated in the recommended manner. Other countries also adopted this method as a means for exemption of casings from import bans, like Austria before joining the EU.

In April 2001 the recommended treatment using 0.5% citric or lactic acid for 5 minutes was presented during the OIE / FAO conference on foot-and-mouth disease. The conference subsequently recommended for natural casings stricter rules for controls and methods for inactivation of the virus in order to protect all countries against risks arising from the international trade in casings and carry-over via travellers.

At the Annual General Meeting of the OIE in May 2001 the recommendation was transformed into a request to the FMD Working Group of the OIE to come up with a draft for new control requirements on casings. However, the chairman of this Working Group was at that point already informed that the international casing industry would perform tests on the usability of casings after citric / lactic acid treatment. Therefore the chairman agreed to wait with publication of any proposals until the results of this project became available (See also Chapter 4.2).

---

<sup>18</sup>Bundesforschungsanstalt für Viruskrankheiten der Tieren, Tübingen, Germany

## 3.2

---

### **Removal of foot-and-mouth disease virus infectivity in salted natural sausage casings by minor adaptation of standardized industrial procedures**

**Authors** J.J. Wijnker, B. Haas<sup>19</sup>, B.R. Berends

#### **Journal**

International Journal of Food Microbiology, 115 (2007) 214–219

#### **Summary**

Intestines are used for the production of natural casings as edible sausage containers. Derived from animals (pigs and sheep) experimentally infected with FMDV (initial dosage  $10^{7.3}$  pfu / ml, strain O<sub>1Kaufbeuren</sub>), these casings were treated with salt (NaCl) or phosphate supplemented salt and the residual FMDV titres measured. After storage at about 20 °C for 30 days, no remaining infectivity was found after either treatment, whereas casings stored at 4 °C still contained infectivity. Storage of salted casings at about 20 °C for 30 days is already part of the Standard Operating Procedures (included in HACCP) of the international casing industry and can therefore be considered as a protective measure for the international trade in natural sausage casings.

#### **Implementation**

The inactivation of FMDV during 30 days of storage in salt has been regarded as an effective method to prevent the spread of virus via cleaned and scraped casings. This method has been included in the Council Directive 2003/85/EC on Community measures for the control of foot-and-mouth disease (annex VII, part A, point 9) and the OIE Terrestrial Animal Health Code (v. 2013), article 8.6.41.

---

<sup>19</sup>Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Insel Riems, Germany

#### **Inactivation of classical swine fever virus in porcine casing preserved in salt**

**Authors** J.J. Wijnker, K.R. Depner<sup>20</sup>, B.R. Berends

#### **Journal**

International Journal of Food Microbiology, 128 (2008) 411-413

#### **Summary**

Pig intestines used for the production of natural sausage casings may carry classical swine fever (CSF) virus. Feeding pigs with human food waste that contains porcine (hog) casings may then spread the virus to CSF-free animals. Casings derived from a pig experimentally infected with CSF by dosing with  $10^6$  tissue culture infectious doses (TCID<sub>50</sub>) of the highly virulent CSF virus strain "Koslov", were treated with phosphate supplemented or citrate supplemented NaCl, instead of with NaCl alone, which is the standard preservation treatment for casings. Treated casings were stored for 30 days at either 4 °C or 20 °C. After storage the casings were fed to 16 susceptible pigs. CSF infection was confirmed in the four animals that had been fed casings treated with citrate supplemented salt and stored at 4 °C. All other animals remained healthy. It is therefore possible to avoid the inadvertent spread of CSF virus via porcine sausage casings by treating casings with phosphate supplemented salt and storing them for 30 days at temperatures over 4 °C.

#### **Implementation**

The inactivation of CSFV during 30 days of storage in phosphate supplemented salt has been regarded as an effective method to prevent the spread of virus via cleaned and scraped casings. This method has been included in OIE Terrestrial Animal Health Code (v. 2013), article 15.2.14.

---

<sup>20</sup>Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Insel Riems, Germany



### 3.4

---

#### **Virus inactivation by salt (NaCl) and phosphate supplemented salt in a 3D collagen matrix model for natural sausage casings**

**Authors** T. Wieringa-Jelsma<sup>21</sup>, J.J. Wijnker, E. M. Zijlstra-Willems<sup>21</sup>, A. Dekker<sup>21</sup>, N. Stockhofe-Zurwieden<sup>21</sup>, R. Maas<sup>21</sup>, H.J. Wisselink<sup>21</sup>

#### **Journal**

International Journal of Food Microbiology, 148 (2011) 128-134

#### **Summary**

Due to possible presence and spread of contagious animal viruses via natural sausage casings the international trade in these food products is subject to veterinary and public health requirements. In order to manage these restrictions we determined the effect of casing preservation on four highly contagious viruses for livestock: foot-and-mouth-disease virus (FMDV), classical swine fever virus (CSFV), swine vesicular disease virus (SVDV) and African swine fever virus (ASFV). We used an *in vitro* 3D collagen matrix model in which cells, infected with the four different viruses were embedded in a bovine collagen type I gel matrix and treated with either saturated salt (NaCl) or phosphate supplemented saturated salt at four different temperatures (4, 12, 20 & 25 °C) during a period of 30 days. The results showed that all viruses were faster inactivated at higher temperatures, but that stability of the various viruses at 4 °C differed. Inactivation of FMDV in the 3D collagen matrix model showed a clear temperature and treatment effect on the reduction of FMDV titres. At 4 and 12 °C phosphate supplemented salt showed a very strong FMDV inactivation during the first hour of incubation. Salt (NaCl) only had a minor effect on FMDV inactivation. Phosphate supplemented salt treatment increased the effect temperature had on inactivation of CSFV. In contrast, the salt (NaCl) treatment only increased CSFV inactivation at the higher temperatures (20 °C and 25 °C). Also SVDV inactivation was increased by phosphate supplemented salt, but salt (NaCl) treatment only resulted in a significant decrease of SVDV titre at a few time points. The ASFV results showed that both salt (NaCl) and phosphate supplemented salt were capable to inactivate ASFV within 48 hours. In contrast to the other viruses (FMDV, CSFV and SVDV), ASFV was the most stable virus even at higher temperatures. The results obtained in this *in vitro* model underline the efficacy of a combined treatment using phosphate supplemented salt and storage at 20 °C or higher for a period of 30 days. This treatment may therefore be useful in reducing the animal health risks posed by spread of contagious animal viruses by international trade of natural sausage casings.

---

<sup>21</sup>Central Veterinary Institute of Wageningen UR, P.O. Box 65, NL-8200 AB Lelystad, The Netherlands

### **Inactivation of foot-and-mouth disease virus in various bovine tissues used for the production of natural sausage casings**

**Authors** J.J. Wijnker, B. Haas, B.R. Berends

**Journal**

International Journal of Food Microbiology, 153 (2012) 237-240

**Summary**

Bovine intestines, bladders and oesophagus are used for the production of natural casings ("beef casings") as edible sausage containers. Derived from cattle experimentally infected with FMDV (initial dosage  $10^4$  TCID<sub>50</sub> / mL, strain A Iran 97), these beef casings were treated with sodium chloride (NaCl) or phosphate supplemented salt (P-salt). In addition, different *in-vitro* experiments using beef casings were done on a small scale with other FMDV strains (A Turkey 06, C-Oberbayern and O<sub>1</sub> Manisa) as "proof of principle".

Based on the combined results of the *in-vivo* and *in-vitro* experiments, it can be concluded that the storage period of 30 days at 20 °C in NaCl is sufficiently effective to inactivate a possible contamination with FMDV in beef casings and that the usage of P-salt does not clearly enhance the inactivation of FMDV infectivity.

Storage of salted beef casings at about 20 °C for 30 days is already part of the Standard Operating Procedures (included in HACCP) of the international casing industry and can therefore be considered as a protective measure for the international trade in natural casings.

### **Scientific Opinion on animal health risk mitigation treatments as regards imports of animal casings**

**Authors** EFSA Panel on Animal Health and Welfare (AWAH)

Hearing expert: Dr. J.J. Wijnker

#### **Journal**

EFSA Journal, 10(7) (2012) 2820

#### **Summary**

Salting with NaCl for 30 days is a well-established and accepted procedure in the casings industry and it has been the standard animal health risk mitigation treatment prescribed in EU legislation for many years. This opinion reviews (i) improvements in the NaCl treatment that would lead to an increased level of safety to avoid transmission of animal pathogens, (ii) alternative treatments that could have been developed giving equivalent or better results in the inactivation of relevant pathogens, and (iii) provides an assessment of the phosphate-salt treatment recommended by OIE for foot and mouth disease virus, in particular if it could be considered safe as regards the elimination of other animal pathogens. The rate of inactivation of viruses was highly dependent on temperature for both NaCl and phosphate-NaCl treatment. Treatment with phosphate-NaCl mixture leads to faster inactivation than treatment with NaCl salt alone. *Brucella* species are readily inactivated by NaCl salting, but mycobacteria may survive beyond 30 days in intestines in conditions similar to those used for salting of casings.

#### **Recommendation**

It is recommended that casings should be treated at 20 °C for 30 days to achieve effective inactivation of animal pathogens. Several other treatments have been applied to casings with the aim of inactivating infectious agents, but none of them have been extensively investigated with viruses relevant for animal health.

### Implementation

OIE Terrestrial Animal Health Code

#### Source

Code online: <http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/>

#### Summary

All papers covers in paragraphs 3.2 – 3.6 have been submitted to the OIE Code Commission after approval by the OIE Scientific Commission.

Subsequently in September 2013 and February 2014, the following amendments to the Code have been proposed by the Commission and submitted to the OIE members for final adoption:

**1) Foot-and-mouth disease – Procedures for the inactivation of the FMD virus in casings of ruminants and pigs**

*For the inactivation of viruses present in casings of ruminants and pigs, the following procedures should be used: salting for at least 30 days either with dry salt (NaCl) or with saturated brine ( $A_w < 0.80$ ), or with phosphate supplemented dry salt or saturated brine ( $A_w < 0.80$ ) containing 86.5% NaCl, 10.7%  $Na_2HPO_4$  and 2.8%  $Na_3PO_4$  (weight/weight/weight), and kept at a temperature of greater than 20 °C during this entire period.*

**2) *Brucella* – Procedures for the inactivation of *Brucella* in casings of bovids, sheep and goats, and pigs**

*For the inactivation of *Brucella* in casings of bovids, sheep and goats, and pigs, the following procedures should be used: salting for at least 30 days either with dry salt (NaCl) or with saturated brine ( $A_w < 0.80$ ), and kept at a temperature of greater than 20 °C during this entire period.*

**3) Peste des petits ruminants – Procedures for the inactivation of the PPRV virus in casings of sheep and goats**

*For the inactivation of viruses present in casings of sheep and goats, the following procedures should be used: salting for at least 30 days either with dry salt (NaCl) or with saturated brine ( $A_w < 0.80$ ), and kept at a temperature of greater than 20 °C during this entire period.*

**4) African swine fever – Procedures for the inactivation of the ASF virus in casings of pigs**

*For the inactivation of viruses present in casings of pigs, the following procedures should be used: salting for at least 30 days either with dry salt (NaCl) or with saturated brine ( $A_w < 0.80$ ), or with phosphate supplemented dry salt or saturated brine ( $A_w < 0.80$ ) containing 86.5% NaCl, 10.7%  $Na_2HPO_4$  and 2.8%  $Na_3PO_4$  (weight/weight/weight), and kept at a temperature of greater than 20 °C during this entire period.*

**5) Classical swine fever – Procedures for the inactivation of the CSF virus in casings of pigs**

*For the inactivation of viruses present in casings of pigs, the following procedures should be used: salting for at least 30 days either with phosphate supplemented dry salt or saturated brine ( $A_w < 0.80$ ) containing 86.5% NaCl, 10.7%  $Na_2HPO_4$  and 2.8%  $Na_3PO_4$  (weight/weight/weight), and kept at a temperature of greater than 20 °C during this entire period.*

These proposed amendments will be included in the OIE Code as of September 2013 or 2014, depending on approval procedures.

## *CHAPTER 4*

---

### **TECHNOLOGICAL DEVELOPMENTS**

### **Effect of initial mild curing, with additives, of hog and sheep sausage casings on their microbial quality and mechanical properties after storage at difference temperatures**

**Authors** W.A.M. Bakker<sup>22</sup>, J.H. Houben, P.A. Koolmees<sup>23</sup>, U. Bindrich<sup>24</sup>, L. Sprehe<sup>24</sup>

#### **Journal**

Meat Science, 51 (1999) 163-174

#### **Summary**

Sausage containers, derived from animal intestines, are usually preserved by salting and/or drying. Adequately salted final products are microbiologically fully acceptable. However casings, even those packed in dry salt, sometimes deteriorate in quality.

Experiments were performed to improve salting procedures by adding food-grade additives to the salt to improve the microbiological and mechanical properties of the casings.

Before storage, casings were cured by slush- or dry-salting for three weeks with mixtures of citric acid/ $\text{Na}_3\text{-citrate}$ , lactic acid/ $\text{Na-lactate}$  or salt supplemented with phosphates ( $\text{Na}_3\text{PO}_4/\text{Na}_2\text{HPO}_4$ ). After rinsing and re-salting (dry- or slush-salting), these casings were stored for 6 months at different temperatures (10, 20 and 40 °C).

Results showed that all treatment types improved the hygienic aspects of the casings compared to the untreated group, with the best results for both acids. Mechanical properties, including slipperiness, were best preserved by using salt supplemented with phosphates.

This article presented the results of the CRAFT project (no. BRE2.CT94.1495) entitled: "*Improved treatment of natural sausage casings for quality improvement in automated stuffing processes*".

---

<sup>22</sup>Intravacc, P.O. Box 450, 3720 AL Bilthoven, The Netherlands

<sup>23</sup>Institute for Risk Assessment Sciences, Division Veterinary Public Health, P.O. Box 80.175 NL-3508 TD, Utrecht University, The Netherlands

<sup>24</sup>Deutsches Institut für Lebensmitteltechnik e.V., P.O. Box 1165, D-49601 Quackenbrück, Germany

## 4.2

---

### **Effect of different curing treatments on the usability of beef, hog and sheep casing**

**Authors** T.J. Verkleij<sup>25</sup>, G. Keizer<sup>26</sup>, W. Oostrom<sup>25</sup>, J.H. van Helvoirt<sup>25</sup>, J.H. Houben

#### **Report**

TNO report V5070, 2003

#### **Summary**

As a result of different studies on the inactivation of foot-and-mouth disease virus and classical swine fever virus in animal casings, the effect of the inactivating agents used on the usability of casings needed to be determined.

Salted beef, hog and sheep casings were obtained and treated with 0.5% / 1.0% citric or lactic acid for 5 minutes or stored in phosphate supplemented salt for 30 days. After treatment the casings were stuffed with meat emulsions under circumstances equivalent to regular commercial practice. The effect of the treatment on the usability was subsequently recorded.

The outcome shows that both citric and lactic acid had a negative influence on the usability of sheep and hog casings during the stuffing process, whereas beef casings remained unaffected. Especially the slipperiness of the casings was reduced due to the pH reduction.

The treatment with phosphate supplemented salt did not affect the usability of the hog casings.

#### **Recommendation**

Based on the outcome of this study, the 2001 OIE recommendation to treat casings with 0.5% citric or lactic acid for 5 minutes to prevent the spread of foot-and-mouth disease was not confirmed as it would reduce the usability of the treated casings. Such measures would be contra-productive for the international trade in natural casings and alternatives were therefore needed (See also Chapter 3.1).

---

<sup>25</sup>TNO Nutrition and Food Research, P.O. Box 360, 3700 AJ Zeist, The Netherlands

<sup>26</sup>Institute for Risk Assessment Sciences, Division Veterinary Public Health, P.O. Box 80.175 NL-3508 TD, Utrecht University, The Netherlands



### **Residues of curing agents in natural sausage casings previously subjected to anti-viral treatments - Hog and sheep casings treated with either lactic acid, citric acid or orthophosphates**

**Author** J.H. Houben

**Journal**

Fleischwirtschaft, 11 (2003) 42-48

**Summary**

Different studies were done to determine the efficacy of various agents on the inactivation of foot-and-mouth disease virus and classical swine fever virus in animal casings and to test the effect of these agents on the usability of casings. This study focused on the confirmation that an antiviral treatment of the casings had taken place. The study showed that it was possible to determine the presence of the agents after treatment, although several technical requirements needed to be taken into account.

### **Effect of trisodium phosphate on slip and textural properties of hog and sheep natural sausage casings**

**Authors** J.H. Houben, W.A.M. Bakker, G. Keizer

**Journal**

Meat Science, 69 (2005) 209-214

**Summary**

This study can be seen as a direct follow-up of the 2003 study by Verkleij et al. (TNO report V5070), showing that the effect of phosphate supplemented salt did not negatively influence the usability of hog casings.

Reduced slipperiness of hog and sheep casings during the stuffing process is regarded as a major problem and, apart from changing the surface of the stuffing horn or adjustment of the stuffing process, a solution may be found by treating the casings.

The gliding behavior of hog and sheep casings was assessed, both mechanically and manually during the stuffing of sausages. Casings were treated with 0.01 M trisodium phosphate (TSP); control casings were untreated. Cooked and smoked sausages were made in hog casings treated with phosphate or untreated and subjected to compression tests. In all cases the treatment with phosphate clearly facilitated the gliding of the casings over the test pipes, as compared to the control casings. The instrument to measure the casing gliding properties did not provide reliable information about the actual stuffing of sausages. The phosphate-treated casings had a lower shear force than the control casings after being used as skins for cooked and smoked sausages. If confirmed, the finding that mild phosphate treatment can diminish the force required to shear a casing will be of interest to the sausage industry because the toughness of certain hog casings is considered a problem.

A study by Nakae et al. (Fleischwirtschaft International 1/2008, 44-46) confirmed how the maximum force and breaking strain of hog casings treated with TSP were reduced, effectively improving the tenderness of tough casings.

### **Biochemical and microbiological changes in natural hog casings treated with ozone**

**Authors** H. Benli<sup>27</sup>, B.S. Hafley<sup>28</sup>, J.T. Keaton<sup>27</sup>, L.M Lucia<sup>27</sup>, E. Cabreza-Diaz<sup>27</sup>, G.R. Acuff<sup>27</sup>

#### **Journal**

Meat Science, 79 (2008) 155-162

#### **Summary**

The intended purpose of this study was to determine whether casings could be treated with ozonated water during a certain period of time as an alternative method of preservation and possible microbial inactivation (pathogenic bacteria, viral animal diseases such as FMD and CSF).

Exposure of casings to ozonated water longer than 2 hours at ~7 mg ozone per litre water weakened the casings substantially and this clearly limited the maximum time of exposure.

An inoculation study using *Escherichia coli* as test bacterium showed incomplete inactivation of the bacteria after 2 hours of ozone treatment.

Based on the results from this study, it became clear that treatment of casings with ozonated water cannot be regarded as an effective decontamination method and thereby replacing traditional preservation using salt.

#### **Recommendation**

No clear recommendation could be made to the casing industry based on the outcome of this study. However it did show that even though an original project proposal may look sound and promising, the final results of such a study will determine whether a clear conclusion is possible, preferably combined with a practical application.

---

<sup>27</sup>Department of Animal Science, 2471 TAMU, Texas A&M University, College Station, TX77843-2471, USA

<sup>28</sup>Tyson Foods Inc., 1825 Ford Avenue, Springdale, AR 72764, USA

### **Phosphate analysis of natural sausage casings preserved in brines with phosphate additives as inactivating agent – Method validation**

**Authors** J.J. Wijnker, J.L.M. Tjeerdsma-van Bokhoven<sup>29</sup>, E.J.A. Veldhuizen<sup>29</sup>

#### **Journal**

Meat Science, 81 (2009) 245-248

#### **Summary**

Certain phosphates have been identified as suitable additives for the improvement of the microbial and mechanical properties of processed natural sausage casings. When mixed with NaCl (sodium chloride) and used under specific treatment and storage conditions, these phosphates are found to prevent the spread of foot-and-mouth disease and classical swine fever via treated casings.

The commercially available Quantichrom™ phosphate assay kit has been evaluated whether it can serve as a reliable and low-tech method for routine analysis of casings treated with phosphate. The outcome of this study indicates that this particular assay kit has sufficient sensitivity to qualitatively determine the presence of phosphate in treated casings without interference of naturally occurring phosphate in salt used for brines in which casings are preserved.

#### **Implementation**

In comparison to the techniques described by Houben in 2003, this new method is more suitable for routine analysis on the presence of phosphates in treated casings.

---

<sup>29</sup>Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, P.O. Box 80.163 NL-3508 TD, University of Utrecht, The Netherlands

### **Implementation**

Application for the approval in EU legislation of sodium phosphates E339 as food additive with acidity regulating properties in the production of natural sausage casings

Note: Although the text below was used for the approval under European legislation, the backgrounds and arguments described are generic and can be applied for any other official application.

**Author** J.J. Wijnker

### **Usage**

Natural sausage casings ("casings") are traditional products that have been used in the production of meat specialties for centuries, and have remained virtually unchanged in function and appearance. A large variety of high-quality sausage is produced world-wide using the processed intestines of pigs, sheep, goats and cattle (and sometimes horses) as edible envelope. The main preservative for natural casings is salt (NaCl), either as dry salt or as fully saturated brine (Aw 0.75). This preservation method has been found to be highly effective against all vegetative bacteria (Gabis and Silliker, 1974; Houben 2005; Wijnker et al., 2006).

Subsequent studies have focussed on the usability of sodium phosphates as food additives (Bakker et al., 1999; Verkleij and Keizer, 2003; Houben et al., 2005; Schwanz and Schnäckel, 2007a, 2007b; Nakae et al., 2008). The overall conclusion of these studies is that when sodium phosphates are applied in combination with salt (NaCl), a clear improvement on different microbial and especially mechanical properties of the natural casings is achieved.

The different treatments of natural casings using either salt or phosphate supplemented salt and their efficacy have been discussed and included in the 2012 EFSA Scientific Opinion on animal health risk mitigation treatments as regards imports of animal casings.

Based on the response received from DG SANCO / E3 – Chemicals, Contaminants and Pesticides, d.d. May 27<sup>th</sup> 2010, a report is drafted answering the questions of the EU Commission Services.

These questions were:

- 1) how much sodium phosphate E339 is added to the natural sausage casings;
- 2) how much still remains in the final product (produced sausage);
- 3) will the phosphates still have a technological effect on the final product?

### **Treatment with phosphate supplemented salt**

Saturated (NaCl) brine is used as a standard preservation technique for processed natural casings (Wijnker et al., 2006).

In this particular situation phosphate supplemented salt is used to produce saturated brine as a Standard Operating Procedure (SOP) to remove all free

water in the casing as a preservative measure against microbiological deterioration.

This mixture consists of:

- |  |       |
|--|-------|
| - Sodium Chloride, NaCl  | 86.5% |
| - Sodium phosphate (TSP), $\text{Na}_3\text{PO}_4$ (Mw 164)          | 2.8%  |
| - Sodium hydrogen phosphate (DSP), $\text{Na}_2\text{HPO}_4$ (Mw142) | 10.7% |

Both sodium phosphates are approved food additives included under E339.

As an SOP natural casings are submerged in brine in order to ensure complete removal of all free water, while the saturation level of the brine is maintained above a specific minimum concentration. This is an identified Critical Control Point for the standard processing of natural casings.

There is no specific volume of brine indicated per kg casing, as the volume of brine needed to achieve the goal of free water removal depends on the actual water content of the casings. This parameter is not recorded as it bears no relevance to the final outcome of having natural casings saturated in a stable brine concentration.

Therefore, in response to question No 1, it is not possible to determine how much phosphates are added to the natural casings during the processing stage.

Exposure of the natural casings to the phosphate supplemented saturated brine will be at least 30 days, which can be regarded as sufficient time for maximum saturation of the natural casings with phosphates.

In addition, natural casings will not be consumed in this preserved state. Any consumption will take place only after sufficient salt removal and, more in general, when the natural casing is used for sausage production.

Therefore, a more specific answer on the phosphate content after the exposure period is of greater importance.

### **Phosphate content of treated natural casings**

A study has been done by Wijnker et al. (2009) to determine the actual phosphate content of treated natural casings. In this study sections natural casings were stored in excess phosphate supplemented saturated brine for a period of 52 days, to ensure maximum saturation. According to this study the average phosphate content of treated hog and sheep casings was 35  $\mu\text{M}$  (Fig.4, page 247).

In order to interpret the data from this study, the following explanation is given:

- M (Molarity) is the molar concentration ( $\text{mol (dissolved)} / \text{volume (solution)}$ ); the S.I. unit is  $\text{mol/m}^3$  or more commonly used  $\text{mol/L}$  ( $1\text{M} = 1000 \text{ mol/m}^3 = 1 \text{ mol/L}$ );
- The molecular weight (1 mol) of  $\text{PO}_4^{3-}$  is 95 gram and consists of 1 mol P and 4 mol O. As 1 mol  $\text{PO}_4^{3-}$  (phosphate) corresponds to 1 mol P (phosphorous), the actual P content of the treated casings can be calculated as follows:
  - Average P content casing sample:  $35 \mu\text{M} = 35 \cdot 10^{-6} \text{ mol/l}$  ;
  - 1 mol of P = 31 gram;
  - $35 \cdot 10^{-6} \times 31 = 0.0011 \text{ g/l} = 1.1\text{mg/l}$
- Each sample in the referred-to study was 2 cm in length, therefore, per meter treated casings a concentration of 55mg P is reached;

- Sheep and hog casings weigh approximately 4 to 10 grams per meter (depending on species and calibre);
- The upper concentration limit is therefore 55 mg P / 10 g of casing;

Per kg of casing:  $100 * 55 \text{ mg} = 5500 \text{ mg P}$

Expressed as  $\text{P}_2\text{O}_5$ :  $5500 * 2.29^{30} = 12595 \text{ mg / kg casing}$

- On average, a produced sausage with an average weight of 200 gram consists for 1-2% of natural casings, resulting in a weight of 2-4 g of natural casings per sausage produced;
- The upper concentration of P per sausage produced is  $55 \text{ mg P} * 0.4 = 22 \text{ mg P}$ , as carry-over from the treated natural casings.

The following calculation will provide a clear indication on the P (phosphorus) content in sausages due to its presence in treated casings and its impact on the Acceptable Daily Intake (ADI) of P.

- The ADI of trisodium phosphate is 70 mg/kg body weight (bw), expressed as P / phosphorus<sup>31</sup>:
  - average body weight of 60 kg (adult): ADI = **4200 mg P**
  - average body weight of 15 kg (child): ADI = **1050 mg P**
- Estimated contribution of P via treated natural casings per day per meal of 200 g sausage:
  - Adult:  $(22/4900 =) 0.5\%$  of the ADI (as carry-over from the natural casing);
  - Child:  $(22/1050 =) 2.1\%$  of the ADI (as carry-over from the natural casing).

In order to propose a specific level for category 8.2.3 (casings and coatings and decorations for meat), Part E, Annex II, Commission Regulation (EC) No 1129/2011, the phosphorus contribution needs to be expressed as  $\text{P}_2\text{O}_5$ :

- $22 * 2.29 = 50 \text{ mg / 200 g sausage}$
- **250 mg / kg sausage as carry-over from the natural casings**

### **Residual technological effect**

In response to question 3, the addition of sodium phosphates to natural casings is very low when compared to, for example the maximum level of various phosphates (E38-452) in breakfast sausage, being 5000 mg/ kg product (Category 8.1.2 meat preparations, Part E, Annex II).

Therefore it can be stated that the technological effect of the added sodium phosphates in natural casings is negligible to the overall technological effect of phosphates in the produced sausage.

<sup>30</sup>2.29 is factor when molecular weight of  $\text{P}_2\text{O}_5$  (142) is divided by contribution P ( $31 * 2 = 62$ ) to molecule weight

<sup>31</sup>Joint FAO/WHO Expert Committee on Food Additives, January 27<sup>th</sup> 2006. Trisodium phosphate E339



**Conclusion and implementation**

The usage of phosphate supplemented salt is a relevant addition to the standard preservation methods of natural sausage casings as an acidity regulator.

A proposal for the use of sodium phosphates (E339) in natural casings for sausages in the European Union was adopted and implemented as Commission Regulation (EU) No 1069/2013.

In 2014, a report was released by the ISWG, entitled "Phosphate supplemented salt treatment of natural casings", as a guidance document for the use sodium phosphates under practical conditions. This report can be found in Annex I.



## *CHAPTER 5*

---

### **BSE /TSE RESEARCH**

### **Natural sausage casings and the BSE / TSE risk**

#### **Bovine Spongiform Encephalopathy**

BSE was first officially diagnosed in the United Kingdom in November 1986. BSE or Bovine Spongiform Encephalopathy became quickly known as “mad cow disease” due to the typical movement and behaviour of animals suffering from the disease. Since the first BSE outbreak in 1986, more than 180,000 cases in cattle have been reported in the UK alone. Epidemiological models predicted a rapid dissemination of the disease between animals, and a considerable increase in number of affected consumers. As a consequence, drastic measures had to be taken by the EU Authorities to ensure consumer’s safety. In contrast to these first predictions, the number of reports of BSE in the UK began to decline in 1992 and continuously declined year by year since then.

The most likely route of exposure was through animal-derived feed materials such as Meat and Bone Meal (MBM) made from BSE-infected animals which lead to a subsequent ban on the use of MBM in animal feeds. Since 1989, when the first BSE case was reported outside the UK, relatively small numbers of BSE cases have also been reported in cattle in most EU Member States and countries like Japan, Canada and the United States. The occurrence of these BSE cases is most likely due to the exports of live animals and the use of animal feed from Europe. The International Office for Epizootic Diseases (OIE) reports these cases on their website ([www.oie.int](http://www.oie.int)).

#### **New variant Creutzfeldt-Jakob disease**

Although characteristic lesions, similar to BSE, were found to exist in scrapie in sheep and Kuru in humans, initially no direct link of cross-species infectivity was found to exist. Only by direct injection of BSE contaminated material into the brains of experimental animals could the disease be reproduced. However, a new disease in humans was first diagnosed in 1996, called new variant Creutzfeldt-Jakob disease (vCJD), a rare and fatal neurodegenerative condition affecting particularly elderly people. As with Creutzfeldt-Jakob disease, vCJD had to be classified as a Transmissible Spongiform Encephalopathy (TSE) because of the characteristic spongy degeneration of the brain. Similarly to BSE, vCJD cannot be transmitted directly from person to person, but can be spread via infected materials such as blood transfusion or other implantation material. In contrast to the long-known CJD, vCJD affected younger patients (average age 29 years, as opposed to 65 years) and has a relatively longer duration of illness (median of 14 months as opposed to 4.5 months). Moreover, evidence was accumulating that vCJD is linked to the exposure from cattle affected by BSE, probably through meat-based food.

The hypothesis of a link between vCJD and BSE was first raised because of the association of these two TSEs in time and place. More recent evidence supporting this link includes identification of pathological features similar to vCJD in brains of macaque monkeys inoculated with BSE contaminated material. A vCJD-BSE link is further supported by the demonstration that vCJD is associated with a molecular marker that distinguishes it from other forms of CJD and which resembles the marker seen in BSE transmitted to a number of other species.

### **Preventive measures taken by the EU Authorities**

As a result of the initial epidemiological assessment and in consideration of the possible link between BSE and vCJD, restrictive measures were put in place. Because of the lack of sufficient scientific information the "Precautionary Principle" **had** to be applied in order to comply with the consumers' demands for safe food and the protection of the consumers, overruling any prevailing economic argument. The Scientific Steering Committee (SSC) of the European Commission was given the task to assess all available scientific evidence, which was subsequently summarised in many Opinions. These Opinions formed the basis for European Regulations, Decisions and Directives.

In July 1988, the UK banned the use of ruminant proteins in the preparation of animal feeds. The use in the food chain of bovine offals, which were considered to pose a potential risk to humans, was banned in the UK in 1989. The list of Specified Risk Material (SRM) was revised and expanded on several occasions, whenever new information became available. In 1994, the EU banned the feeding of all mammalian MBM to ruminants. However, the date of implementation and the extent of enforcement varied from country to country within the EU. In 2001, EU Authorities introduced a total ban on feeding MBM to all farm animals.

With regard to natural casings, beef casings produced from cattle intestines were designated SRM and banned for human consumption completely in 1997. This terminated the production of beef casings in Europe and allowed only imports into the EU from countries regarded as BSE-free.

At the meeting of the Scientific Steering Committee on April 4 & 5 2002, an opinion was presented on the safe sourcing of small ruminant materials.

In this opinion the following was stated: *"Whether or not casings obtained from sheep intestines can be considered as representing a negligible risk will depend upon the reduction resulting from the casing production process, the potential presence of infectivity in the parts of the intestine used for casing production and the age of the animal. Relevant data and information to assess the risk possibly posed by casings are currently being collected by a number of research bodies and a final conclusion should await the outcome of this exercise".*

These referred-to studies are described in detail below. However, after being submitted and evaluated, the Scientific Steering Committee presented during its meeting on September 12 & 13 2002 the complement to the SSC opinion on the safe sourcing of small ruminant materials, with special reference to the safety with regard to BSE risks of sheep intestines and casings.

In this opinion the following was stated: *"As far as casings are concerned, the available data permit to conclude that the amount of infectivity that would be present in the intestine would be reduced by a factor of at least 100, possibly more than 1000, during the casings production processes. Some residual infectivity may nevertheless remain present which may pose a risk should the presence of BSE in sheep become probable or proven".*

As BSE was not found to be present in any national small ruminant flock, sheep intestines remained exempt from the SRM list and could still be produced in Europe.

### **Studies in sheep casings**

#### **Assessment of the risk of exposure to the BSE agent through the use of natural sausage casings**

**Author** P. Comer<sup>32</sup>

#### **Report**

DNV Report No. 716146, 2002

#### **Conclusions**

1. The processing of the small intestine of sheep into sausage casings reduces the weight by a factor of 9.
2. As most lymphatic tissue is removed during processing it is suggested that the average infectivity in casings should be reduced by an additional factor of 10. Thus the infectivity in the casings is assumed to be 100 times less than that in the unprocessed intestine assuming that this has the same infectivity as the Ileum.
3. It is estimated that Peyer's patches account for less than 1% of the area of the casings. If the Peyer's patches are assumed to have the same infectivity as the ileum, and the rest of the casing the same infectivity as the stomach, then the average infectivity of the casings would be a factor of about 900 less than that of the unprocessed intestine assuming that this is all at the same infectivity as the ileum.
4. With a processing factor of 100, intestines contribute about 20% of the overall infectivity consumed compared to 76% for lymph nodes.
5. If the processing factor is less than 30, Intestines would contribute more than lymph nodes, but this is considered to be unlikely.
6. The total infectivity consumed in a meal of sausages made with casings from an animal infected with BSE is estimated to be less than one hundredth of an infectious unit, even if the casings are made from an older sheep with clinical levels of infectivity.
7. At the maximum prevalence of BSE in Sheep in the UK (2% of scrapie cases are BSE) the chance that any one sheep is infected is  $2 \times 10^{-5}$  (2 in 100,000).
8. It has been estimated that the infectivity due to the Popliteal lymph node in a leg of lamb is 5 times greater than the infectivity in a 250 g meal of sausages, assuming that both are from an animal with ovine BSE.

---

<sup>32</sup>Somerset Risk Management, United Kingdom

## **Note on weights of intestine in small ruminants and expected risk reduction**

**Authors** P. Comer, R. Bradley<sup>33</sup>

### **Report**

Report 2002

### **Summary**

The Food Standards Agency (FSA) of the UK had proposed to the European Commission that the intestine of small ruminants is made SRM throughout the European Union as a result of a recommendation in the Report of the Core Stakeholder Group on BSE and Sheep, May 2002. The Report was endorsed by the FSA Board on 13 June 2002. This paper was intended to provide some additional factual information on typical weights of intestine in sheep and the proportion of this used in food for human consumption.

The paper concluded that:

1. The only part of the small ruminant intestine used for natural casing production is the duodenum and jejunum;
2. The proportion that the prepared natural casing forms of the total intestinal mass is about 7%. The remaining 93% does not enter the human feed chain. Furthermore this 93% is not permitted in the animal feed chain under current European legislation. In practice the material is rendered to produce meat-and-bone-meal (MBM) and tallow and the MBM is prohibited for use in food animal feed;
3. Current SRM controls would reduce the overall exposure to infectivity (were any to be present in sheep) by about 53%; however this reduction is only from older sheep;
4. Including intestine from all sheep as SRM would increase this overall reduction by 12%, from 53% to 65%. The measure would reduce the exposure from lamb (sheep less than 1 year old) by 25%, leaving 75% of the infectivity to be consumed;
5. The FSA claim that including intestine from all sheep as SRM would increase the risk reduction from one third to two thirds is an oversimplification and highly misleading.

---

<sup>33</sup>Private BSE Consultant, Guildford, United Kingdom



## Risk assessment of the use of sheep natural casings and legs of lamb

Authors P.A. Koolmees, B.R. Berends, M.H.G. Tersteeg<sup>34</sup>

### Report

VVDO Report No. H0204, 2002

### Summary

This study, conducted in 2002 at Utrecht University, The Netherlands, gave a clear indication on the risk reduction due to the cleaning process of sheep intestines in comparison to meat-on-the-bone, such as a leg of lamb. Some conclusions made:

- *"When a butcher prepares a leg of lamb for sale, most of the lymph nodes are probably being removed. However, this cannot be stated with certainty, since European law does not consistently require the removal of lymph nodes from slaughtered animals or meat, and dressing habits may vary from country to country. This means that at least about 0.05% of the total weight of a consumed leg of lamb consists of lymphatic tissue, which may contain BSE".*
- *"The removal of the different tissue layers from intestines during the processing into natural casings means a weight-based reduction of a factor of about 11-12, which is approximately the same as the reduction in thickness of layers. If it is assumed that at most 10% of all lymph nodes present in the intestinal wall stay firmly attached to the remaining layer during processing into natural sausage casings, the overall weight-based reduction of lymph nodes is at least a factor of 120. When an even more likely 99% reduction of the lymph nodes is assumed, the **weight-based reduction of infectivity would be at least a factor of 1200**".*
- *"If the consumption of a portion of leg of lamb from a BSE infected animal is compared with the consumption of an equivalent amount of sausages made from the intestine of that same animal, the sausages would lead to an **exposure to potentially infectious tissues which is 7 to 10 times lower than to a portion of leg of lamb**".*

### Implementation

These conclusions were incorporated into a risk assessment written by Dr. Ray Bradley entitled: *"Report on the safety of sheep intestine and natural casings derived therefrom in regard to risks from animal TSE and BSE in particular"*.

As indicated already, when the SSC published its complement to the original opinion on safe sourcing of small ruminant materials in September 2002, it acknowledged that sheep and goat casings are safe for human consumption in regard to BSE.

---

<sup>34</sup>Institute for Risk Assessment Sciences, Division Veterinary Public Health, P.O. Box 80.175 NL-3508 TD, Utrecht University, The Netherlands

## 5.3

---

### **Comparative histological studies of mechanically versus manually processed sheep intestines used to make natural sausage casings**

**Authors** P.A. Koolmees, M.H.G. Tersteeg, G. Keizer, J. van den Broek<sup>35</sup>, R. Bradley

#### **Journal**

Journal of Food Protection, 67 (2004) 2747-2755

#### **Summary**

This study was intended as a complement to the previous reports and showed conclusively that the traditional manual cleaning and the mechanical processing of sheep intestines completely removes all lymphoid tissue which could be associated with BSE. In addition it showed that there was no difference in cleaning efficacy between the manual and mechanical cleaning process.

#### **Implementation**

Having made a thorough risk assessment on the safety of sheep casings, the industry was confronted with the notification of a BSE case in a goat (France, 2004). As this case was confined to one individual animal, the European Commission and European Food Safety Authority (EFSA) did not implement new regulations but called for an increase in the number of animals to be tested for BSE, in order to determine whether more BSE cases in small ruminants could be detected. Fortunately this was not the case. Accordingly, EFSA published their opinion (June 2005) on *"A quantitative assessment of risk posed to humans by tissues of small ruminants in case BSE is present in these animal populations"*. In the preparation of this opinion ENSCA was consulted to provide information on the safety of natural casings from sheep and goats. As this information was already available (see the above mentioned studies) it was presented to EFSA, who then confirmed the previous statement of the SSC that sheep casings are safe for human consumption.

#### **SWG statement on the definition of ileum**

In addition to the reports and scientific papers, the SWG issued a statement in 2003 on the anatomical definition of the ileum according to the *Nomina Anatomica Veterinaria* (NAV). The NAV definition of ileum is:

*"The short terminal part of the small intestine to which the Plica ileocaecalis is attached"*.

The complete statement is included in annex III.

---

<sup>35</sup>Centre for Biostatistics, Utrecht University, Padualaan 14, 3583 CH Utrecht, The Netherlands

### **Studies in beef casings**

#### **Histology of bovine natural casings**

**Author** P.A. Koolmees

#### **Report**

VVDO Report No. 9806, 1998

#### **Conclusion**

Aggregations of lymph nodes or patches of Peyer are mainly present in the ileum part of the bovine small intestine. In practice the ileum is not used as sausage container. Isolated lymph nodes and some patches of Peyer also occur in the duodenum and jejunum part of bovine intestines. After mechanical cleaning the lymph nodes were removed from the intestine wall for the most part. Although the amount of lymphatic tissue was significantly reduced by the cleaning process, some remnants of lymph nodes were still present at spots on the casings showing the phenomenon of "goose-skin". Since this was not the case with pork and sheep casings, the remnants of lymphatic tissue in bovine casings may be due to the fact that these casings are turned inside out, and are thicker because they contain more tissue layers.

#### **Implementation**

This study provided the first information on what tissue layers remained present in beef casings after the cleaning process. As only a small number of samples were included in this study, no quantitative analysis or statistical evaluation was possible on its results, limiting the impact of its conclusions. However, as the aim of this pilot experiment was to determine whether lymph nodes (patches of Peyer) remained present after cleaning, its conclusion could not be ignored.

#### **The TSE roadmap: the situation in 2005**

In 2005, a big step forward was taken by the European Commission in its approach to BSE. In the introduction of the TSE Roadmap document the Commission states the following: *"We have come to the stage that amendments of certain measures could be envisaged without endangering the health of the consumer or the policy of eradicating BSE, provided that the positive trend continues and scientific conditions are in place".*

With this current change in BSE policy an amendment of the SRM-list now becomes possible which could allow the production of beef casings in Europe in the near future.

### **Quantitative histological analysis of bovine small intestines before and after processing into natural sausage casings**

**Authors** J.J. Wijnker, M.H.G. Tersteeg, B.R. Berends, J.C.M. Vernooij<sup>36</sup>, P.A. Koolmees

#### **Journal**

Journal of Food Protection, 71 (2008) 1199–1204

#### **Summary**

A histological study was undertaken to determine the efficiency in the removal of the mucosa and Peyer's patches by standard processing of bovine intestines into natural sausage casings. The second objective was to calculate the quantity of lymphoid and nervous tissue per consumable sausage.

The histological analysis indicate that a weight reduction of about 50% during standard cleaning procedures is achieved, as 90% of the mucosa and 48% of the lymphoid tissue are removed.

Based on the quantitative histological image analysis, it was calculated that 1 m of cleaned casings, weighing on average 64 g, contains about 2.8 g of mucosa, 0.3 g of lymphoid tissue, and 0.1 g of neural tissue.

Assuming that, in a worst-case scenario, the sausage casing is ingested when consuming 200 g of sausage at one meal, this consumption includes 0.09 g of lymphoid tissue and 0.02 g of neural tissue as part of the sausage casing. These data can be included in a risk assessment on the potential exposure of consumers to bovine spongiform encephalopathy infectivity after eating sausages in beef casings.

#### **Implementation**

Prior to its publication in a scientific journal in 2008, this study had been presented to EFSA in 2007 in view of the possible relaxation of BSE restrictive measures indicated in the 2005 TSE Roadmap. However, the EFSA opinion, on "*quantitative histological studies and the re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings*" from 2007 did not result in any change in the current SRM status of cattle intestines (See Chapter 5.6).

---

<sup>36</sup>Department of Farm Animal Health, Division of Epidemiology, Faculty of Veterinary Medicine, P.O. Box 80.175 NL-3508 TD, University of Utrecht, The Netherlands

### **Opinion on quantitative histological studies and the re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings**

**Author** EFSA Panel on Biological Hazards (BIOHAZ)

**Journal**

EFSA Journal, 464 (2007) 1-14

**Summary**

The Institute for Risk Assessment Sciences (IRAS) and the Department of Farm Animal Health of Utrecht University produced a report on quantitative histological studies of bovine small intestine before and after processing into natural sausage casings. The report concludes that commercially processed casings do not pose a measurable risk, in terms of BSE, for consumers.

EFSA was asked by the European Commission to provide an opinion on the evaluation of the design of the study and, if needed, to issue recommendations on the topics that should be addressed in future studies on the subject. Furthermore EFSA was asked to evaluate the conclusions of the report and, if it was considered necessary based on the report and any other new relevant scientific information, to re-assess the BSE related risk of bovine intestines after processing into natural sausage casings.

EFSA's Scientific Panel on Biological Hazards (BIOHAZ) analysed the design of the study in detail and considered the IRAS study inadequate for the purpose of demonstrating the safety of bovine casings of cattle originating from BSE risk countries. A number of recommendations were made on the topics that should be addressed in future studies on the subject. Moreover, the BIOHAZ Panel did not consider valid, from a scientific point of view, the conclusions of the report and did not consider necessary, based on the information received and on currently available scientific information, to re-assess the BSE related risk of bovine intestines after processing into natural sausage casings.

### **TSE risk assessment for use of bovine casings**

**Author** P. Comer

#### **Report**

DNV Report No. 22926377, 2008

#### **Summary**

This study presents the results of a risk based approach to estimate the potential exposure to BSE infectivity from the consumption of sausage casings made from bovine intestine, assuming that the casings are sourced from within the EU.

Its results show that the exposure for a person with a relatively high consumption of sausages made with bovine casings (based on consumption of sausages in Germany) would be  $1 \times 10^{-6}$  cattle oral ID50 units per year (range  $4 \times 10^{-8}$  to  $7 \times 10^{-6}$ ) based on BSE prevalence in The Netherlands. This is a very low exposure, and taking account of the species barrier between humans and cattle, would represent an extremely low risk.

It is concluded that the risks from BSE infectivity associated with the use of bovine casings is extremely small and that changes to the SRM regulations could be considered without any significant increase in the risk to consumers.

Risk comparisons made between bovine casings and a T-bone steak or meat pie, show clearly that the potential exposure to BSE infectivity from bovine casings is significantly less than that from either a T-bone steak containing neural tissue or from a meat product derived from head meat where it is possible that there is CNS contamination. The potential risk from both of these products is accepted in the EU by both the legislators and the public.

#### **Implementation**

The final report was submitted by the Swiss Task Force Cervelas to the European Commission Services in December 2008. Subsequently, it was forwarded to the EFSA with a mandate to form an official opinion on this new report (See Chapter 5.8).

### **Scientific Opinion on BSE risk in bovine intestines**

**Author** EFSA Panel on Biological Hazards (BIOHAZ)

**Journal**

EFSA Journal, 1317 (2009) 1-19

**Summary**

According to Regulation (EC) No 999/2001 certain ruminant tissues, designated as Specified Risk Material (SRM), must be removed from the food and feed chain to protect the health of consumers against the risk of BSE. The intestines, from the duodenum to the rectum, of bovine animals of all ages are currently included in the list of SRM. The "TSE roadmap" of the European Commission (EC) foresees amendments of the current SRM list based on newly evolving scientific knowledge while ensuring and maintaining a high level of consumer protection. EFSA was requested by the EC to evaluate the current risk of BSE linked with the use of bovine casings for the production of sausages. EFSA's assessment was based on new but limited experimental scientific data demonstrating that in addition to ileum, also jejunum may harbour infectivity when a large BSE inoculum dose was used to experimentally infect cattle, and a recent report that attempted to quantify the BSE infectious load in bovine sausage casings if produced in the EU from intestines excluding ileum. The calculated results considered in that report show that the individual human exposure from bovine casings, excluding ileum, produced in the EU (based on the calculated BSE prevalence in 2007) were "very low". When the total human exposure in the EU per year is considered by the Panel, the obtained figures cannot be considered negligible, even when ileum is excluded. Several input assumptions of the report bore considerable uncertainties, in particular with regard to the amount of tissue calculated for production of bovine casings and the amount of infectivity potentially present in bovine intestine. EFSA concludes that the previous assessment of the BSE related risk of bovine intestines after processing into natural sausage casings remains valid.



### **Experimental bovine spongiform encephalopathy: detection of PrP<sup>Sc</sup> in the small intestine relative to exposure dose and age**

**Author** M.J. Stack<sup>37</sup>, S.J. Moore<sup>37</sup>, A. Vidal-Diez<sup>37</sup>, M.E. Arnold<sup>37</sup>, E.M. Jones<sup>37</sup>, Y.I. Spencer<sup>37</sup>, P. Webb<sup>37</sup>, J. Spiropoulos<sup>37</sup>, L. Powell<sup>37</sup>, P. Bellerby<sup>37</sup>, L. Thurston<sup>37</sup>, J. Cooper<sup>37</sup>, M.J. Chaplin<sup>37</sup>, L.A. Davis<sup>37</sup>, S. Everitt<sup>37</sup>, R. Focosi-Snyman<sup>37</sup>, S.A.C. Hawkins<sup>37</sup>, M.M. Simmons<sup>37</sup>, G.A.H. Wells<sup>37</sup>

#### **Journal**

Journal of Comparative Pathology, 145 (2011) 289-301

#### **Report**

VLA Report No. FT1394, 2009

#### **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<sup>Sc</sup>) 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<sup>Sc</sup> 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<sup>Sc</sup> 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<sup>Sc</sup>-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<sup>Sc</sup> 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<sup>Sc</sup> 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.

#### **Implementation**

This study was originally conducted as an ISWG project and subsequently reported in 2010. Together with other information it was submitted to the European Commission Services and forwarded to EFSA with a mandate to form an official opinion on this new paper (See Chapter 5.10).

---

<sup>37</sup>Animal Health and Veterinary Laboratories Agency - Weybridge, Addlestone, United Kingdom

### **Scientific Opinion on a review of the BSE-related risk in bovine intestines**

**Author** EFSA Panel on Biological Hazards (BIOHAZ)

**Journal**

EFSA Journal, 9(3) (2011) 2104

**Summary**

The opinion reviews a 2007 opinion of the French Food Safety Agency (AFSSA), which, referring to the current situation in France, concluded that the current removal of the whole intestine from bovine animals of all ages as specified risk material could be limited to the ileum. It is concluded that the model used in the AFSSA opinion is an appropriate tool to estimate the Classical BSE prevalence in cattle cohorts in countries with extensive surveillance systems and the maximum number of undetected Classical BSE cases that could enter into the food chain in a particular country. It is concluded that the AFSSA methodology cannot be used to make inference to situations other than the French situation to assess the Classical BSE-related risk in bovine intestines. The new scientific data available are reviewed. These data, which add some new elements, concur and confirm the presence of limited amounts of PrP<sup>Sc</sup> and/or infectivity in parts of the intestine other than ileum of Classical BSE infected cattle under experimental inoculation (jejunum) and natural exposure (distal jejunum and colon). The new scientific information further confirms the presence of consistent amounts of PrP<sup>Sc</sup> and infectivity in the ileum of Classical BSE infected cattle under experimental inoculation and natural exposure. Due to limitations in the data currently available, an accurate quantification of the amount of infectivity in the intestinal parts other than ileum of Classical BSE infected cattle at different stages of the incubation period cannot be provided. It is also concluded that, due to the continuous decline of the Classical BSE epidemic, the current Classical BSE exposure risk from bovine intestines has declined correspondingly. Exposure to Atypical BSE from the consumption of bovine intestines cannot be assessed at this stage and research on the pathogenesis of Atypical BSE is recommended.

**Evaluation**

In the period 2007-2011, three EFSA opinions have been published focussing on the potential BSE risk of the bovine intestinal tract. The basis for all three opinions were studies done on behalf of or in close relationship with the ISWG. Unfortunately these opinions did not have an outcome that could serve as a basis to amend the list of Specified Risk Material included in the European BSE / TSE legislation.

However, in February 2012 a formal request was made by the European Commission Services to EFSA for a scientific opinion on a quantitative evaluation of the BSE risk in bovine intestines and mesentery. A different approach was used as EFSA will now make its own assessment based on available data, not review submitted reports or papers prepared by other research and assessment groups.

In preparation of this opinion, ENSCA has submitted extensive responses to various questionnaires sent by EFSA on the production of beef casings.

### **Scientific Opinion on BSE risk in bovine intestines and mesentery**

**Author** EFSA Panel on Biological Hazards (BIOHAZ)

**Journal**

EFSA Journal, 12(2) (2014) 3554

**Summary**

Bovine intestines and mesenteries in the European Union (EU) have to be removed from the food and feed chain. The opinion provides a quantitative assessment of the Bovine Spongiform Encephalopathy (BSE) infectious load that might enter the food and feed chain yearly if bovine intestine and mesentery from animals born and raised in the EU would be re-allowed for consumption. Data on the evolution of the BSE infectious titre and of the weight of histological structures accumulating BSE infectivity, were collected. The Cattle TSE Monitoring Model (C-TSEMM) was used to estimate the number of BSE infected cattle entering undetected in the food and feed chain yearly. A model named TSEi was developed to estimate the BSE infectious load in tissues from infected animals at different ages and the total yearly infectious load that could enter the food and feed chain in the EU27. In BSE infected cattle, the infectivity associated with intestine and mesentery reaches its maximum in animals younger than 18 months and then progressively declines to a minimum value in animals older than 60 months. Due to the decline of the BSE prevalence in the EU, between 2007 and 2012, the yearly amount of BSE infectivity associated with intestine and mesentery (sent to destruction) from animals entering the food and feed chain was reduced by a factor of 10. However, over this period, the maximum level of exposure to the BSE agent for individuals that would have consumed these tissues remained stable. Finally, the TSEi model indicated that the removal of the last four metres of the small intestine and of the caecum from the food and feed chain would result in a major reduction of the BSE exposure risk associated with intestine and mesentery in cattle.

**Implementation**

As the Opinion was presented shortly before the finalisation of this book, it is unknown at this moment if the European list of Specified Risk Material will be amended. Should this be the case then the door is (re)opened for the production of beef casings in Europe.



## *CHAPTER 6*

---

### **SPECIAL PROJECTS**

## 6.1

---

### **Improved treatment of natural sausage casings for quality improvement in automated stuffing processes**

EU – CRAFT – Project (BRC 2.CT 94.1495)

#### **Partners**

Charles Frères, France  
Combinatie Teijssen & van den Hengel, The Netherlands  
DAT-Schaub, Denmark  
Peter Gelhard, Germany  
Van Hessen bv, The Netherlands  
Jürging, Germany  
Boekos, The Netherlands,  
Kemper, Germany  
VEMAG, Germany  
Deutsches Institut für Lebensmitteltechnik e.V., Germany  
Utrecht University, The Netherlands

#### **Summary**

An overall description of the project and the main results were distributed to all companies in INSCA and ENSCA in 1998. In the accompanying letter, the acting chairmen of both associations at that time, Mr. Grundt and Mr. Jürging, stressed the general value of the project. *“This project has made an extremely valuable contribution towards the declared objective of this industry, i.e. to move away from waste utilization and towards food industry. It has provided data which indicate that the prospects for continuing in this direction is very promising from a financial and economical point of view, that is to say with the aim of reducing production costs.”*

Major results and consequences of the six sections of the project were:

1. Inventory: The chemical analysis of all parts of the intestinal tract of sheep and hog gave a total new analysis aspect for protein, fat ash and moisture content. These results were used in 1999 for an attempt to exempt casings from the Belgian export ban due to dioxin findings in meat and meat product of Belgian origin. Because of the scientific findings, which showed a very low fat content in casings, they were exempted from the ban. This information was still valid in 2006 and 2008 when dioxin contamination of pig feed and subsequently meat and meat products again threatened the international trade in casings. As before import bans were lifted or never implemented due to available data specific for casings.

Through microscopic examinations, the histological investigations made evident for the first time the transformation process during de-sliming from the intestine to the casing, which leaves only the submucosa layer of the intestinal wall. These results were presented to the EU Commission as early as 1998 when the discussion on defining sheep and beef casings as possible specified risk material (SRM) started. This was done together with a comprehensive argumentation paper showing the risk reduction potential of the processing of sheep casings. Additional research papers have subsequently solidified the argument, limiting the sheep's ileum to the SRM

list. However, the intestinal tract of cattle could not escape the "Precautionary Principle" and was designated as SRM from the duodenum up to rectum in 2000. Efforts are currently ongoing to amend this situation allowing for beef casings to be produced once again in Europe.

The original inventory part is included in annex II.

2. Pulling – Cleaning: A prototype of an improved pulling knife was presented, which needed further investigation as it was stated: *"These ways of technological improvement during their initial processing of casings must be left to the individual discretion of casing establishments prepared to take the risk"*. However, it has shown the direction in which to proceed.
3. Salting – Curing: In this part, intensive analysis of microbial counts of casings, using acidic and alkaline additives to the salt after various period of storage, resulted in a recommendation on "Microbiological Standards for casings at the point of delivery to sausage makers / meat industry".
4. Storage and transport: The results of the thoroughly performed test on microbial and mechanical properties of the casings after different times and different temperatures are used as argumentation for temperature requirements during the transport of raw material from the slaughterhouse to the processing plant, to demonstrate the risk reduction for casings at different temperatures.
5. Filling: The objective to come up with an improved filling technology, e.g. coated filling pipes, was not sufficiently reached. However, first ideas were presented for further activities of casing companies together with machine producing companies.
6. Methods: The elaboration of recommended sampling and salting / curing procedures and methods for the examination of natural casings, process water and brines were distributed as a leaflet to the industry. This report enabled standardized methods for sampling and subsequent analysis would permit a comparison of results assessing quality in general in a pre-competitive stage. This also has made it possible to avoid misinterpretation of the term "hygienic processing".

The results from the CRAFT project were summarized and drafted into the article by Bakker et al. (1999) entitled: *Effect of initial mild curing, with additives, of hog and sheep sausage casings on their microbial quality and mechanical properties after storage at difference temperatures*.

More importantly the CRAFT project showed the potential of the casing industry to provide clear scientific information when needed on important subjects. In addition, the CRAFT project has triggered various other projects intended to clarify specific questions left open or to expand on knowledge gained.

### **HACCP manual for processing natural sausage casings**

**Authors** A. Fischer, B. Krol (first edition 1997) / H.L. Heeres<sup>38</sup>, N.B. Lucas Luijckx<sup>38</sup>, F.K. Stekelenburg<sup>38</sup>

#### **Report**

TNO report V7169, 2009

#### **Summary**

In 1997, immediately after the finalization of the CRAFT project, Prof. B. Krol and Prof. A. Fischer presented the HACCP manual for processing natural sausage casings within a timeframe of only two months. By their involvement, ENSCA, INSCA and NANCA demonstrated that they have fulfilled their obligations regarding the introduction of a HACCP system for the production and handling of casings.

The compilation of such a HACCP manual became necessary due to the new mandatory legislation implemented world-wide regulating the hygiene rules for meat, meat products and other products of animal origin, including casings. As of January 1<sup>st</sup> 2006, the requirement for each food producing establishment to have a HACCP system became mandatory under EU law. Not only European companies but also companies registered for the export of natural casings to Europe were faced with this requirement.

In anticipation of this mandatory requirement, the ISWG had contracted TNO Quality of Life to update the existing manual from 1997. A first version was issued in October 2006, followed by a second version in June 2007. In 2009 the third version was issued, keeping the manual up to date with new legal developments.

The 1997 HACCP manual was one of the first manuals of its kind for the food industry world-wide. This version and its successors have been distributed by the different associations included in the ISWG and the manual has served since then as a useful guidance document for a company-based HACCP system.

Apart from being globally implemented by the natural casing industry, it has also gained the approval of the veterinary competent authorities in many countries.

In 2011 the ENSCA Community Guide to Good Practice (cGGP) for hygiene and the application of the HACCP principles in the production of natural sausage casings was finalised and presented. This cGGP was based on the existing HACCP manual but now also included an comprehensive Hazard Identification and Analyses section. Originally presented as a European guide, it was approved and adopted by the European Commission's Standing Committee on the Food Chain and Animal Health as a reference document. Several translations were made including a Chinese version, which has been implemented by the Chinese Natural Sausage Casing Association CNSCA. In 2013 it was decided to redraft the ENSCA cGGP into a generic INSCA HACCP manual, with a formal release in 2014.

---

<sup>38</sup>TNO quality of Life, P.O. Box 360, 3700 AJ Zeist, The Netherlands



## 6.3

### The Road Map: Consumer safety of natural sausage casings

**Authors** J. Fink-Gremmels, J. A. Schrickx<sup>39</sup>, S. Teunissen<sup>39</sup>, H. Morales<sup>39</sup>, M. van der Doelen<sup>39</sup>, G. de Vrieze<sup>39</sup>

#### Report

ISWG report October 2008

#### Summary

##### 1. Microbiological risks inherent to current processing techniques

An inventory of micro-organisms occurring in the intestines of slaughter animals such as pigs and ruminants (sheep, goats, cattle) indicated the presence of more than 200 species of microbiota belonging to the genera bacteria, fungi imperfecti and yeasts, and comprising pathogenic and non-pathogenic organisms. However, *no data are available on individual growth characteristics of these organisms*, and hence a formal microbiological assessment remains pending.

However, previous investigations confined to major pathogens (spoilage organisms were not included here) showed that common pathogens such as *Salmonella*, *E. coli* are intolerant to water activity ( $a_w$ ) values below 0.9, particularly after exposure over several days, confirming the efficacy of commonly used procedures of salt preservation even at ambient temperatures. Brine, with an  $a_w$  of 0.86, is equal to approximately 20 °Baumé at 20 °C or a 22% salt solution; dry salt reduces the  $a_w$  to approximately 0.75.

$a_w$	Micro-organisms generally inhibited by $a_w$ at this point
0.950	<i>Pseudomonas</i> , <i>Escherichia</i> ( <i>E. coli</i> ), <i>Proteus</i> , <i>Shigella</i> , <i>Klebsiella</i> , <i>Bacillus</i> , <i>Clostridium perfringens</i> , some yeasts
0.910	<i>Salmonella</i> , <i>Vibrio parahaemolyticus</i> , <i>C. Botulinum</i> , <i>Serratia</i> , <i>Lactobacillus</i> , <i>Pediococcus</i> , some moulds, <i>Rhodotorula</i> , <i>Pichia</i>
0.870	Many yeasts ( <i>Candida</i> , <i>Torulopsis</i> , <i>Hansenula</i> ), <i>Micrococcus</i>
0.800	Most moulds (mycotoxigenic penicillia), <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> , most <i>Saccharomyces (bailii) spp.</i> , <i>Debaryomyces</i>
0.750	Most halophilic bacteria, mycotoxigenic asperilli
0.650	Xerophilic moulds ( <i>Aspergillus chevalieri</i> , <i>A. Candidus</i> , <i>Wallemia sebi</i> ), <i>Saccharomyces bisporus</i>
0.600	Osmophilic yeasts ( <i>Saccharomyces rouxii</i> ), few moulds ( <i>Aspergillus echinulatus</i> , <i>Monascus bisporus</i> )
0.500	No microbial proliferation

Table 6: micro-organisms inhibited at various  $a_w$  values

Table 6 illustrates the practical usability of water activity as quality parameter to assess the potential risk of different micro-organisms in salted casings. These

<sup>39</sup>Department of Veterinary Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

data indicate that most bacterial species, including the ones that can be found in the gastrointestinal tract of ruminants and pigs, cannot survive a water activity level below 0.91.

At the same time these data indicate that incorporation of repeated  $a_w$  measurements in every HACCP protocol could be recommended and replace to a large extent microbiological testing at different production steps.

The traditional salt preservation remains, however, insufficient to guarantee the absence of spores from *Clostridium* or *Bacillus* species (Wijnker et al. 2006) as well as the absence of certain moulds (fungi imperfecti) in the final product. The latter two species impair the shelf life of the product.

### **Conclusion and recommendations**

In a HACCP protocol, measurement of the water activity ( $a_w$  values) at all preservation steps can replace at many production stages a detailed microbiological control.

Spore-forming organisms are obviously insufficiently inactivated by common salting procedures. While the actual public health risk associated with *Clostridium* spores is presumably low in fresh or canned (sterilized) sausages, modern vacuum packing may increase the risk for *Clostridium* growth in the ready-to-eat products. Therefore it is recommended to initiate further experimental studies to identify preservatives that could improve (and/or partly replace) traditional salting techniques. This recommendation is also made in consideration of the adverse environmental impact of the large quantities of salt and water (for desalting) used by the casing industry.

## **2. Verotoxin in processed casings**

Verotoxin is a bacterial toxin produced by certain strains of *Escherichia coli* (*E. coli*), particularly by strains of the O:H serotype of which O157:H7 is most frequently reported to be associated with human diseases. Symptoms in humans associated with VTEC infections range from mild to bloody diarrhoea and in some cases of systemic infections to hemolytic uremic syndrome. Animals are a natural reservoir for VTEC (verotoxin producing *E. coli* strains), which reside in the intestines of healthy ruminants.

Model experiments indicated that neither saturated brine, nor dry salt reduces the amount of verotoxin as measured by immunochemical methods (ELISA). However, the total verotoxin concentrations remained low and bioassays with two different cell types indicated only an inhibition of cell proliferation rather than a significant cytotoxicity. Temperatures above 80 °C inactivated the toxin.

### **Conclusions and recommendations**

The experimental findings suggest that residues of verotoxin in natural casings do not represent a public health risk. However, continuing attention (see HACCP Protocol) is needed to guarantee the absence of living VTEC bacteria in consumable products, as these present a health risk, especially for children.

### 3. Antimicrobial Resistance

Antimicrobial resistance and the transmission of resistant micro-organisms is one of the emerging global public health concerns. Public interest in Europe is focusing on MRSA (*Methicillin-Resistant Staphylococcus aureus*), which is transmitted from animals (pigs, companion animals) to humans and vice versa. In the United States the FDA has banned the use of certain antimicrobials in the poultry industry in consideration of the possible transmission of resistant *Salmonella* and *Campylobacter* strains to humans, comparable precautionary measures cannot be excluded for the pig industry. Of additional concern are vancomycin-resistant enterococci (VREs) as well as vancomycin-resistant *Clostridium difficile*. The complete ban of antimicrobial growth promoters in the EU is considered as a successful measure to reduce the prevalence of vancomycin-resistant organisms in animals.

Despite these precautionary measures, monitoring of antimicrobial resistance has become mandatory following an amendment of Directive 2003/99/EC (Zoonoses Directive) and the EFSA (European Food Safety Authority) has very recently expressed its concerns about the likely transmission of antimicrobial resistance (resistant organisms of genes/plasmids) via food. Moreover, plasmids can be transmitted from pathogenic to non-pathogenic bacterial strains, which underline the concerns related to plasmid resistance to current food preservation methods.

Model experiments using an *E. coli* strain carrying a plasmid that harbours the most prominent resistance genes for penicillins (*bla*), tetracyclins (*tet*) and chloramphenicol (*cat*) revealed indeed that this plasmid is salt resistant and remains intact even following exposure to varying salt concentrations for an extended period of 33 days.

As yet, these experiments were conducted only with isolated plasmids, and not with plasmids rescued from *E. coli* infected casings. Under natural conditions, plasmids might be even more protected and resistant against any deteriorating effects of brine and salt.

### Conclusions and recommendations

Transmission of resistance-genes located on plasmids by animal-derived foods is of emerging concern and it cannot be excluded that legal requirements (absence of plasmids on ready-to-eat foods) will be defined in the future. It is recommended to include in the evaluation of advanced preservation techniques (as discussed under point 1) for natural casings also the issue of bacterial plasmids carrying antimicrobial resistance genes.

Moreover, as various Asian countries (including China) are recognized as geographical areas with a high prevalence of antimicrobial resistance in the human and animal population and transmission of resistance plasmids from animals to food or humans to food and water (aquacultures) is likely to occur, all measures should be taken to exclude a secondary contamination of products during the sorting and handling procedures.

### 4. Toxicological risk factors

A large number of toxic compounds originating from the animals' environment or being used intentionally during the production phase (antibiotics, antiparasitics) may form residues in animal tissues, including the intestines and subsequently

the casings. In a comprehensive pharmacokinetic (PK) modeling study, 33 unrelated compounds, including hormones (banned in the EU) and medicinal products listed in Annex IV of Council Regulation (EEC) No 2377/90 (not allowed to be used in food producing animals such as nitrofurans, 5-nitroimidazoles, K-agonists and chloramphenicol) were evaluated. Results show that residues in casings equal closely those that are measured in muscle tissue. Considering the general food basket, which uses a consumption of 300 g muscle tissue (meat), the contribution of casings, which would represent approximately 1-1.5% of the meat consumption, is negligible.

### **Conclusions and recommendations**

The current PK modeling studies confirmed the previous assumption that residues in casing contribute only to a very minor amount to the overall risk of consumers of exposure to undesirable residues in foods. At present, casings remain to be controlled and subsequently discarded in the event of the presence of residues of any compound that is not licensed in the EU (see chloramphenicol, nitrofurans,  $\beta$ -agonists (ractopamine, clenbuterol). The current report (draft publication) should be used to re-open the discussion with the competent authorities, explaining that exposure to the mentioned compounds occurs in the life-phase of the animal, which is beyond the responsibility of the casing industry.

At the same time members of the casing industry should request guarantees from the primary producers that none of these compounds that are banned in the EU have been used in the living animals that served as slaughter animals.

### **5. Residue analyses in casings**

The analyses of residues in animal tissues are a Community priority, and harmonized and validated methods are applied in most of the local control institutions. However, at present none of the official residue methods - neither for veterinary medicinal products, nor for other contaminants - have been validated particularly for natural casings, despite the fact the MRPL levels (minimum required performance limits) have been set for nitrofurans and their metabolites as well as for chloramphenicol and other antibiotics (see Commission Decision 2002/657/EC).

A major obstacle in the analysis of casing (particularly sheep casings) was its difficulty to homogenize the material prior to extraction or enzymatic digestion. Own laboratory experiments revealed that a very simple freezing step with liquid nitrogen entirely solves this problem, as nitrogen-frozen casing can be micro-pulverized easily with simple laboratory equipment. This method can be easily combined with current microwave extraction procedures, enzymatic digestion and all modern analytical techniques validated according to Commission Decision 2002/657/EC. This implies that it remains unnecessary to develop specific methods of analysis for natural casings.

### **Conclusions and recommendations**

A very simple step in the sample clean-up procedure can be recommended to be included in any protocol that aims to analyse natural casings for the presence of residues. Together with the results under point 4 (toxicological risks) these data should be discussed with the competent authorities to emphasize the fact that casings **are unlikely to contribute significantly to human exposure** to residues of undesirable/toxic compounds in animal products.

### **The Road Map 2: Safety and quality of natural casings**

**Authors** J. Fink-Gremmels

#### **Report**

ISWG report October 2013

#### **Background and terms of reference**

With a letter dating back to October 2008, a proposal was made to continue some of the work that was conducted in the frame of the Road Map 1 project. As already stated, a number of these projects were closely related to activities of the European Food Safety Authority, establishing new guidelines for the risk assessment of chemical and microbiological contaminants in foods of animal origin. Some of these provisions are of relevance to the casing industry. The aim of this reports is to provide a brief overview about the different subjects and to present in an Annex some detailed information.

#### **Toxic contaminants**

##### *Veterinary medicinal products*

The Road Map 1 presented a statistical model for Veterinary Medicinal Products. This model can be applied to different substances concerning possible chemical residues in animal casings. It has been applied by INSCA/ENSCA for example in the discussion of the safety of new  $\beta$ -agonists (for example ractopamine), which are licensed in the US and some other countries, but prohibited for use in animals in the EU. Other substances that will remain of relevance for the casing industry are residues of chloramphenicol and the nitrofurane group (including their metabolites) in all animal species. Although the residual amounts of these substances present in casings are of negligible human health concern, their use in food producing animal species is prohibited and will in turn result in the rejection of contaminated casings as being non-compliant with EU legislation. The ban of chloramphenicol has been endorsed by an increasing number of countries and is controlled by almost all competent authorities.

Moreover, the use of substances of the group of 5 nitro-imidazoles (metronidazole, ronidazole and others) is prohibited in Europe, but licensed in various other countries. Their use is mainly in poultry and only for some indications in sheep and goats. As these compounds have a short half-life, they are of lower relevance to the casing industry. This applies also to phenylbutazone that is exclusively used in horses and has in this animal species a comparable long persistence after treatment.

A clear increase is observed in the use of non-steroidal drugs to control pain and discomforts in animals and the number of positive residue findings in meat is significantly increasing. However, as these substances have a clear dose-related toxicity and human exposure to residues in casings is negligible, these substances are of low concern for the casing industry and are covered in the statistical model presented already. This applies also for and parasitic drugs.

### *Contaminants*

As already indicated, EFSA has presented modified risk assessment strategies for persistent organic pollutants and toxic metals that may enter the food chain. The major changes in the EFSA risk assessment approach are:

- A full integration of data from the entire food chain, starting with the analysis of contaminants in animal feed, the assessment of possible animal health and welfare concern following exposure to such contaminants and finally the transfer (carry-over) into animal derived products such as meat, milk and eggs. Where appropriate other by-products of special preparation techniques of carcasses (for example sheep and goat meat following ritual slaughter processes) will be considered.
- A complex analysis of human exposure assessment for any compound that enters the food chain. To this end, EFSA had invited all European Member states to compile a food consumption data base (by means of total diet analyses) for different age groups including infants, toddlers, adults, elderly and very elderly people. All recent and forthcoming risk assessments use these Comprehensive Food Consumption Data Base in the form of exposure assessment.
- A quantitative hazard characterization (dose-response assessment) considering the above mentioned details exposure assessment, followed by an uncertainty analysis indicating potential needs for additional data of corrective measures. In cases where no safe dose can be defined, a risk estimate using the margin-of-exposure (MoE) is applied to set priorities for intervention. A default value of > 10 000 for the MoE is considered to be of negligible concern for human health.

This approach has been applied in many recent opinions including critical substances such as polychlorinated and polybrominated hydrocarbons including dioxins, dioxin-like PCBs, nondioxin-like PCBs (polychlorinated biphenols), and perfluorated compounds. **This quantitative exposure assessment confirms that human exposure due to potential residues of these compounds in casings is negligible, and no testing is required.**

In the light of these developments, the original proposal (as part of RoadMap 2) to present a second review on chemical contaminants (like the one for veterinary medicinal products, can be omitted.

### *Expected changes in European legislation following the mandate for "Revision of Meat Inspection"*

In 2008 the European Commission requested EFSA to prepare Scientific Opinions addressing all aspects of meat inspection of slaughter animals. The mandate was divided into several Opinions addressing the animal categories, bovines, small ruminants, pigs, poultry, solipeds (horse and related species) and wild game including rabbits.

The approach including microbiological as well as chemical contaminants, considering the findings of epidemiological studies (zoonosis reports) as well as the outcome of the national residue control programs of 25 (27) Member States during the recent 5 years. All individual documents are available on the EFSA Website ([www.efsa.europa.eu](http://www.efsa.europa.eu)) under the special section: Meat Inspection.

The forthcoming implementation of the recommendation of EFSA into European legislation has started in July 2013. The impact of these changes are expected to be minor for the casing industry, but it cannot be excluded that tracking (and



labelling) of materials from slaughter animals will reassessed and amended as well. Furthermore, it can be expected that food controls to ensure the absence of Verotoxin-producing *Escherichia coli* (VTEC) will be intensified, as these bacteria have been classified as being of concern in products derived from ruminants, sheep and goats (see also original research proposal 3 and 4 in Road Map 2 that remain of interest but should be specified according to the forthcoming EU legislation).

### **Packaging Materials**

During processing and transport, foods come into contact with diverse materials including equipment during processing and transport such as containers, tubes and plastic bags and any other type of packaging.

Such food contact materials for all application are generally divided into the following categories:

- plastics;
- metals;
- glass and ceramics;
- paper and board;
- regenerated cellulose film;
- elastomers.

Migration compounds present in these materials can influence food quality by changing organoleptic and/or nutrition characteristics. Moreover these materials may contain undesirable and toxic substances that migrate into food, any residual amounts in foods have to comply with food safety standards.

The principles of harmonization of European provision regarding such food contact materials are presented in Commission Regulation (EC) No 1935/2004 for all food contact materials. This regulation requires also the establishment of a list of all potential migrating substances and the establishment of maximum permissible levels in foods (see Annex 1 of the original report for more details). Certain amendments for plastic materials were made in Commission Regulation EU 10/2011 (OJL12, 15.1.2011) concerning plastic materials and articles to come into contact with food.

As suggested, a comprehensive list of packaging materials, with reference to the individual EFSA opinions have been compiled and is attached as annex to the report. It should be noted that this is by definition an "open list" and further substances can be added at any time.

In the USA a list of materials that are permitted for use for food products is available (Materials listed on CFR21). In Japan, the Food Sanitation Law and The Food Safety Basic Law regulate food contact materials. The regulations in Japan are close to CFR21-USA and hence differ in some details from the EU guidance documents. Information from other countries is scattered, and often only addressed in the General Food Laws following the principle approach of the EU and USA.

### Aspects of quality assurance in processing natural sausage casings

**Author** J.J. Wijnker

#### **PhD thesis**

Utrecht: Utrecht University, Faculty of Veterinary Medicine, The Netherlands

ISBN: 978-90-393-4932-8

(<http://dspace.library.uu.nl/handle/1874/31822>)

PhD degree obtained on January 8<sup>th</sup> 2009

#### **Summary**

Natural sausage casings are produced from the intestines of various species and used as edible containers for many different types of sausage around the world. Casings must therefore be fit for human consumption and must meet all food safety and hygiene requirements that apply to food of animal origin. As a hazard analysis of sausages would either focus on the final product or on the meat batter inside the casing, it becomes clear that many studies were done on sausages and only a handful on casings.

Salt has been in use for centuries as the main preservative agent of casings and a study was done to determine whether this technique can meet the current microbiological criteria. Results indicate that the antimicrobial properties of salt used for the preservation of casings are sufficient to reduce the bacterial contamination (except for *Clostridium* spores) well below acceptable levels at a water activity level of 0.85 or lower during a 30-day storage period.

To prevent the outgrowth of bacterial spores, additional preservation measures should be implemented. In the experiments described the use of the bacteriocin nisin was evaluated to reduce outgrowth of spores in desalinated casings. Additionally, the binding of nisin to casings, using <sup>14</sup>C-labeled nisin Z and subsequent bioavailability of nisin were evaluated. Results demonstrate that nisin is bound to casings but if sufficient nisin was present the outgrowth of *Clostridium* spores in this model was reduced.

Neural and lymphoid tissue can be regarded as marker tissues for the potential presence of Bovine Spongiform Encephalopathy (BSE) disease-specific prion protein (PrP<sup>Sc</sup>). These tissue types can also be present in cleaned beef casings. Based on the results from a quantitative histological analysis it was calculated that a standard 200 g sausage produced in beef casings contains 0.09 g lymphoid and 0.02 g neural tissue originating from the casing. Using these quantitative histological results a BSE infectivity risk - consumer exposure assessment was made, indicating that the potential risk when consuming a sausage produced in beef casings is far less than consuming a T-bone steak from the same animal.

Casings are produced and shipped all over the world and this trans-global movement of casings means that restrictive measures, implemented to prevent the spread of contagious animal diseases, are also applicable.



Two studies describe the efficacy of FMD and CSF virus inactivation in cleaned casings, either by salt (NaCl) or by using phosphate supplemented salt.

After storage at about 20 °C for 30 days, no remaining FMDV infectivity was found after either treatment, whereas casings stored at 4 °C still contained infectivity. CSFV infectivity was no longer present after treatment with phosphate supplemented salt and storage for 30 days at either 4 °C or 20 °C. To verify that phosphate supplemented salt was used, a commercially available phosphate assay kit was validated for the qualitative determination of phosphate present in treated casings without interference of naturally occurring phosphate in salt used for brines in which casings are preserved.

The general discussion of the thesis was published as a review article in 2 parts in Fleischwirtschaft International:

- Part 1, Safety measures are mandatory now, FLWI 1/2009, 50-58;
- Part 2, Viruses inactivation through salt preservation, FLWI 2/2009, 36-41.

In 2013 an article was published by the author in Fleischwirtschaft International, "It's all about natural", describing how the history of sausage production is closely linked to natural casings. However, it also underlines how the ancient technology of salting natural casings is still up-to-date with current requirements on hygiene and food safety.



**PHOSPHATE SUPPLEMENTED SALT TREATMENT OF  
NATURAL SAUSAGE CASINGS**

## CONTENTS

---

i	General introduction	89
ii	Treatment protocol	91
	Method description	
	Flow chart	
	Description of phosphates E339 used for treatment	
iii	Treatment verification	97
	Method description	
iv	EU approval as food additive	98

## GENERAL INTRODUCTION

---

Foot-and-mouth disease (FMD), classical swine fever (CSF), African swine fever (ASF) and swine vesicular disease (SVD) are highly contagious diseases of cloven-hoofed animals (e.g. pigs, cattle, sheep and goats) and continuing outbreaks incur significant social and economic costs on a global scale (World Animal Health Information Database, accessed December 2013). Affected countries are limited in their ability to trade, with subsequent reductions in the value of their meat commodities. As a result, various measures are put in place on (inter-) national levels to prevent the spread of these diseases either in live animals or its products.

Natural sausage casings ("casings") are traditional products that have been used in the production of meat specialties for centuries, and have remained virtually unchanged in function and appearance. A large variety of high-quality sausage is produced world-wide using the processed intestines of pigs, sheep, goats and cattle (and sometimes horses) as edible envelope.

The main preservative for natural casings is salt (NaCl), either as dry salt or as fully saturated brine (Aw 0.75). This preservation method has been found to be highly effective against all vegetative bacteria (Gabis and Silliker, 1974; Houben 2005; Wijnker et al., 2006).

Subsequent studies have focussed on the usability of phosphates as food additives (Bakker et al., 1999; Verkleij et al., 2003; Houben et al., 2005; Nakae et al., 2008), showing a clear improvement on different microbial and mechanical properties of the natural casings.

As salt and phosphates have shown their usability in the standard processing of natural casings, the efficacy of these treatments against the afore mentioned viruses was studied (Wijnker et al., 2007; Wijnker et al., 2008; Wieringa et al., 2011; Wijnker et al., 2012).

The results from these studies show how specific combinations of salt, phosphate supplemented salt, temperature and treatment time can remove the infectivity risk of different viruses from treated natural casings.

Dry-salting or storage in fully saturated brine using sodium chloride (NaCl) is still the industry's Standard Operating Procedure (SOP) for the preservation of natural casings. Therefore, the specific treatment with phosphate supplemented salt and / or certain minimum storage temperatures during the treatment period should only be part of an emergency protocol in case of an outbreak of a contagious animal disease for which salt alone is not sufficient.

The European Food Safety Authority (EFSA) has published an opinion on this particular subject in 2012 evaluating the different combinations on their efficacy against certain animal diseases. Its recommendations are now included in EU legislation relevant for the import of natural casings.

The actual treatment of natural casings of ruminant or porcine origin, using either salt or phosphate supplemented salt to prevent the spread of different contagious animal diseases, is described in various articles of the OIE's Terrestrial Animal Health Code.

The following combinations are applicable:

<b>Disease</b>	<b>Species</b>	<b>Treatment</b>	<b>Temp</b>	<b>Time</b>
<b>FMDV</b>	Ruminant	Salt (NaCl) only	≥ 12 °C	≥ 30 Days
	Porcine	Phosphate supplemented salt	≥ 12 °C	≥ 30 Days
<b>PPRV</b>	Ruminant	Salt (NaCl) only	≥ 12 °C	≥ 30 Days
<b>CSFV</b>	Porcine	Phosphate supplemented salt	≥ 20 °C	≥ 30 Days
<b>ASFV</b>	Porcine	Salt (NaCl) only	≥ 20 °C	≥ 30 Days
		Phosphate supplemented salt	≥ 20 °C	≥ 30 Days
<b>SVDV</b>	Porcine	Phosphate supplemented salt	≥ 20 °C	≥ 30 Days
<b>Brucellosis</b>	Ruminant	Salt (NaCl) only	≥ 20 °C	≥ 30 Days
	Porcine			

Notes:

1. FMDV – foot-and-mouth disease virus / PPRV – peste des petits ruminants virus / CSFV – classical swine fever virus / ASFV – African swine fever virus / SVDV – swine vesicular disease virus
2. Any treatment can only be done on cleaned and scraped natural casings;
3. Any treatment can be done either via dry salting or in saturated brine;
4. Any treatment other than standard preservation in salt can be replaced by an official declaration issued by the local / national competent authorities stating that the natural casings are derived from animals not affected by the outbreak and no limitations on processing or transport need therefore apply;
5. Application of any treatment applies to natural casings which have not been in salt before, longer than 30 days at the temperature specified for a certain disease;
6. Application of the treatment using phosphate supplemented salt also applies to natural casings which have been stored in salt already but now require additional treatment to prevent transport restrictions from areas affected by an outbreak of CSFV or SVDV.

Modifications to the table and notes above may be applicable due to newly available information.

## **TREATMENT PROTOCOL - METHOD DESCRIPTION**

### **§ 1 Objective**

To adequately treat natural casings with phosphate supplemented salt (P-salt) and / or store at a specified temperature in order to remove a potential infectious animal disease.

### **§ 2 CCP identification**

The following Critical Control Points can be identified in the treatment of natural sausage casings with P-salt or storage at a specified temperature:

	Measure	Critical Limits
<b>01</b> pH of P-brine* used for treatment	All P-brine used during rinsing and storage must remain on or above the critical limit	pH $\geq$ 9.2
<b>02</b> P-salt used for treatment	Usage of sufficient P-salt during production	Visible salt crystals spread evenly over product
<b>03</b> Storage temperature of casks after treatment	Continuous measurement of storage temperature	$\geq$ 12 °C for FMDV, PPRV  $\geq$ 20 °C for CSFV, ASFV, SVDV and Brucellosis
<b>04</b> Storage period	All P-brine treated goods must meet critical limit before they are shipped to a third party	$\geq$ 30 days stored in P-brine

\*P-brine is saturated brine made from P-salt

### **§ 3 Requirements**

- Natural casings
- Container with potable water at ambient temperature
- Container with saturated brine made from P-salt (P-brine)
- Sufficient supply of P-brine to fill-up casks
- Clean casks
- New cask liners

### **§ 4 Instructions**

The instructions listed below include different options, depending on type of product (raw material, finished goods) and storage (dry salt or brine). On a company level the applicable steps need to be identified and implemented.

### 1. P-brine production

- P-salt consists of (weight/weight/weight): 1) sodium chloride (NaCl), 86.5%; 2) trisodium phosphate  $\text{Na}_3\text{PO}_4$  (TSP), 2.8; 3) disodium phosphate  $\text{Na}_2\text{HPO}_4$  (DSP), 10.7%.
- Maximum solubility of salt (NaCl) in water is 350 g/l → 35 kg per 100 l.  
Per 100 l P-brine: 1) TSP, 1.0 kg; 2) DSP, 3.75 kg; 3) NaCl, 30,25 kg;
- Mix different components in potable water at ambient temperature until completely dissolved and check pH (lower limit 9.2 – upper limit 10). Start by dissolving TSP and DSP in water before NaCl;
- Alternatively: use available P-salt premix in same ratio to make a saturated P-brine. The same solubility as salt (350 g/l) is applicable.

### 2. Treating unsalted natural casings (raw material)

- Raw material available in water or unsaturated (NaCl) brine;
- Fill container with sufficient P-brine;
- Check pH prior and during rinsing step (lower limit 9.2). If pH is below 9.2 replace P-brine;
- Rinse individual bundles / tubes / pipes in P-brine for 10 minutes or until all visible salt is removed;
- The company SOP can be used for (dry-) salting raw material, only the standard salt (NaCl) needs to be replaced with P-salt.

### 3. Treating salted natural casings (finished goods)

- Finished goods available in dry salt or saturated (NaCl) brine;
- Check pH prior and during rinsing step (lower limit 9.2). If pH is below 9.2 replace P-brine;
- Rinse individual bundles / tubes / pipes in P-brine for 10 minutes or until all visible salt is removed;
- The company SOP can be used for re-salting finished goods (either dry salt or in saturated brine), only the standard salt (NaCl) needs to be replaced with P-salt.

### 4. Packing treated natural casings (raw materials) and storage

- Pack natural casings treated with P-salt in clean cask & liner according to company SOP;
- Store casks at temperatures over 12 or 20 °C for at least 30 days;
- Label casks containing natural casings treated with P-salt clearly and keep them quarantined until 30-day period has elapsed.

### 5. Repacking treated natural casings (finished goods) and storage

- Repack finished goods soaked in P-brine in clean cask & liner according to company SOP;
- Fill cask with P-brine. Prior to usage, check pH of P-brine (lower limit 9.2). If pH is below 9.2 replace P-brine;
- In case of dry-salted bundles:  
The company SOP can be used for salting finished goods, only the standard salt (NaCl) needs to be replaced with P-salt;
- Store casks at temperatures over 12 or 20 °C for 30 days;



- Label casks containing natural casings treated with P-brine or P-salt clearly and keep them quarantined until 30-day period has elapsed.

#### 6. Treatment verification

- If required, the treatment of the natural casings can now be verified by the competent authorities using the methodology described in Chapter 3 of the ISWG booklet *"Phosphate supplemented salt treatment of natural sausage casings"*;
- After verification, approval and registration, the natural casings can either remain in P-brine or return to the item's original type. This could be dry salted, stored in saturated brine using only NaCl and storage without specified temperatures.

### **§ 5 P-salt variants**

Due to differences in local availability, different variants of the described phosphate salts may be used. The differences lie in the inclusion of H<sub>2</sub>O in the molecule. Although TSP and DSP are available in many variants, only two variants should be considered for this protocol. Other variants will reduce the amount of NaCl on the overall mix to such an extent that this will have a negative effect on the preservation of natural casings and pH buffering capacity necessary for the treatment.

As with NaCl, only food grade phosphate salts shall be used for the production of P-salt or P-brine.

	Na <sub>3</sub> PO <sub>4</sub>	Na <sub>3</sub> PO <sub>4</sub> ·2H <sub>2</sub> O	Na <sub>2</sub> HPO <sub>4</sub>	Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O	NaCl
Mw*	164	200	142	178	58
ratio(%)	2.8	3.7	10.7	13.4	82.9 - 86.5
P-mix (KG)	1	1.3	3.75	4.7	29 - 30.25

\*Mw = molecular weight

The % of NaCl included depends on the specific phosphate salt combination used and its related ratio. NaCl can be added to complete 100% of P-salt mix.

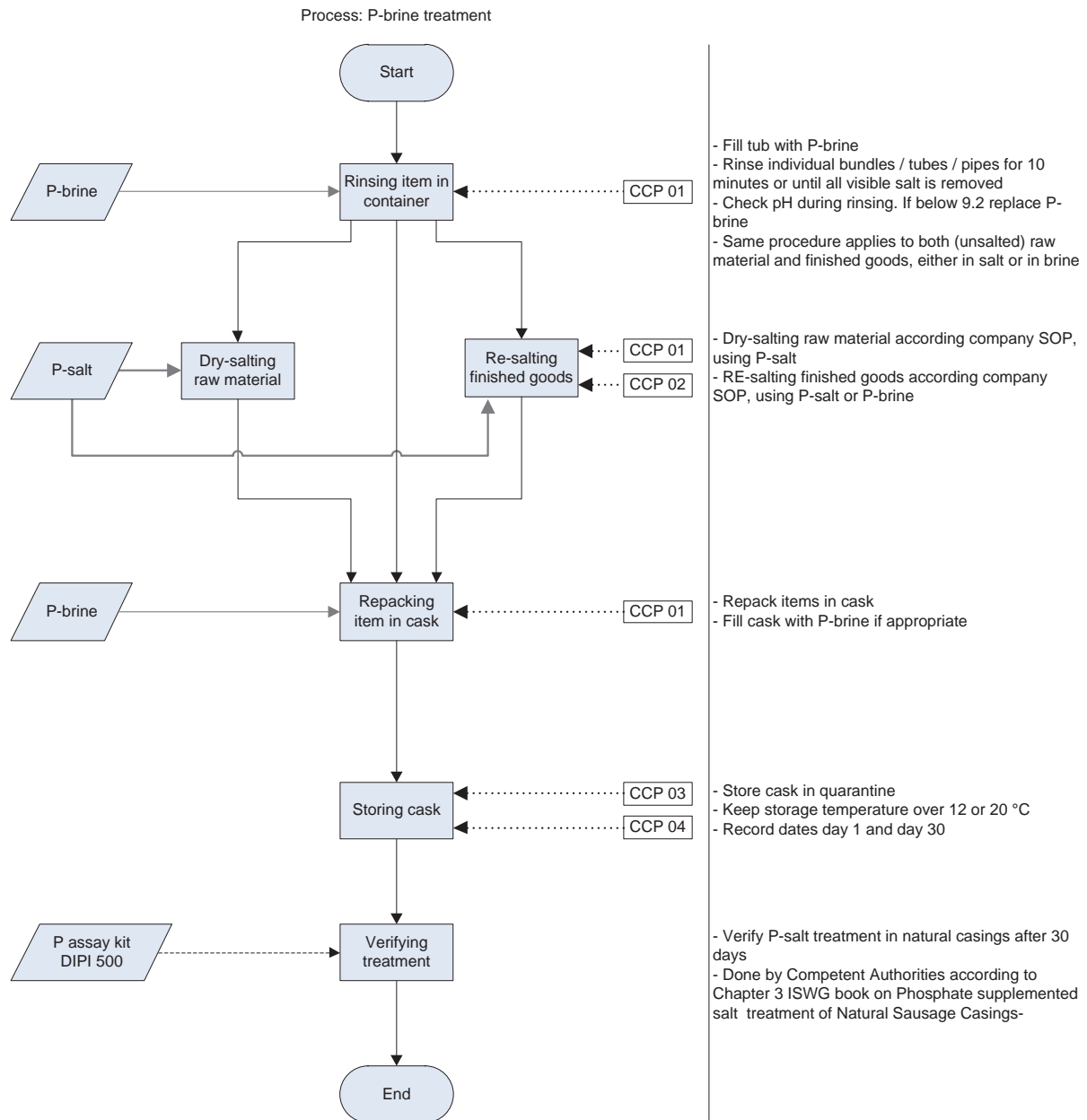
### **§ 6 References**

- OIE Terrestrial Animal Health Code
- All studies referred to in Chapter 5 of the ISWG booklet *"Phosphate supplemented salt treatment of natural sausage casings"*

### **§ 7 Registrations**

- P-brine treatment form (containing info on cask ID, pH measurements, storage temperature, storage period)

## FLOW CHART



### Flowchart validation

Measured results:

- Hog casing raw material samples, cleaned and either unsalted or salted (NaCl), were rinsed in P-brine and subsequently stored in P-salt directly or after overnight drain. Samples were stored at ambient temperatures for over 60 days.

Results: Microbiological status well below industry requirements;

P-salt treatment had no negative effect on quality parameters of casings;

Outside of casings felt softer, lumpy salt crystals.

- Hog casing dry-salted (NaCl) finished goods samples were rinsed in P-brine and subsequently stored in P-brine or P-salt, directly or after overnight drain. Samples were stored at ambient temperatures for over 60 days.

Results: Microbiological status well below industry requirements;

P-salt treatment had no negative effect on quality parameters of casings;

Samples stored in P-salt had lumpy salt crystals, good outer slip, poor inner slip and outside of casings felt softer. However, the poor inner slip is most likely due to the original quality of the hog casing sample used for this test;



Samples stored in P-brine had crystalized salt crusts on outer surface, good inner and outer slip and outside of casings felt softer.

- Sheep casing dry-salted (NaCl) finished goods samples were rinsed in P-brine and subsequently stored in P-brine or P-salt, directly or after overnight drain. Samples were stored at ambient temperatures for over 60 days.

Results: Microbiological status well below industry requirements;

P-salt treatment had no negative effect on quality parameters of casings;



Samples stored in P-salt had lumpy salt crystals, good inner and outer slip and outside of casings felt softer. However, the good inner slip is most likely due to the original quality of the sheep casing used for this test;

Samples stored in P-brine had crystalized salt crusts on outer surface, good inner and outer slip and outside of casings felt softer.

#### Personal comments:

- Extra effort was required to remove crystalized P-salt, which may reduce handling speed when goods are desalinated, either for selection or sausage production;
- Salt crystals and “milky” aspect of water after rinsing are likely to be caused by calcium-phosphate complexes. The amount of calcium present in the potable water could be of influence on this phenomenon. Usage of demineralized water may prevent this phenomenon although no negative effects of the calcium-phosphate complexes in water is presently known.

#### Acknowledgements

The skilled assistance of Jop Abendanon and Matthijs Knetsch (Van Hessen bv, The Netherlands) was highly appreciated in the completion of this method description.

## DESCRIPTION OF PHOSPHATES (E 339)

---

### § 1 Trisodium phosphate (TSP)

TSP is a white, granular or crystalline solid, highly soluble in water producing an alkaline solution. The item of commerce is often partially hydrated and may range from anhydrous trisodium phosphate,  $\text{Na}_3\text{PO}_4$ , to the dodecahydrate,  $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ . Most often found in white powder form, it can also be called trisodium orthophosphate or just plain sodium phosphate.

- Molecular formula:  $\text{Na}_3\text{PO}_4$
- Molecular weight: 164 g / mol
- CAS number: 7601-54-9
- EINECS number: 231-509

Note: the phosphate supplemented salt mixture is based on a weight-to-weight %, using the anhydrous TSP. Therefore if any  $\text{H}_2\text{O}$  containing TSP is to be included in the mixture, the actual amount of TSP must be compensated for the additional weight of the  $\text{H}_2\text{O}$  molecules (see table in Chapter 2.1, paragraph 2).

For more information: [http://en.wikipedia.org/wiki/Trisodium\\_phosphate](http://en.wikipedia.org/wiki/Trisodium_phosphate)

### § 2 Disodium phosphate (DSP)

DSP ( $\text{Na}_2\text{HPO}_4$ ) is a [sodium salt](#) of [phosphoric acid](#). It is a white powder that is highly [hygroscopic](#) and water soluble. It is therefore used commercially as an anti-caking additive in powdered products. It is also known as disodium hydrogen orthophosphate, sodium hydrogen phosphate or sodium phosphate dibasic. It is commercially available in both the hydrated and anhydrous forms. pH of disodium hydrogen phosphate water solution is between 8.0 and 11.0.

- Molecular formula:  $\text{Na}_2\text{HPO}_4$
- Molecular weight: 142 g / mol
- CAS number: 7558-79-4
- EINECS number: 231-448-7

Note: the phosphate supplemented salt mixture is based on a weight-to-weight %, using the anhydrous DSP. Therefore if any  $\text{H}_2\text{O}$  containing DSP is to be included in the mixture, the actual amount of DSP must be compensated for the additional weight of the  $\text{H}_2\text{O}$  molecules (see table in Chapter 2.1, paragraph 2).

For more information: [http://en.wikipedia.org/wiki/Disodium\\_phosphate](http://en.wikipedia.org/wiki/Disodium_phosphate)

### § 3 Global availability of TSP and DSP

Due to its common nature and application in many different products, both TSP and DSP are readily available world-wide.



# TREATMENT VERIFICATION - METHOD DESCRIPTION<sup>40</sup>

## BioAssay Systems

## Phosphate

DIP1006.pdf

### QuantiChrom™ Phosphate Assay Kit (DIP1-500) Quantitative Colorimetric Phosphate Determination at 620nm

#### DESCRIPTION

Phosphate (Pi) is one of the most important ion species in nature. Phosphate is present in all biological systems. It is a major constituent in minerals and fertilizers, and is a component of industrial wastewater. Thus accurate determination of phosphate concentration finds numerous applications in pharmacology, biomedical research, clinical chemistry, industrial process monitoring and environmental monitoring.

Simple, direct and automation-ready procedures for measuring phosphate concentration in biological and environmental samples are becoming popular. BioAssay Systems' phosphate assay kit is designed to measure phosphate ion directly in samples without any pretreatment. The improved Malachite Green method utilizes the malachite green dye and molybdate, which forms a stable colored complex specifically with inorganic phosphate. The intensity of the color, measured at 620nm, is directly proportional to the phosphate concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

#### KEY FEATURES

**Sensitive and accurate.** Linear detection range 0.30 µM (0.0028 mg/dL) to 50 µM (0.47 mg/dL) phosphate in 96-well plate assay.

**Simple and high-throughput.** The procedure involves addition of a single working reagent and incubation for 30 min. Can be readily automated as a high-throughput assay for thousands of samples per day.

**Improved reagent stability and versatility.** The optimized formulation has greatly enhanced reagent and signal stability. Assays can be executed in cuvet or 96-well plate.

**Low interference in biological samples.** No pretreatments are needed. Assays can be directly performed on raw biological samples i.e., in the presence of lipid, protein and minerals.

#### APPLICATIONS

**Direct Assays:** Pi in serum, urine, saliva, sweat, tissue culture etc.

**Drug Discovery/Pharmacology:** effects of drugs on Pi metabolism.

**Food and Beverages:** Pi determination.

**Environment:** Pi determination in water, soil and fertilizer.

#### KIT CONTENTS (500 tests in 96-well plates)

Reagent: 50 mL      Pi standard: 14 mL 0.28 mg/dL (30 µM)  
Blank Control: 14 mL

**Storage conditions.** The kit is shipped at room temperature. Store all components at 4°C. Shelf life of at least 6 months (see expiry dates on labels).

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

##### Reagent Preparation:

Important: bring reagents to room temperature and shake before use.

##### Procedure using 96-well plate:

1. Set up standards and samples. Transfer 50 µL distilled water ("Blank"), Standard and samples in duplicate wells of a clear bottom 96-well plate.
2. Add 100 µL Reagent and tap lightly to mix.
3. Incubate 30 min at room temperature and read optical density at 620nm (600-660nm).

##### Procedure using cuvette:

1. Set up test tubes labeled Blank, Standard, Samples. Transfer 400 µL Water, Standard and samples to appropriately labeled tubes.
2. Add 800 µL Reagent and tap lightly to mix.
3. Incubate 30 min at room temperature, transfer to cuvet and read optical density at 620 nm (600-660nm).

**Important:** (1) if sample OD is higher than the OD for standard, dilute samples in distilled water and repeat the assay. (2) It is not necessary to

prepare a calibration curve, because the concentration of the provided standard lies within the linear range. (3) Precipitation may occur at high concentrations of phosphate (>100 µM), or in the presence of high concentrations of e.g. proteins and metals. In this case, dilute samples in distilled water and repeat the assay.

#### CALCULATION

The phosphate concentration of Sample is calculated as

$$= \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{OD_{\text{STANDARD}} - OD_{\text{BLANK}}} \times 0.28 \text{ (mg/dL)}$$

OD<sub>BLANK</sub>, OD<sub>STANDARD</sub> and OD<sub>SAMPLE</sub> are OD<sub>620nm</sub> values of Blank, Standard and Sample, respectively.

**Conversions:** 1 mg/dL Pi equals 105.3 µM, 0.001% or 10 ppm.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories.

##### Procedure using 96-well plate:

Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

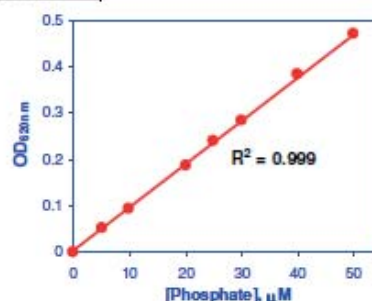
##### Procedure using cuvette:

Spectrophotometer and cuvetts for measuring OD 620nm.

#### EXAMPLES (96-well plate assay):

	Pi (mg/dL)
1	6.4 ± 0.6
2	2.2 ± 0.1
3	3.5 ± 0.1
4	0.081 ± 0.003
5	0.003 ± 0.001
6	0.02 ± 0.001
7	1.10 ± 0.01
8	0.56 ± 0.06
9	0.19 ± 0.03

**Biological Samples:** 1. Commercial 2% reduced fat milk (Kirkland). 2. Invitrogen fetal bovine serum. 3. Fresh human urine. **Water samples:** 4. Tap water (Hayward, CA). 5. Tap water (San Bruno, CA). **Food and Beverages:** 6. Crystal Geyser natural alpine spring water. 7. Coca-cola® classic coke. 8. Lipton Lemon iced tea. **Environmental:** 9. Soil extract. 5.6 g of soil (Hayward, CA) was extracted with 10 mL MilliQ water. The supernatant was centrifuged to remove any insoluble particles. Clear supernatant was assayed.



Standard Curve in 96-well plate assay

#### PUBLICATIONS

1. Abranches, J. (2008). CcpA regulates central metabolism and virulence gene expression in *Streptococcus mutans*. J Bacteriol. 190(7):2340-9.
2. Hildebrand, J. et al (2009) Functional and energetic characterization of P-gp-mediated doxorubicin transport in rainbow trout. Comp Biochem Physiol C Toxicol Pharmacol. 149(1):65-72.
3. Dunbar, D.R. et al. (2010). Transcriptional and physiological responses to chronic ACTH treatment by the mouse kidney. Physiol Genomics 40(3): 158-166.

<sup>40</sup>See Chapter 4.6 for a more detailed description of the method validation in natural sausage casings by Wijnker et al., 2009

## EU APPROVAL AS FOOD ADDITIVE

The use of food additives in the European Union is governed by Regulation (EC) No 1333/2008. Annex II of this regulation, listing all food additives approved for use in foods and conditions of use, is governed by Commission Regulation (EC) No 1129/2011.

On October 31<sup>st</sup> 2013, the following publication in the Official Journal of the European Union was made:

*Commission Regulation (EC) No 1069/2013, of 30 October 2013, amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of sodium phosphates (E339) in natural casings for sausages.*

As of November 20<sup>th</sup> 2013, phosphates (E 339) may now be used as acidity regulator during the production of natural casings. The following arguments were listed:

- *The maximum tolerable daily intake (MTDI) of phosphates established by the Scientific Committee for Food is 70 mg/kg body weight. The maximum level proposed by the applicant is 12600 mg/kg of casings leading to a maximum carry-over of phosphates from casings in the final sausage of 250 mg/kg. The highest contribution of phosphates via treated natural casings will be 2,1 % of the MTDI. It is therefore appropriate to allow the use of sodium phosphates as an acidity regulator to improve the mechanical properties of casings for sausages;*
- *Pursuant to Article 3 of Regulation (EC) No 1331/2008, the Commission is to seek the opinion of the European Food Safety Authority in order to update the Union list of food additives set out in Annex II to Regulation (EC) No 1333/2008, except where the update in question is not liable to have an effect on human health. Since the authorisation of use of sodium phosphates (E 339) for the improvement of the mechanical properties of natural casings for sausages constitutes an update of that list which is not liable to have an effect on human health for the aforesaid reasons, it is not necessary to seek the opinion of the European Food Safety Authority.*

In Part E of Annex II to Commission Regulation (EC) No 1333/2008, the following entry is inserted in the food category 08.2.3 "casings and coatings and decorations for meat":

E 339	Sodium phosphates	12600	(4) (80)	Only in natural casings for sausages
	(4): The maximum level is expressed as P <sub>2</sub> O <sub>5</sub>			
	(80): Carry-over in the final product shall not exceed 250 mg/kg.			

**EU – CRAFT – Project (BRC 2.CT 94.1495)**  
**Improved treatment of natural sausage casings for quality**  
**improvement in automated stuffing processes**

**INVENTORY PART**  
**HISTOLOGY AND MICROBIOLOGY OF**  
**HOG AND SHEEP CASINGS**



# **EU - CRAFT - Project**

BRE 2.CT 94. 1495

**Improved treatment of natural casings  
for quality improvement in automated  
filling processes.**

## **INVENTORY PART HISTOLOGY AND MICROBIOLOGY OF HOG AND SHEEP CASINGS**

### **Industrial partners**

#### *Casing companies*

CHARLES FRÈRES, France; COMBINATIE TEIJSEN & VAN DEN HENGEL, the Netherlands  
DAT-SCHAUB, Denmark; PETER GELHARD, Germany;  
VAN HESSEN, the Netherlands; JÜRGING, Germany.

#### *Sausage manufacturers*

BOEKOS, the Netherlands; KEMPER, Germany.

#### *Manufacturer of stuffing machines*

VEMAG, Germany.

### **R&D-Performers**



UTRECHT UNIVERSITY



Deutsches Institut  
für Lebensmitteltechnik e.V.



## HISTOLOGY OF HOG CASINGS

### AIM

The aim of this inventory project was to obtain basic information on the microstructure of unprocessed and processed hog small intestines

An inventory was made of the anatomy of unprocessed hog small intestines (Figures A-C)

### ANATOMY OF THE HOG SMALL INTESTINE

Fig. A.  
Hog viscera illustrating the way in which the small intestine (top) is connected to the mesenterium.

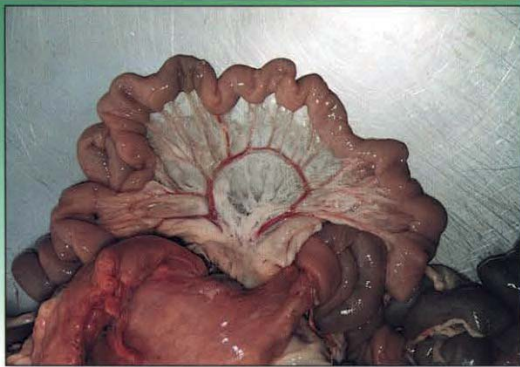


Fig. B.  
Small intestine (jejunum) with attached mesenterium. Blood vessels from the mesenterium penetrate into the submucosa layer. Some parts of these blood vessels may remain after pulling (so-called "whiskers")



Fig. C.  
Diagrams (A and B) of the distribution of blood vessels in the small intestine. B: The mucous layer (villus and crypt layers) and the muscle layers (CM and LM) are removed by pulling. The natural casing only consists of the submucosa layer (Sub). (Source: W. Bloom & D.W. Fawcett, *A textbook of Histology*, Philadelphia 1969).

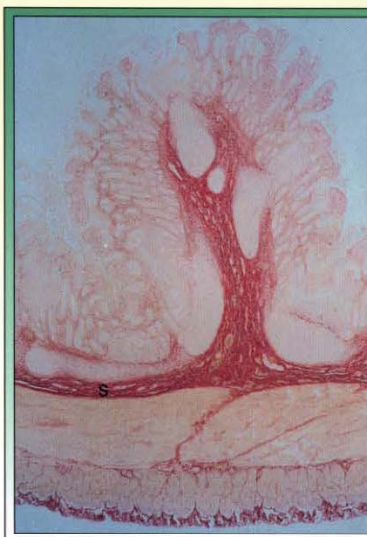
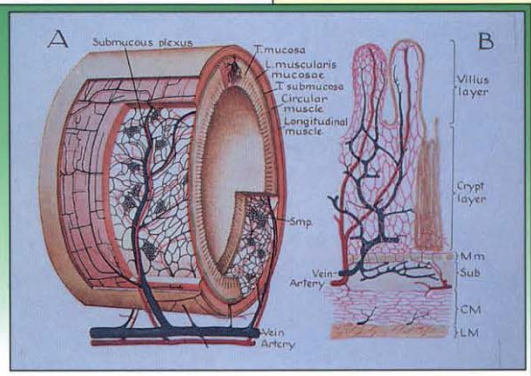


Fig. D.  
Micrograph of hog jejunum, taken before the pulling knife. The intestine is still intact. The submucosa (S) is stained red. Mucous layer on top, muscle layer below, bar = 0.5 mm.

### RESULTS

A closer look at the anatomy of hog intestines resulted in a better understanding of the process of separating the intestine from the mesenterium and explained the phenomenon of "whiskers", which may show up after pulling.



## MICROSTRUCTURE OF HOG CASINGS AFTER EACH STEP IN THE PROCESS LINE.

### METHODS

Histological sections were made of hog small intestines, taken at different sampling locations of the process line. These sections were examined microscopically. Micrographs illustrating the main differences were taken (Figures D-H).



Fig. E.  
Micrograph of hog jejunum, sampling location 1. The muscle layers (below) and submucosa (S) are still intact. The mucous layer (top) is largely removed.

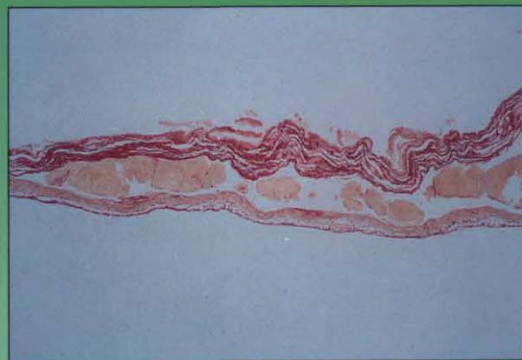


Fig. F.  
Micrograph of hog jejunum, sampling location 2. The mucous layer is almost completely removed, while the submucosa remains intact.

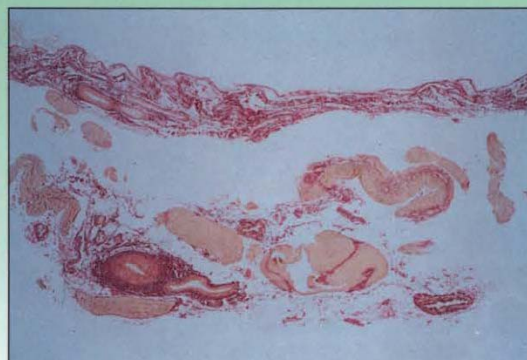
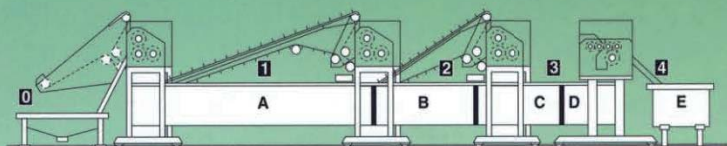


Fig. G.  
Micrograph of hog jejunum, sampling location 3. The mucous layer is removed. The muscle layer is separated from the intact submucosa.



Fig. H.  
Micrograph of hog jejunum, sampling location 4. All layers are removed, while a clean submucosa remains.

### RESULTS

The histological examination revealed that hog intestines were cleaned thoroughly without damaging the tissue of the submucosa layer, which essentially comprises the basic structure of hog natural casings. The mucous layer of the jejunum is gradually removed in different steps; the muscle layer is removed by the finisher. The microstructure of natural casings is characterized by a network of collagen and elastin fibres and blood vessels of different sizes. The thickness of the clean casing is on average 0.32 mm.

## MICROSTRUCTURE OF SHEEP CASINGS AFTER EACH STEP IN THE PROCESS LINE.

### AIM

The aim of this inventory project was to obtain basic information on the microstructure of unprocessed and processed sheep small intestines.

### METHODS

Histological sections were made of sheep small intestines, taken at different sampling locations of the process line. These sections were examined microscopically. Micrographs illustrating the main differences were taken (Figures I-L).



Fig. I.  
Micrograph of an intact sheep intestine, sampling location 0, taken before manual pulling. All layers are still intact. The submucosa (S) is stained red. Mucous layer on top, muscle layer below, bar = 0.5 mm.



Fig. J.  
Micrograph of sheep intestine, sampling location 1, after manual pulling, roller 1 and the additional soaking process.

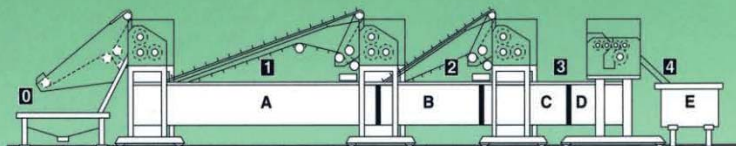


Fig. K.  
Micrograph of sheep intestine, sampling location 2. The muscle layer is damaged, while the mucous layer is partly removed.

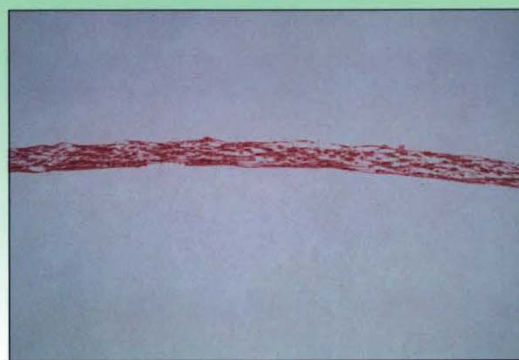


Fig. L.  
Micrograph of sheep casing, sampling location 4. Only the submucosa layer is left.

### RESULTS

The micrographs show that the cleaning process of sheep casings is quite similar to that of hog casings. However, an interim soaking period may result in some tissue denaturation. The thickness of the clean casing is on average 0.11 mm.



## HISTOLOGY OF CASINGS

The phenomena of "whiskers", "veiny" casings and "Patches of Peyer" (Figures, M-O).

Fig. M.  
Stereo microscopy of a natural hog casing  
with an attached remainder of a blood vessel  
(so-called "whisker").

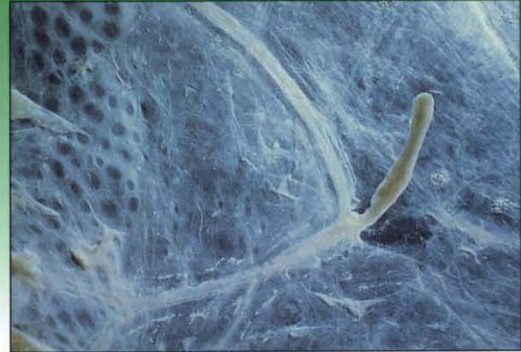


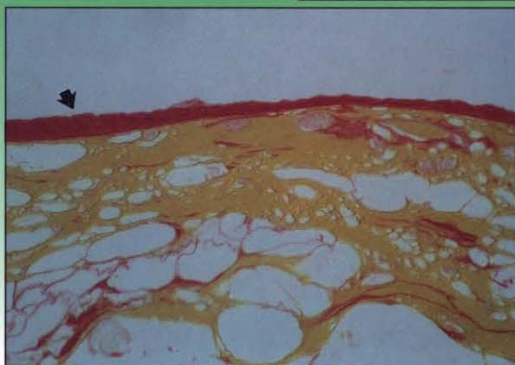
Fig. N.  
Stereo microscopy of a hog natural casing in  
which light spots are present. In practice,  
these spots are erroneously called "worm"-  
holes. Microscopically these spots were  
identified as the points where lymph nodes  
(so-called "Patches of Peyer") were originally  
present in the intestines. During the cleaning  
process these nodes are removed together  
with the mucous layer, thus causing the  
presence of light spots in the natural casing.



Fig. O.  
Stereo microscopy of "veiny" casings. In some  
casings a pattern of blood vessels is visible  
(see also Fig. N, left side). The veins and  
arteries are well visible because of the white  
substance that surrounds them. This  
substance consists of fat pockets which  
disappear upon cooking.



Fig. P.  
Micrograph of a hog natural casing  
surrounding a fermented sausage.



## CONCLUSION

The additional information about the microstructure of hog and sheep small intestines and natural casings provided by histological examination can be usefully applied by experts in process control and for improving processing machinery and methods.

## EXPLANATION TO TABLES

< = Smaller than

<u>Count / gram</u>	<u>Log Count / gram</u>
10-100	1.00-2.00
example 30	1.48
example 50	1.70
example 80	1.90
100-1,000	2.00-3.00
example 300	2.48
example 500	2.70
etc.	
1,000-10,000	3.00-4.00
example 3,000	3.48
etc.	
10,000-100,000	4.00-5.00
100,000-1,000,000	5.00-6.00
1,000,000-10,000,000	6.00-7.00
etc.	

[ In formula:  $^{10}\text{Log } a = b \leftrightarrow 10^b = a$  ]



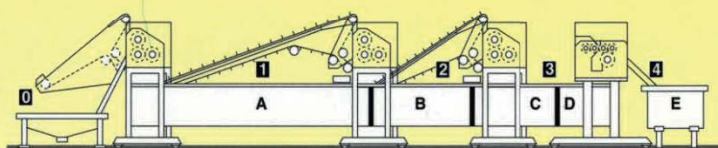
## MICROBIOLOGY OF HOG AND SHEEP CASINGS

### AIM

The aim of this inventory project was to obtain basic information on the microbiology of unprocessed hog and sheep small intestines and of casings and water samples taken during and after cleaning operations.

### RESULTS

BACTERIOLOGICAL RESULTS HOG LINE (LOG COUNTS/G ; AVERAGES PRESENTED)								
	SAMPLE LOCATION IN CLEANING LINE					SAMPLED AFTER SALTING		
	0	1	2	3	4	SAME DAY	AFTER 24h	AFTER 72h
Total aerobic count	5.70	6.13	6.50	6.63	5.79	5.77	6.03	6.40
Enterobacteriaceae	4.75	5.19	5.34	5.36	4.43	<2.5	<2.5	<2.5
Staphylococcus aureus*	3.46	3.53	2.97	3.45	3.35	5.13	3.50	4.52
Salt tolerant organisms	3.18	3.41	2.76	3.17	3.46	4.08	4.34	4.95
Lactic acid bacteria	5.76	5.89	6.12	6.17	4.68	5.31	5.33	5.04
Bacterial spores	if detected, than at insignificant levels (<100/g)							
* BPM counts; all appeared coagulase negative								



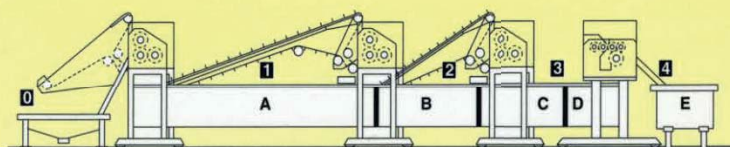
BACTERIOLOGICAL EXAMINATION PROCESS WATER HOG LINE (LOG COUNTS/G ; AVERAGES PRESENTED)				
	SAMPLE LOCATION IN CLEANING LINE			
	A	B	C	D
Total aerobic count (20°C)	6.69	6.14	5.27	5.94
Enterobacteriaceae	5.04	4.57	4.36	4.83

BACTERIOLOGICAL RESULTS WET SALTED HOG CASINGS - SORTING / GRADING PLANT - (LOG COUNTS/G ; AVERAGES PRESENTED)					
	At receival	After calibration	Before 'piping'	After 'piping'	At dispatch to sausage factory
Days p.m.	2	2	44	45	51
Total aerobic count	7.25	6.82	5.60	5.28	4.90
Enterobacteriaceae	6.27	6.65	2.64	3.88	2.77
Staphylococcus aureus*	<2.00	<2,00	<2,00	2,13	<2,00
Salt tolerant organisms	6.16	5.69	3.55	3.47	4.70
Lactic acid bacteria	6.46	6.52	4.62	4.29	4.65
Bacterial spores	if present, than at insignificant levels (<100/g)				
* BPM counts; all appeared coagulase negative					

## MICROBIOLOGY OF HOG AND SHEEP CASINGS

### RESULTS

BACTERIOLOGICAL RESULTS SHEEP LINE (LOG COUNTS/G ; AVERAGES PRESENTED)								
	SAMPLE LOCATION IN CLEANING LINE					SAMPLED AFTER SALTING		
	0	1	2	3	4	SAME DAY	AFTER 72h	AFTER 4wk
Total aerobic count	5.71	5.74	6.34	6.45	6.54	4.23	5.91	4.74
Enterobacteriaceae	4.04	4.17	4.27	4.51	4.26	< 2.5	< 2.5	< 2.5
Staphylococcus aureus*	2.69	3.49	4.33	3.94	4.31	2.70	--	<2.30
Salt tolerant organisms	2.96	3.45	3.20	3.66	3.39	6.88	5.18	6.82
Lactic acid bacteria	5.44	5.43	6.09	6.10	5.48	3.62	5.56	4.15
Bacterial spores	if detected, than at levels ranging from insignificance to occasionally 1000/g							
* BPM counts; all appeared coagulase negative								



BACTERIOLOGICAL EXAMINATION PROCESS WATER SHEEP LINE (LOG COUNTS/G ; AVERAGES PRESENTED)					
	SAMPLE LOCATION IN CLEANING LINE				
	A	B	C	D	E
Total aerobic count (20°C)	6.13	5.62	6.50	5.88	5.37
Enterobacteriaceae	4.08	3.04	3.28	3.37	2.46

### CONCLUSIONS

Casings as investigated come from the animal in a moderately contaminated way with respect to total aerobic counts, Enterobacteriaceae, lactic acid bacteria and salt tolerant bacteria. During cleaning operations these counts sometimes increase, except for the salt tolerant organisms. Bacterial spores were detected at levels ranging from insignificance to thousand/g for sheep casings (mainly *Bacillus* species). Overall, final products were fully acceptable. Pathogens were not detected any more in adequately salted products.

### RECOMMENDATIONS

- # maintain a high standard of equipment cleaning and disinfection procedures;
- # safeguard a water supply to the cleaning line of a high hygienic quality;
- # try to limit increases in bacterial counts during cleaning operations as much as possible;
- # reduce additional treatments after salting to the essential minimum.





**INSCA – ENSCA – NANCA  
SCIENTIFIC WORKING GROUP**

**STATEMENT ON THE DEFINITION OF ILEUM**

## STATEMENT ON THE DEFINITION OF ILEUM

On October 1<sup>st</sup>, 2003 Commission Regulation (EC) No 1139/2003 amending Regulation (EC) No 999/2001 was implemented, which related to the classification of sheep and goat ileum as SRM for animals of all ages slaughtered on and after 1 October 2003. In response the following statement was issued by the SWG in order to clarify certain points for the benefit of the natural casing industry worldwide and those responsible for risk management and enforcement of the Regulations.

### Anatomical definition

At the request of the natural casing industry, three internationally recognized veterinary anatomists were consulted on the anatomical definition of the ileum. It has been recommended that the definition in *Nomina Anatomica Veterinaria* (NAV) is used (I.C.V.G.A.N., 2005). The official body that authorizes acceptable veterinary anatomical terms is the World Association of Veterinary Anatomists and their list of official terms is published in NAV. The NAV definition is:

*"Ileum: the short terminal part of the small intestine to which the Plica ileocaecalis is attached"*.

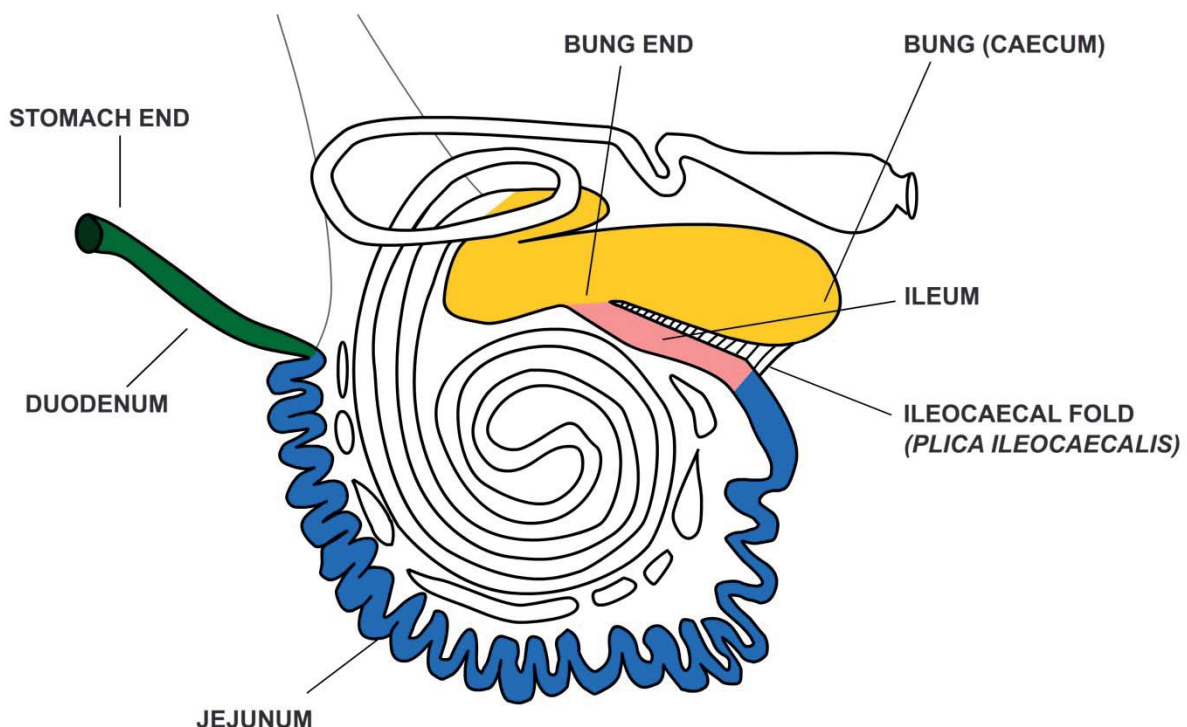


Figure 7: Small ruminant intestinal tract

The meat industry in general and the natural casing industry in particular, have adopted a range of terms to describe various parts of the intestine (Figure 7), not all of which correspond with the internationally agreed veterinary anatomical terms. When legislation was adopted (as of October 1<sup>st</sup>, 2003 in the EU) it was essential that the industry, regulators and inspectors had a common understanding of the law and how it should be applied. It was the purpose of this statement to assist in achieving a common understanding of some additional terms that have led to a degree of misunderstanding / confusion.

The following appellations are commonly used in the natural casing industry:

- Bell ends: found according to season and age of the animals, such special parts are usually 0.5 to 1.5 m long. Such portion is the distal part of the jejunum, just before the ileum;
- New Zealand or Australian Cuts: such specific processing methodology has been widely used in those 2 countries and by extension adopted in many factories. Here three cuts of the small intestine / casing are defined, starting from the distal (bung) end of the small intestine:
  - 1<sup>st</sup> Cut: from the terminal (distal) jejunum (junction with the ileum) and extending forwards between 7.5 and 12 m;
  - 2<sup>nd</sup> Cut: the middle part of the jejunum of unspecified length;
  - 3<sup>rd</sup> Cut: the proximal jejunum (junction with the duodenum) of unspecified length.



## REFERENCES

---

- Alkema, W., Mierau, I., Stringer, S., 2013. Establishing safe thresholds for spore formers in the casings industry. ISWG report (Project No. 1301) July 2013.
- Bakker, W.A.M., Houben, J.H., Koolmees, P.A., Bindrich, U., Sprehe, L., 1999. Effect of initial mild curing, with additives, of hog and sheep sausage casings on their microbial quality and mechanical properties after storage at different temperatures. *Meat Science* 51, 163-174.
- Bartenschlager-Blässing, E.-M., 1979. Technische, organoleptische und mikrobiologische Eigenschaften sowie histologische Merkmale von Darmsaitlingen in Hautfasersaitlingen. *Fleischwirtschaft* 59-3-293-300.
- Benli, H., Hafley, B.S., Keeton, J.T., Lucia, L.M., Cabrera-Diaz, E., Acuff, G.R., 2008. Biomechanical and microbiological changes in natural hog casings treated with ozone. *Meat Science* 79, 155-162.
- Berends, B.R., Bergwerf, A.A., Houben, J.H., 2000. Substances and residues in natural sausage casings. Department of the safety of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
- Berends, B.R., 2011. Bacterial spores on natural casings and means of control or elimination. ISWG report (Project No. 1002) September 2011.
- Blackwell, J.H., 1984. Foreign animal disease agent survival in animal products: Recent developments. *Journal of the American Veterinary Medical Association* 184, 674-679.
- Bockemühl, J., 2000. Studies on emerging pathogens in natural sausage casings, final report. Hygiene Institut Hamburg.
- Böhm, H.O., Krebs, H., 1974. Nachweis von Maul- und Klauenseuche-Virus in Organen krank geschlachteter Schafe. *Berliner und Münchener Tierärztliche Wochenschrift* 87, 410-412.
- Botka-Petrak, K., Hraste, A., Petrak, T., 2001. Influence of different treatment procedures on morphological structure of beef intestines. *Veterinarski Arhiv* 71, 1, 1-9.
- Brown, D.R., Wong, B.S., Hafiz, F., Clive, C., Haswell, S.J., Jones, I.M., 1999. Normal prion protein has an activity like that of superoxide dismutase. *Biochemical Journal* 344, 1-5.
- Buschmann, A., Groschup, M.H., 2005. Highly Bovine Spongiform Encephalopathy-sensitive transgenic mice confirm the essential restriction of infectivity to the nervous system of clinically diseased cattle. *Journal of Infectious Diseases* 192, 934-942.
- Byun, M.W., Lee, J.W., Jo, C., Yook, H.S., 2001. Quality properties of sausages made with gamma-irradiated natural pork and lamb casing. *Meat Science* 59, 223-228.
- Chawla, S.P., Chander, R., Sharma, A., 2006. Safe and shelf-stable natural casing using hurdle technology. *Food Control* 17, 127-131.
- Codex Alimentarius, 2001. Codex standard for food grade salt, CX STAN 150-1985, Rev. 1-1997, Amend. 1-1999, Amend. 2-2001.
- Comer, P., 2002. Assessment of the risk of exposure to the BSE agent through the use of natural sausage casings. DNV Report No. 716146. DNV Consulting, Palace House, London, UK.
- Comer, P., Bradley, R., 2002. Note on weights of intestine in small ruminants and expected risk reduction. DNV Consulting, Palace House, London, UK.
- Comer, P., 2008. TSE risk assessment for use of bovine casings. DNV Report No. 22926377. Det Norske Veritas LTD, Palace House, London, UK.
- DasSarma, S., Arora, P., 2001. Halophiles. *Encyclopedia of Life Sciences* 1-9.
- Doherr, M.G., 2007. Brief review on the epidemiology of transmissible spongiform encephalopathies (TSE). *Vaccine* 25, 5619-5624.
- EFSA Panel on Animal Health and Welfare, 2012. Scientific Opinion on the animal health risk mitigation treatments as regards imports of animal casings. *EFSA Journal* 10(7), 2820.
- EFSA Panel on Biological Hazards, 2007. Opinion on quantitative histological studies and the re-assessment of the BSE related risk of bovine intestines after processing into natural sausage casings. *EFSA Journal* 464, 1-14.
- EFSA Panel on Biological Hazards, 2009. Scientific Opinion on BSE risk in bovine intestines. *EFSA Journal* 1317, 1-19.
- EFSA Panel on Biological Hazards, 2011. Scientific Opinion on a review of the BSE-related risk in bovine intestines. *EFSA Journal* 9(3), 2104.
- EFSA Panel on Biological Hazards, 2014. Scientific Opinion on BSE risk in bovine intestines and mesentery. *EFSA Journal* 12(2), 3554.
- Farez, S., Morley, R.S., 1997. Potential animal health hazards of pork and pork products. *Revue scientifique et technique (International Office of Epizootics)* 16, 65-78.
- Fink-Gremmels, J., 2008. The Road Map, Consumer safety of natural sausage casings. Division Veterinary Pharmacology, Pharmacy and Toxicology (VFFT), Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

- Fink-Gremmels, J., 2013. The Road Map 2, Safety and quality of natural casings. Division Veterinary Pharmacology, Pharmacy and Toxicology (VFFT), Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
- Fischer, A., Schweglinghaus, M., 1988. Naturdärme 1: Anatomie und Gewinnung. Fleischerei 39, 10-14.
- Fischer, A., Krol, B., 1997. HACCP Manual for Processing of Natural Sausage Casings. Institute for Risk Assessment Sciences, Department of Public Health and Food Safety, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
- Gabis, D.A., Silliker, J.H., 1974. *Salmonella* in natural animal casings. Applied Microbiology 27, 66-71.
- Gröning, G., 1905. Aus der Auslandfleischschau – Gesalzene Därme. Zeitschrift für Fleisch- und Milchhygiene XV, 12, 357-359.
- Haritova, A.M., Fink-Gremmels, J., 2010. A simulation model for the prediction of tissue:plasma partition coefficients for drug residues in natural casings. The Veterinary Journal 185, 278-284.
- Heeres, H.L., Lucas Luijckx, N.B., Stekelenburg, F., 2009. HACCP Manual for Processing of Natural Sausage Casings (third edition). TNO report V7169. TNO Quality of Life, Zeist, The Netherlands.
- Heiss-Straubig, 1902. Die Darmputzmaschine nach Nägele. Zeitschrift für Fleisch- und Milchhygiene XII, 4, 210-211.
- Helwig, D.M., Keast, J.C., 1966. Viability of virulent swine fever virus in cooked and uncooked ham and sausage casings. Australian Veterinary Journal 42, 131-135.
- Hoffmann, C., Ziegler, U., Buschmann, A., Weber, A., Kupfer, L., Oelschlegel, A., Hammerschmidt, B., Groschup, M.H., 2007. Prions spread via the autonomic nervous system in cattle incubating bovine spongiform encephalopathy. Journal of General Virology 88, 1048-1055.
- Houben, J.H., 2003. Residues of curing agents in natural sausage casings previously subjected to anti-viral treatments - Hog and sheep casings treated with either lactic acid, citric acid or orthophosphates. Fleischwirtschaft 11, 42-48.
- Houben, J.H., 2005. A survey of dry-salted natural casings for the presence of *Salmonella* spp., *Listeria monocytogenes* and sulphite-reducing *Clostridium* spores. Food Microbiology 22, 221-225.
- Houben, J.H., Bakker, W.A.M., Keizer, G., 2005. Effect of trisodium phosphate on slip and textural properties of hog and sheep natural sausage casings. Meat Science 69, 209-214.
- International Committee on Veterinary Gross Anatomical Nomenclature (I.C.V.G.A.N.), 2005. Nomina Anatomica Veterinaria, 5<sup>th</sup> Edition. Published by the Editorial Committee Hannover, Columbia, Gent, Sapporo.
- Jo, C., Lee, J.W., Cho, K.H., Yook, H.S., Byun, M.W., 2002. Quality properties of sausage made with gamma irradiated natural casing from intestine of pork or lamb. Radiation Physics and Chemistry 63, 365-367.
- Koolmees, P.A., Houben, J.H., 1997. Inventory part: histology and microbiology of hog and sheep casings. Colour print leaflet EU—CRAFT Project BRE 2. CT 94. 1495: Improved treatment of natural sausage casings for quality improvement in automated stuffing processes. Deutsches Institut für Lebensmitteltechnik eV, Quackenbrück.
- Koolmees, P.A., 1998. Histology of bovine natural casings. VVDO report 9806, Utrecht University.
- Koolmees, P.A., Berends, B.R., Tersteeg, M.H.G., 2002. Risk assessment of the use of sheep natural casings and legs of lamb. VVDO Report No. H0204. Department of Public Health and Food Safety, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
- Koolmees, P.A., Tersteeg, M.H.G., Keizer, G., van den Broek, J., Bradley, R., 2004. Comparative histological studies of mechanically versus manually processed sheep intestines used to make natural sausage casings. Journal of Food Protection 67, 2747-2755.
- Labots, H., 1967. "Rode Hond" bij natuurdarmen. Vleesdistributie en Vleestechnologie, 1- 2e jaargang, 1967.
- Labots, H., Krol, B., 1964. Enkele eigenschappen van bacteriën die "rode hond" veroorzaken bij de opslag van natuurdarmen. Report R.1787. TNO, Utrecht.
- McKercher, P.D., Hess, W.R., Hamdy, F., 1978. Residual viruses in Pork Products. Applied and Environmental Microbiology 35, 142-145.
- McKercher, P.D., Morgan, D.O., McVicar, J.W., Shuot, N.J., 1980. Thermal processing to inactivate viruses in meat products. Proceedings, Annual Meeting US Animal Health Association 84, 320-328.
- Nakae, S., Oshida, T., Yoon, H., Sakata, R., 2008. Maximum force and breaking strain decrease. Fleischwirtschaft International 1, 44-46.
- Nishiumi, T., Sakata, R., 1999. Histological and biochemical evaluation of connective tissue of natural hog and sheep casings. Proceedings 45<sup>th</sup> ICoMST, 174-175.
- Nishiumi, T., Nojiri, T., Yoshihara, T., Ichinoseki, S., Suzuki, A., Tanabe, M., Sakata, R., 2005. Proceedings 51<sup>st</sup> ICoMST, 1138-1141.

- Ockerman, H. W., and C. L. Hansen (ed.). 2000. Sausage Containers, p. 285-323. In: Animal Byproduct Processing and Utilization. CRC Press, Boca Raton, FL.
- von Ostertag, R., 1905. Zur Beurteilung von Därmen, die mit parasitären Knötchen behaftet sind, im Inlandverkehr. Zeitschrift für Fleisch- und Milchhygiene XVI, 1, 1-5.
- Panina, G.F., Civardi, A., Massirio I., Scatozza, F., Baldini, P., Palmia, F., 1989. Survival of foot-and-mouth disease virus in sausage meat products (Italian salami). International Journal of Food Microbiology 8, 141-148.
- Panina, G.F., Civardi, A., Cordioli, P., Massirio, I., Scatozza, F., Baldini, P., Palmia, F., 1992. Survival of hog cholera virus (HCV) in sausage meat products (Italian salami). International Journal of Food Microbiology 17, 19-25.
- Panzer, G., 1977. Der Naturdarm – seine wirtschaftliche Bedeutung und seine Zukunftschancen. Fleischerei 28, 51-53.
- Prusiner, S.B., 1998. Prions. Proceedings of the National Academy of Sciences USA 95, 13363-13383.
- Reichert, J.E., 1996. Methods of tenderizing edible sausage casings of animal origin. Fleischwirtschaft 76, 392-393.
- den Reijer, M.H.W., 1996. Stugheid van darmen bij de produktie van rookworst. Report V 96.184. TNO-Voeding, Zeist.
- Riha, W.E., Solberg, M., 1970. Microflora of fresh pork sausage casings. 2. Natural casings. Journal of Food Science 35, 860-863.
- Rust, R.E., 1988. Production of edible casings, p 261-274. In: Pearson, A.M., Dutson, T.R. (Eds), Edible meat by-products. Elsevier Science Publishers, London.
- Sakata, R., Segawa, S., Morita, H., Nagata, Y., 1998. Tenderization of hog casings - application of organic acids and proteases. Fleischwirtschaft International 4, 20-21.
- Savic, Z., Savic, I., 2002. Sausage Casings, p. 25-31, 121-126. Victus Vienna.
- Schmidt, H., 2001. Report on the analysis of natural casings from third countries (non EU countries) to detect residues of pharmacological substances. Tiergesundheitsdienst Bayern e.V., Animal Hygiene Department, Poing, Germany.
- Schwanz, S., Schnäbel, W., 2007a. Untersuchungen zur Festigkeit von Naturdärmen -1. Optimierung von Messtechnologien – Wunsch nach einheitlichem Prüfverfahren. Fleischwirtschaft 87, 220-223.
- Schwanz, S., Schnäbel, W., 2007b. Untersuchungen zur Festigkeit von Naturdärmen -2. Brühwürste – Optimierung von Textureigenschaften. Fleischwirtschaft 87, 99-103.
- Schweigmann, A., Seeger, H., 1988. Untersuchungen zur Normbeschaffenheit von Naturdärmen. Fleischwirtschaft 68, 970-978.
- Smits, J., Keizer, G., 2003. Verpakken – Natuurdarmen. B.V.I. 1-10.
- Stack, M.J., 2009. Immuno-histochemical and immuno-biochemical PrP<sup>d</sup> analysis of BSE infected small intestinal tissues used for sausage casings. VLA Report No. FT1394. Veterinary Laboratories Agency - Weybridge, Addlestone, UK.
- Stack, M.J., Moore, S.J., Vidal-Diez, A., Arnold, M.E., Jones, E.M., Spencer, Y.I., Webb, P., Spiropoulos, J., Powell, L., Bellerby, P., Thurston, L., Cooper, J., Chaplin, M.J., Davis, L.A., Everitt, S., Focosi-Snyman, R., Hawkins, S.A.C., Simmons, M.M., Wells, G.A.H., 2011. Experimental bovine spongiform encephalopathy: detection of PrP<sup>Sc</sup> in the small intestine relative to exposure dose and age. Journal of Comparative Pathology 145, 289-301.
- Terry, L.A., Marsh, S., Ryder, S.J., Hawkins, S.A.C., Wells, G.A.H., Spencer, Y.I., 2003. Detection of disease-specific PrP in the distal ileum of cattle exposed orally to the agent of bovine spongiform encephalopathy. Veterinary Record 152, 387-392.
- Trigo, M.J., Fraqueza, M.J., 1998. Effect of gamma radiation on microbial population of natural casings. Radiation Physics and Chemistry 52, 125-128.
- Wells, G.A.H., Dawson, M., Hawkins, S.A.C., Green, R.B., Dexter, I., Francis, M.E., Simmons, M.M., Austin, A.R., Horgan, M.W., 1994. Infectivity in the ileum of cattle challenged orally with bovine spongiform encephalopathy. Veterinary Record 135, 40-41.
- Verkleij, T.J., Keizer, G., 2003. Effects of different curing treatments on the usability of beef, hog and sheep casing. TNO report V5070. TNO Nutrition and Food Research, Zeist.
- Wieringa-Jelsma, T., Wijnker, J.J., Zijlstra-Willems, E.M., Dekker, A., Stockhove-Zurwieden, N., Maas, R., Wisselink, H.J., 2011. Virus inactivation by salt (NaCl) and phosphate supplemented salt in a 3D collagen matrix model for natural sausage casings. International Journal of Food Microbiology 148, 128-134.
- Wijnker, J.J., 2006. Casings and the TSE / BSE risk: is it safe? Fleischwirtschaft International 1, 34-37.
- Wijnker, J.J., Koop, G., Lipman, L.J.A., 2006. Antimicrobial properties of salt (NaCl) used for the preservation of natural sausage casings. Food Microbiology 23, 657-662.

- Wijnker, J.J., Haas, B., Berends, B.R., 2007. Removal of foot-and-mouth disease virus infectivity in salted natural sausage casings by minor adaptation of standardized industrial procedures. *International Journal of Food Microbiology* 115, 214-219.
- Wijnker, J.J., Depner, K.R., Berends, B.R., 2008. Inactivation of classical swine fever virus in porcine casing preserved in salt. *International Journal of Food Microbiology* 128, 411-413.
- Wijnker, J.J., Tersteeg, M.H.G., Berends, B.R., Vernooij, J.C.M., Koolmees, P.A., 2008. Quantitative histological analysis of bovine small intestines before and after processing into natural sausage casings. *Journal of Food Protection* 71, 1190-1204.
- Wijnker, J.J., 2009. Aspects of quality assurance in processing natural sausage casings. PhD thesis. Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
- Wijnker, J.J., 2009. Safety measures are mandatory now. *Fleischwirtschaft International* 1, 50-58.
- Wijnker, J.J., 2009. Virus inactivation through salt preservation. *Fleischwirtschaft International* 2, 36-41.
- Wijnker, J.J., 2009. Shelf life of natural sausage casings. ISWG report May 2009.
- Wijnker, J.J., Tjeerdsma-van Bokhoven, J.L.M., Veldhuizen, E.J.A., 2009. Phosphate analysis of natural sausage casings preserved in brines with phosphate additives as inactivating agent – Method validation. *Meat Science* 81, 245-248.
- Wijnker, J.J., Weerts, E.A.W.S., Breukink, E.J., Houben, J.H., Lipman, L.J.A., 2011. Reduction of *Clostridium sporogenes* spore outgrowth in natural sausage casings using nisin. *Food Microbiology* 28, 974-979.
- Wijnker, J.J., 2012. Nutritional value of natural sausage casings. ISWG report April 2012.
- Wijnker, J.J., Haas, B., Berends, B.R., 2012. Inactivation of foot-and-mouth disease virus in various bovine tissues used for the production of natural sausage casings. *International Journal of Food Microbiology* 153, 237-240.
- Wijnker, J.J., 2013. It's all about natural. *Fleischwirtschaft International* 6, 16-17.
- Wirth, F., 1990. Microbiological investigation of natural casings. Invest. No. 60-81. Federal Office for Meat Research, Institute for Technology, Kulmbach.
- Wirth, F., 1994. Microbiological investigation of natural casings. Invest. No. 34-55. Federal Office for Meat Research, Institute for Technology, Kulmbach.