European Food Safety Authority

EFSA Journal 2012;10(7):2820

SCIENTIFIC OPINION

Scientific Opinion on animal health risk mitigation treatments as regards imports of animal casings[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

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. 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. © European Food Safety Authority, 2012

KEY WORDS

Intestinal casings, pathogens, inactivation, salting, phosphate, temperature.

[1] On request from the European Commission Question No EFSA-Q-2011-01255, adopted on 22 June 2012.

[2] Panel members: Anette Bøtner, Donald Broom, Marcus G. Doherr, Mariano Domingo, Jörg Hartung, Linda Keeling, Frank Koenen, Simon More, David Morton, Pascal Oltenacu, Albert Osterhaus, Fulvio Salati, Mo Salman, Moez Sanaa, James M. Sharp, Jan A. Stegeman, Endre Szücs, Hans-H. Thulke, Philippe Vannier, John Webster and Martin Wierup. Correspondence: ahaw@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the AHAW Working Group on Treatments of Casings, Mariano Domingo (Chair), Bernd Haas and Klaus Depner, for the preparatory work on this scientific opinion and the hearing expert, Joris Wijnker, and EFSA staff, Milen Georgiev and Per Have, for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Animal Health and Welfare (AHAW); Scientific Opinion on animal health risk mitigation treatments as regards imports of animal casings. EFSA Journal 2012;10(7):2820. [32 pp.] doi:10.2903/j.efsa.2012.2820. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Animal Welfare (AHAW) was asked to deliver a scientific opinion on animal health risk mitigation treatments with regard to imports of animal casings. This request is limited to casings produced from intestines and bladders of animals of the bovine, ovine, caprine, porcine and equine species.

Animal casings are imported into the European Union from a variety of third countries with different animal health status, provided that casings have been treated according to EU legislation (Commission Decision 2003/779/EC, laying down animal health requirements and the required veterinary certification for the import of animal casings from third countries). Salting with sodium chloride (NaCl) for 30 days is a well-established and accepted procedure in the casings industry worldwide, and it has been the standard animal health risk mitigation treatment prescribed in EU legislation for many years, in order to eliminate any potential animal health risk from casings. Experience has shown that, under practical conditions, the EU prescribed treatment has effectively prevented the spread or introduction of animal diseases within or into the EU via animal casings.

The request from the European Commission focused on the scientific review of: (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 be developed, giving equivalent or better results in the inactivation of relevant pathogens; and (iii) assessment of the modified phosphate salt treatment recently recommended by the World Organisation for Animal Health for foot and mouth disease virus (FMDV), in particular whether it could be considered safe with regard to the elimination of animal health risks posed by pathogens other than FMDV possibly present in casings, taking into account the effects of both temperature and duration of treatment.

The list of the infectious agents of concern for the import of casings into the EU was derived from legislation on import of fresh meat (if not treated, intestines are considered fresh meat), and comprised, among others, viruses causing FMD, classical swine fever (CSF) and African swine fever (ASF), as well as bacterial infections such as brucellosis, tuberculosis and glanders. Available practices and treatments in the production of casings were reviewed, and information on general biological characteristics related to survival or inactivation of the pathogens was collected. Scientific information dealing with the effect of treatments on the survival of the relevant pathogens in casings or in a collagen-matrix model was reviewed.

For most of the infectious agents investigated, salting either with NaCl or with phosphate-supplemented NaCl showed higher efficacy at the upper range of temperature (about 20 ºC) than at 4 ºC.

FMDV in casings derived from experimentally infected animals was inactivated after treatment with NaCl for a period of 30 days at 20 ºC. However, after 30 days at 4 °C, some FMDV still remained infectious. Similar results were obtained with casings spiked with FMDV. In a three-dimensional (3D) matrix model the inactivation kinetics of FMDV in untreated and NaCl-salted samples was similar at the different temperatures tested, which emphasises the important role of temperature in inactivation.

Treatment with a phosphate-supplemented NaCl led to faster inactivation of FMDV than treatment with NaCl salt alone, both in casings derived from experimentally infected animals as well as in spiked casings. For both NaCl and phosphate-supplemented NaCl treatment a temperature of 20 ºC was more effective than lower temperatures for inactivation.

Salting of casings derived from experimentally infected animals with NaCl for a period of 30 days at room temperature (~20 °C) leads to inactivation of CSFV. However, the same treatment at low temperature (4 ºC) does not fully inactivate the virus. In a 3D matrix model an effect of NaCl salting on virus inactivation compared with the untreated controls was evident at 20 °C and 25 °C but not at

4 °C and 12 °C. Treatment with a phosphate-supplemented NaCl leads to complete inactivation of CSFV in casings derived from experimentally infected animals within 30 days.

Regarding ASFV, no studies were found dealing with inactivation on experimentally infected or spiked casings. In a 3D matrix model ASFV was readily inactivated by NaCl salting at all temperatures (4, 12, 20 and 25 °C) after 21 days. However, from its general characteristics, ASFV can be regarded as more stable than CSFV. Therefore, these results should be interpreted with caution.

There are no data available on inactivation of peste des petits ruminants virus and rinderpest virus on casings from experimentally infected animals, nor in spiked casings or in a 3D matrix model. From the general characteristics of these viruses it was assumed that they will be inactivated within 30 days at room temperature.

For swine vesicular disease virus (SVDV) there are no studies on inactivation performed with NaCl salting at room temperature. Survival of virus for at least 200 days at 4 °C has been reported in porcine casings preserved with NaCl. In a 3D matrix model SVDV showed a temperature-dependent inactivation, but, even at 20 °C with NaCl salting or 25 °C in untreated controls, some virus survived until day 30. Based on the general characteristics of SVDV, it is possible that NaCl salting for 30 days at room temperature will not completely inactivate the virus.

Brucella spp. are readily inactivated by NaCl salting, but *Mycobacterium avium* may survive far beyond 30 days in intestines in conditions similar to those used for salting of casings. *M. bovis* and other mycobacteria may show similar resistance to the salting treatment in casings, but this should be investigated.

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. No alternative procedures can therefore be recommended as substitutes for current salting treatments based on NaCl and phosphate-supplemented NaCl.

As there is a lack of specific scientific studies on the efficacy of bleaching and drying for pathogen inactivation in casings, these treatments cannot be recommended as stand-alone treatments, i.e. without prior salting for 30 days.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Animal casings[4] are imported into the European Union from a variety of third countries with different animal health status *inter alia* for use in the production of meat products like sausages. The animal health conditions for trade in and imports of casings are set up in Chapter 2 of Annex I to Council Directive 92/118/EEC of 17 December 1992 laying down animal health and public health requirements governing trade in and imports into the Community of products not subject to the said requirements laid down in specific Community rules referred to in Annex A(I) to Directive 89/662/EEC and, as regards pathogens, to Directive 90/425/EEC[5]. Commission Decision 2003/779/EC laying down animal health requirements and the veterinary certification for the import of animal casings from third countries[6] provides that Member States shall authorise the importation of animal casings derived from any species and from any third country accompanied by a health certificate certifying *inter alia* that those casings have been cleaned, scraped and either salted with sodium chloride (NaCl) for 30 days, bleached or dried after scraping. Salting with NaCl for 30 days is a well-established and accepted procedure in the casings industry worldwide and has been the standard animal health risk mitigation treatment prescribed in Union legislation for many years in order to eliminate any potential animal health risk, in particular pathogens of animals of the bovine, ovine, caprine, porcine and equine species, presented by animal casings imported from third countries. Experience has shown that under practical conditions, the EU prescribed treatment has effectively prevented the spread or introduction of animal diseases within or into the EU via animal casings.

In 2008, the World Organisation for Animal Health (OIE) introduced in Chapter 8.5 of its Terrestrial Animal Health Code (Code) a recommendation for the inactivation of foot-and mouth-disease virus (FMDV), if present in casings derived from ruminants and pigs. The recommended treatment consists of the exposure of the casing to either NaCl salt or a phosphate salt / NaCl mixture for at least 30 days at a room temperature of about 20°C. In 2011, the Member States of the EU supported the modification of that phosphate salt treatment and its temperature specification in the foot and mouth disease Code chapter[7]. Furthermore, that phosphate salt treatment was also proposed for introduction in a modified code chapter on Classical Swine Fever (CSF) as a procedure for the inactivation of CSF virus (CSFV) if present in casings derived from pigs. However, that draft-modified chapter was not adopted in 2011 and various issues remain under further discussion.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority (EFSA) to provide a scientific opinion on whether

1. The NaCl treatment has been refined in recent years as regards temperature and/or duration of treatment in a way that would lead to an increased level of safety as regards animal pathogens, and

2. Alternative treatments have been developed that give equivalent or better results in the inactivation of pathogens possibly present in casings derived from animals of the bovine, ovine, caprine, porcine and equine species, taking into account scientific developments and technological progress.

[4] The scope of this request for a scientific opinion shall be limited to casings produced from intestines and bladders of animals of the bovine, ovine, caprine, porcine and equine species.

[5] OJL062, 15.3.1993, p. 49.

[6] OJL285, 1.11.2003, p. 38.

[7] "Article 8.5.41. 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 (Aw < 0.80), or with phosphate supplemented dry salt containing 86.5 percent NaCl, 10.7 percent Na_2HPO_4 and 2.8 percent Na_3PO_4 (weight/weight/weight), and kept at a temperature of greater than 12 ºC during this entire period." [2011 edition of the Code]

Furthermore, considering that the OIE, in its Code chapter on FMD, has recently recommended a modified phosphate salt treatment as an alternative to the standard NaCl treatment for the inactivation of FMDV, if present in casings derived from ruminants and pigs, EFSA is requested to provide a scientific opinion on whether

3. modified phosphate salt treatment, when applied to casings derived from animals of the bovine, ovine, caprine, porcine and equine species, can be considered as an effective and reliable alternative to the standard NaCl treatment currently foreseen in Union legislation, so as to provide at least equivalent animal health guarantees as regards the elimination of animal health risks posed by pathogens other than FMDV possibly present in casings derived from animals of the bovine, ovine, caprine, porcine and equine species, taking into account the effects of both the temperature and the duration of treatment.

ASSESSMENT

1. Introduction

1.1. Focus of the work

This opinion focuses on the treatments for casings and their ability to inactivate relevant infectious agents that could be present in casings. Traditionally, treatments to produce animal casings include cleaning, scraping and salting at room temperature for a period of at least 30 days. Other less frequent treatments also authorised for the import of casings into the European Union and performed after cleaning and scraping are drying (normally done after the salting procedure) or bleaching (referred to as *blanchir* in French with the meaning "to bleach" or "to treat with heat"). Casings may be produced form several hollow organs and from a variety of species, but, for the purpose of this mandate, only casings derived from the intestines and urinary bladder of cattle, sheep, goats, swine and horses are considered. If originating in authorised establishments, and treated by any of the approved procedures (Council Directive 92/118/EEC and Commission Decision 2003/779/EC), animal casings may be imported into the EU regardless of the animal health status of the country of origin or production of casings. Trading of casings under these conditions is considered to be safe, and there is not a single recorded incident of animal pathogens entering the EU farm animal population that is attributable to contaminated casings. Scientific information was reviewed to assess the ability of classic and new alternative treatments (such as the sodium phosphate–NaCl treatment) to inactivate possible infectious agents present in casings. Only relevant infectious agents that are of concern in relation to the trade of fresh meat under EU legislation (Commission Regulation (EU) No 206/2010, Annex II) were considered.

1.2. Work approach

1.2.1. Methodology

In responding to this mandate the AHAW Panel focused on the scientific assessment of information on treatments of casings against specific pathogens causing animal diseases in line with the requested mandate rather than a thorough risk assessment on the risk of their introduction into the EU.

A critical review of the literature obtained by a broad scope search and provided by the hearing experts was used to extract relevant information. The flow of the work followed the structure of this document: (i) determine the list of the pathogens of concern for the EU related to casings with justifiable reasons; (ii) review available practices and treatments for production of casings; (iii) collect information, through literature and other sources, on biological characteristics related to the survival or inactivation of the pathogens; and (iv) assess the effects of available and new treatments on the survival of the relevant pathogens in casings. The basic biological features of the pathogens were collected from well-known/-recognised sources (scientific documents, reference textbooks, World Organisation for Animal Health (OIE) Manual, etc.). Specific outcomes of various treatments were based on information available from published literature. Differentiation was made between studies on casings derived from experimentally infected animals and those involving *in vitro* experiments. Some assumptions were made, and it is stated when information for a pathogen has been extrapolated from other infectious agents with similar physicochemical characteristics.

1.2.2. Identification of relevant infectious agents that might be present in casings derived from animals of the bovine, ovine, caprine, porcine and equine species

There is no specific EU legislation identifying infectious agents that might be present in casings derived from animals of the bovine, ovine, caprine, porcine and equine species. Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community lists in Part A of Annex I the diseases of terrestrial animals that are notifiable. In total, 22 infectious diseases are currently listed, all of which are traditionally considered to have a major impact not only on animal health but also on trade or human health (zoonotic character). The epizootic diseases subject

to mandatory emergency actions with territorial restrictions are listed in Annex C of Council Directive 90/425/EEC of 26 June 1990, concerning veterinary and zootechnical checks applicable in intra-community trade of certain live animals and products with a view to the completion of the internal market. Several of the listed diseases are irrelevant when it comes to the role of casings in their transmission. For example pathogens of poultry, bacterial infections, parasitic agents, vector-transmitted pathogens and zoonotic agents are not transmitted via casings, and animal casings do not contribute to the spread of these infections.

Infectious agents relevant for this opinion are those listed in the EU legislation concerning import of fresh meat (Commission Regulation (EU) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements). This Regulation is an important legal document, which identifies relevant pathogens that might also be introduced by the trade of casings. Annex II of this Regulation lays down model veterinary certificates for the import of fresh meat that indicate potential animal health pathogens that might be present in fresh meat derived from animals of the bovine, ovine, caprine, porcine and equine species are indicated. The same infectious agents identified for fresh meat have been also considered for animal casings.

For bovine, ovine and caprine meat under "Animal Health attestation" the following diseases are included:

- foot and mouth disease (FMD)
- rinderpest
- tuberculosis
- ovine or caprine brucellosis.

For pork under "Animal Health attestation" the following diseases are mentioned:

- FMD
- classical swine fever
- African swine fever
- rinderpest
- swine vesicular disease
- porcine brucellosis.

For equine meat under "Animal Health attestation" the following diseases are mentioned

- African horse sickness
- glanders.

From the above-listed diseases in the certificates for import of fresh meat, rinderpest, African Horse sickness and glanders can be ignored regarding the role of casings in their transmission. Rinderpest was eradicated worldwide in 2011, and therefore it does not play a role any more. However, a close relative of the rinderpest virus (RPV), the peste des petits ruminants virus (PPRV) is still present in many countries of the world and might play a role as an important pathogen in casings. As the general properties of both viruses are more or less identical in respect of their tenacity and survival time, it

appeared appropriate to include peste des petits ruminants instead of rinderpest in the list of diseases that might be transmitted via casings. Therefore, the properties of PPRV will be discussed under the chapter on RPV.

Most bacterial species, including the ones that may be present in the gastrointestinal tract of ruminants, cannot survive a water activity (a_w) less than 0.91 (Wijnker, 2009) The casings industry uses either dry salt (a_w 0.75) or saturated brine (a_w between 0.75 and 0.80) for preservation and a normal storage period of 30 days.

Studies have demonstrated that growth of *Brucella abortus* and *B. melitensis* is completely inhibited at a concentration of 4 % sodium chloride (NaCl), far less than the concentrations of NaCl (10–40 %) in casings traded internationally (Houben, 2005). Furthermore, *B. abortus* and *B. melitensis* have been shown not to survive beyond 25 days in sterilised sea water (Mitscherlich and Marth, 1984), with a salt concentration also less than that used to preserve casings. In a risk assessment from New Zealand (MAF Biosecurity New Zealand, 2010) it was concluded that the likelihood of *Brucella* species entering the country in sheep or goat casings is negligible.

Palásek et al. (1991) reported the survival of mycobacteria in salted pigs' guts for up to 7 months in an experiment in which only the mucosa of the intestines was removed. At +4 °C and near-saturation of salt *Mycobacterium avium* survived for 7 months in salted guts vs. 35 days in unsalted guts. Thus, a six-fold increase in survival time was observed after the salting procedure. If other mycobacteria, like *M. bovis* or *M. avium* subsp. *paratuberculosis*, display a similar stabilising effect after salting, it cannot be ruled out that bovine casings, in which part of the mucosal lymphoid tissue still remains after cleaning and scraping, could pose a potential risk for introduction of these mycobacteria. In general, bovine tuberculosis of the digestive system is uncommon, but in some areas alimentary tuberculosis in cattle may be more common due to husbandry practices (Ameni et al., 2006). Further data on the influence of salting on mycobacteria should therefore be sought in order to be able to assess the risk of their introduction by casings.

African horse sickness (AHS) is a serious arthropod-borne viral disease of horses and other equids, which can result in significant morbidity and mortality in susceptible populations. AHS virus transmission occurs exclusively by the bite of competent arthropod vectors. An exception to this is the infection of dogs via ingestion of meat from infected animals. However, from the pathogenesis of AHS, it is not expected that casings will play a role in the transmission of the virus, and therefore no particular attention has been given to this disease.

Glanders (infection by *Burkholderia mallei*) is mainly a disease of equids, affecting the nasal cavity and lungs, and eventually the skin (farcy). In glanders, the digestive and urinary tracts are not affected, and casings do not pose a risk for transmission of this infection.

Vesicular stomatitis virus (VSV) is not quoted in the above list, but it causes a disease with clinical signs similar to those of FMD. Although in cattle, horses and swine, high titres of VSV are found at the margins of lesions and in vesicular fluids for a short period after infection (Scherer et al., 2007), viraemia is undetectable in other tissues including muscle, brain, liver, spleen, mesenteric lymph nodes, kidneys and spinal cord (USAHA Grey Book, 2008). This indicates that transmission of VSV by meat is not likely. The potential for VSV transmission by other animal products has not been addressed in the literature (EFSA Journal 2012;10(4):2631). Those findings place the virus outwith the scope of the current mandate.

The import of animal casings into the EU also has to fulfil the requirements of the legislation on transmissible spongiform encephalopathies (TSEs), which establishes a country risk classification and related requirements for import and considers certain tissues from bovine, ovine and caprine animals as specified risk material (SRM), having to be removed from the food and feed chain to protect the health of consumers against the risk of bovine spongiform encephalitis (BSE) (Regulation (EC) No

999/2001). It was agreed that TSEs are excluded from the scope of the current work. Furthermore, previous EFSA opinions have assessed the risks of TSEs associated with casings.[8]

2. Production of casings

All domestic ungulates farmed for food purposes can be used as a source of animal casings. However, casings are predominantly produced from pigs ("hog casings"[9]), sheep and goats ("sheep casings") and cattle ("beef casings"). From pigs, cattle and sheep the entire intestinal tract is used for the production of casings, with the exception of the ileum in cattle, which is not used. Beef casings are also produced from the urinary bladder (Ockerman and Hansen, 2000) and from the oesophagus ("weasand"). However, that part is outwith the scope of the current mandate.

After processing, the submucosa of the intestine is the remaining layer in hog and sheep casings. A cleaned sheep casing is on average 0.11 mm thick, whereas a cleaned hog casing is about 0.32 mm (Bartenschlager-Blässing, 1979; Koolmees and Houben 1997). In contrast to hog and sheep casings, beef casings retain all the original intestinal layers after cleaning.

From horses only the large intestines are processed into casings, with the exclusion of the caecum. This is mostly done by hand and all layers remain identifiable (personal communication, ENSCA 2012).

In Europe, the species available for production of animal casings are limited to porcine, ovine, caprine and equine animals, as cattle intestines are listed as a SRM in accordance with TSE Regulation (EC) No 999/2001. Therefore, any animal casings of bovine origin are imported into the EU only from countries with a negligible risk status for BSE, as defined by OIE .

The import of animal casings into the EU is covered by Council Directive 92/118/EEC and Commission Decision 2003/779/EC, laying down the animal health and veterinary certification requirements for the import of animal casings from third countries. All third country establishments from which casings originate have an implemented hazard analysis and critical control point (HACCP) system, are subjected to Food and Veterinary Office (FVO) inspection and are approved for the import of animal casings into the EU.[10] Some of the countries from which significant amounts of animal casings are imported into the EU are endemically infected with FMD with a high disease prevalence, e.g. China, Colombia, Egypt, India, Iran and Pakistan.

In 2011, the European Natural Sausage Casings Association (ENSCA) presented their Community Guide to Good Practice for hygiene and the application of the HACCP principles in the production of natural sausage casings (ENSCA, 2012). This document was prepared in accordance with Article 9 of Regulation (EC) No 852/2004 of the European Parliament and of the Council on the Hygiene of Foodstuffs, and it was subsequently endorsed by the Standing Committee on the Food Chain and Animal Health (SCOFCAH). It is available on the website of DG SANCO (link). Although this document is mainly intended for the European casing industry, it is also used extensively by the international casing industry. Its aim is to assist in the implementation of the European Hygiene Package and HACCP principles according to the Codex Alimentarius.[11]

The treatments prescribed for animal casings imported into the EU are specified in Commission Decision 2003/779/EC and include cleaning, scraping and salting with NaCl for 30 days. In an alternative to the salting procedure, casings may be dried or bleached[12] after cleaning and scraping.

[8] http://www.efsa.europa.eu/en/efsajournal/doc/464.pdf; http://www.efsa.europa.eu/en/efsajournal/pub/1317.htm

[9] The terms in the section are broadly recognised and used by the casings industry and indicated in the ENSCA Guide to Good Practice available on the website of DG SANCO (http://ec.europa.eu/food/food/biosafety/hygienelegislation/good_practice_en.htm).

[10] https://webgate.ec.europa.eu/sanco/traces/output/non_eu_listsPerActivity_en.htm

[11] Accessed online http://www.codexalimentarius.org/ on 29 May.

[12] Referred to as *blanchir* in French with the meaning of "to bleach" or "to treat with heat".

The use of approved procedures other than salting with NaCl is anecdotal and irrelevant for the actual casing industry. There is only one report of animal casings treated by an actual bleaching process, using peracetic acid (US patent 2,966,415), but this procedure was never applied in practice. Recently, a variation of the NaCl salting procedure, with the addition of phosphate salts has been tested and recommended, along with the classic NaCl treatment, to inactivate FMDV present in casings of ruminants and pigs (OIE Terrestrial Animal Health Code, 2011). Therefore, only NaCl salting and the modified phosphate salting procedures will be considered further.

See Annex 1 for a more detailed description of the production of casings.

2.1. Salt treatment

Traditional salting with NaCl for at least 30 days has been the main preservation procedure for animal casings for many years. NaCl is added either as dry salt or using fully saturated brine (a_w 0.75).[13] This treatment is accepted as the industry's standard operating procedure (SOP) for the preservation of animal casings (ENSCA, 2012) and is included in the OIE's Terrestrial Animal Health Code as the reference treatment for casings to avoid the introduction of FMDV via casings. The effectiveness of this treatment has been extensively investigated against the vegetative phase of bacteria (Gabis and Silliker, 1974; Houben, 2005; Wijnker et al., 2006) but information on its effectiveness in inactivating other relevant animal viruses is scarce (see Table 1).

The temperature at which the salted casings are preserved and the duration of the treatment may have an effect on the inactivation of infectious agents, as shown by the different outcomes of treatments in Table 1. Most recent studies (Wieringa-Jelsma et al., 2011) have compared different temperatures representative of refrigeration (4 ºC) and room temperature (20–21 ºC). It is generally accepted that a duration of 30 days is sufficient for inactivation of viruses in casings. For example, FMDV is fully inactivated after this period, provided that preservation is done at room temperature (20 ºC) (Wijnker et al., 2007, 2012). However, inactivation of other viruses such as CSFV was not achieved after 30 days if the temperature of preservation was 4 ºC (Depner et al., 1998). Few or no recent references could be found for other relevant ungulate and equine viruses. For swine vesicular disease virus (SVDV), a classic reference (McKercher et al., 1974) stated that SVDV is still present after NaCl salting after 200 days of storage at 39 °F (4 °C). There are no data on the effect of this standard treatment on ASFV, VSV or PPRV.

Table 1: Salting treatments for casings and their effect on the infectivity of relevant animal viruses

Agent	Animal/ product	Treatment	Result	Reference
FMD	Bovine and porcine intestines (untreated)	Storage at –30 °C Storage at 2–4 °C	Present at day 120 Absent after 48 h	Savi et al., 1961
FMD	Bovine intestine (untreated)	Storage at 4 °C	Absent after day 6	Cottral, 1969
FMD	Sheep casing	Dry salted for 14 days at 4 °C	Present at day 14	Böhm and Krebs, 1974

[13] Brine is a solution of salt (usually sodium chloride) in water. In different contexts, brine may refer to salt solutions ranging from about 3.5 % (a typical concentration of sea water or the lower end of solutions used for brining foods) up to about 26 % (a typical saturated solution, depending on temperature). At 100 °C (373.65 K, 212 °F), saturated sodium chloride brine is about 28 % salt by weight, i.e. 39.12 g salt dissolves in 100 mL of water at 100 °C. At 0 °C (273.15 K, 32 °F), brine can hold only about 26 % salt (Handbook of Chemistry and Physics, 63rd edition 1982–1983).

FMD	Sheep, hog and beef casings	Dry salted for 30 days at 4 or 20 °C	Present at day 30 when stored at 4 °C Absent at day 30 when stored at 20 °C	Wijnker et al., 2007, 2012
SVD	Hog casings	Saturated brine, storage at 4 °C	Present after day 200	McKercher et al., 1975
CSF	Hog casings	Brine soak 4 °C for 20 h, water soak 44 °C, dry salted and storage at 4 °C	Absent at day 12	Helwig and Keast, 1966
		Water soak 21 °C for 3 days, dry salted and storage at 4 °C	Present at day 86	
		Water soak 21 °C for 23 h, +42 °C for 1 h, dry salted and storage at 4 °C	Absent after day 9	
CSF	Hog casings	Saturated brine for 30 days at 4 °C	Present at day 30	Depner, 1998

CSF, classical swine fever; FMD, foot and mouth disease; SVD, swine vesicular disease.

Specific studies on the efficacy of drying or bleaching of casings to inactivate animal viruses were not found.

2.2. Phosphate treatment (salting with the addition of phosphates)

The initial studies using phosphates for treatment of casings (Bakker et al., 1999; Houben, 2005) showed a clear improvement on different microbial and mechanical properties of animal casings, and prompted further studies to show the inactivation properties of this modified salting procedure. Treatment consists of traditional salting with phosphate-supplemented NaCl, either as dry salt or using fully saturated brine. The phosphate-supplemented salt contained 86.5 % NaCl, 10.7 % Na_2HPO_4 (molecular weight 142) and 2.8 %, Na_3PO_4 (molecular weight 164) (wt/wt/wt). The efficacy of this treatment for the inactivation of viruses has been investigated in animal casings for FMDV and CSFV (Wijnker et al., 2007, 2008, 2012; Wieringa-Jelsma et al., 2011). These studies have shown that the correct combination of NaCl plus phosphate salts, temperature and treatment time can remove the risk of infectivity of different viruses from treated animal casings (Table 2). The NaCl–phosphate treatment showed an advantage over the classic salting procedure, being effective against FMDV and CSFV to some extent also at 4 ºC. At present the treatment of casings of ruminant or porcine origin using either salt or phosphate-supplemented salt for a minimum of 30 days and at a temperature above 12 ºC is recommended by the OIE for the inactivation of FMDV present in casings (OIE Terrestrial Animal Health Code, Chapter 8.5—Foot-and-mouth disease, Article 8.5.41).

Table 2: Salting—phosphate salt treatment of casings and effect on infectivity of relevant animal viruses

Agent	Animal/ product	Treatment	Result	Reference
FMD	Sheep, hog and beef casings	Dry salted using phosphate–salt for 30 days at 4 or 20 °C	Absent at day 30 when stored at 4 or 20 °C	Wijnker et al., 2007, 2012
CSF	Hog casings	Dry salted using phosphate–salt for 30 days at 4 or 20 °C	Present at day 15 but absent at day 30 when stored at 4 or 20 °C	Wijnker et al., 2008

CSF, classical swine fever; FMD, foot and mouth disease.

2.3. Other potential alternative treatments

Several treatments have been applied to casings with the aim of inactivating relevant viral pathogens. In a classic experiment, intestines of experimentally FMDV-infected sheep were treated with the NaCl salting procedure, and shown to contain infectious FMDV after 14 days at 4 ºC. The authors then assessed the additional inactivating effect of low pH on FMDV by using citric acid (2 %) or lactic acid (0.5 %) on the 14-day salted casings, obtaining a full inactivation with any of the two treatments even after 5 minutes.

In another study, as a part of a broader experiment, a citrate-supplemented salt solution containing 89.2 % NaCl, 8.9 % trisodium citrate dehydrate and 1.9 % citric acid monohydrate (wt/wt/wt), with pH 4.5 was applied to casing made from pigs infected with CSFV, at two different preservation temperatures (4º C and 20 ºC) and two time points (15 days and 30 days) (Wijnker et al., 2008). The treatment was successful in removing infectivity only after 30 days, and only when casings were preserved at 20 ºC.

Ozone in water has been recently used as an alternative system of treatment of casings with the aim of reducing bacterial contamination (Benli et al., 2008). This treatment was found to be only partially effective, with a reduction in bacterial counts after treatment for 2 hours. Longer treatments reduced the quality and other properties of the casings. The authors concluded that other methods should be investigated (e.g. irradiation, with an electron beam or a gamma radiation source).

Gamma irradiation has been tested with pork and lamb casings for the reduction or elimination of bacterial contamination. The irradiation treatment significantly reduced microbial contamination in a dose-dependent manner (Byun et al., 2001; Jo et al., 2002; Trigo et al., 2003; Chawla et al., 2006), and when combined with a previous washing step with distilled water it eliminated microbial contamination (Byun et al., 2001). In general, it can be concluded from these studies that this treatment is useful to improve the microbial quality of casings, without adverse effects on quality characteristics.

There are many reports on the use of different substances for the treatment of animal casings, e.g. organic acids (Sakata et al., 1998; Nishiumi et al., 2005), phosphate salts (Schwanz and Schnackel, 2007; Sakata et al., 2008), sodium carbonate (Schwanz and Schnäckel, 2007), or beer and brewer's grains (Sakata et al., 2011). The main purpose of these treatments was to improve the casings with regard to quality, e.g. tenderness, resistance or gliding properties, but the studies did not generate information about the microbiological quality of the casings.

There is a patent on the treatment of animal casings by bleaching with peracetic acid and neutralisation with sodium bicarbonate (US patent 2,966,415, Dec. 27, 1960), but no pathogen inactivation data were provided and the procedure was probably never applied in practice owing to concerns about the negative effects of low pH values on the technological properties of the casings.

3. General properties of relevant viral pathogens

3.1. Foot and mouth disease virus

FMD is a highly contagious disease of cloven-hooved animals, which can have devastating economic consequences. FMDV is a member of the family *Picornaviridae*, genus *Aphthovirus*, and can be divided into seven immunologically distinct serotypes: A, O, C, SAT1, SAT2, SAT3, and Asia1, which do not confer cross-immunity.

As a non-enveloped virus it is stable to organic solvents. It is preserved by refrigeration and freezing but progressively inactivated by temperatures above 50 °C. Heating animal products to a minimum core temperature of 70 °C for at least 30 minutes inactivates the virus. It is stable only at a relatively narrow range of pH values and quickly inactivated by pH < 6.0. Values above pH > 9.0 will also lead to inactivation. Therefore suitable disinfectants are, for example, sodium hydroxide (2 %), sodium

carbonate (4 %), citric acid (0.2 %) and acetic acid (2 %). Also sodium hypochlorite (3 %), potassium peroxymonosulphate/sodium chloride (1 %) and chlorine dioxide will inactivate FMDV, but iodophores, quaternary ammonium compounds and phenol will not, especially in the presence of organic matter (OIE FMD Technical Disease Card).

The concentrations and survival of FMDV in animal tissues and, in particular, in animal products was extensively reviewed by Ryan et al. (2008), who emphasized that the inactivation of FMDV by pH and temperature change is biphasic. Bachrach et al. (1957), for example, showed that the initial inactivation followed first-order kinetics, but at 55 ºC, 61 ºC, pH 5 and pH 6, a residual fraction of virus, 10^{-6} of the initial concentration, remained which was very resistant to further inactivation. The inactivation-resistant fraction of FMDV must be taken into account when estimating the efficiency of thermal or pH-dependent reduction of virus load. The significance of this residual fraction of infectious virus is related to the initial virus load in the animal product in question, and may vary considerably depending on the nature of the product and the treatment it undergoes. Proteins and lipids may have a protective effect on the virus. While the virus survives in lymph nodes and bone marrow at neutral pH, it is destroyed in muscle at pH < 6.0 i.e. after rigor mortis. The virus survives drying and may persist for days to weeks in organic matter in moist conditions and cool temperatures.

In order to interpret reports on the survival of FMDV in a particular matrix (buffer, media, organic tissues, etc.) or products, one has to consider the starting titres, which are often not stated. Of course, higher starting concentrations mean that it will take longer until no residual virus can be found. Furthermore, the methods for assaying the infectivity differ. While some authors inoculated the virus intradermally or into the tongue of cattle, most used various cell culture systems and the results are usually given either as tissue culture infective dose ($TCID_{50}$) per gram or mL or as plaque-forming units (PFUs) per gram or mL. Assuming comparable sensitivity of the cell culture system, 1 PFU equals about 1.4 $TCID_{50}$.

Nevertheless, based on the extensive review of virus survival in organs and animal products derived from experimentally infected animals provided by Ryan et al., (2008), it can be stated that the highest titres can be found in affected areas of the skin (clinically infected pigs: up to 10^{10} $TCID_{50}$/g, (Alexandersen et al., 2001), whereas in most tissues, titres in the range of 10^4 to 10^6 $TCID_{50}$/g were found. During the viraemic phase, a blood[14] titre of $10^{5.6}$ PFUs/mL was reported by Cottral (1969) for cattle, while a blood titre of $10^{7.2}$ ID_{50}/g was reported by Sellers (1971) for pigs and a blood titre of $10^{5.0}$ PFUs/mL by Burrows (1968) for sheep. The maximum titres in organs where, to our current knowledge, no significant virus amplification occurs (e.g. the small and large intestines used for the production of casings), is probably dependent on the blood titres during viraemia. From the data compiled by Ryan et al. (2008), it can be concluded that infective virus can still be found in animal tissues and products where high starting titres can be assumed after several months at low temperatures (below 0 ºC or 4 ºC) and after 1 month at ambient temperatures, e.g. 20 ºC.

3.2. Classical swine fever virus

CSFV is a small enveloped positive single-stranded RNA virus that belongs to the genus *Pestivirus* of the *Flaviviridae* family (Becher et al., 1999). As an enveloped virus, CSFV is inactivated by organic solvents (ether or chloroform), detergents (Nonidet P40, deoxycholate, saponin), chlorine-based disinfectants, phenolics and quaternary ammonium aldehydes (formaldehyde, glutaraldehyde) (reviewed by Edwards (2000)). CSFV is known to survive for prolonged periods in a favourable environment—cool, moist, protein-rich—as found, for example, in meat and intestines (casings). The survival of the virus is highly influenced by the environmental temperature and matrix in which it is located/found. In general it can be stated that survival of the virus increases when the temperature is low and where the environment is rich in proteins (such as in casings) and moist. CSFV is relatively stable between pH 5 and 10. Stability is extended at lower pH values, but the degree of stability is, to a certain extent, strain dependent (Depner et al., 1992).

[14] Reported as "blood titres", but tests may have been carried out with serum as usual.

The increased stability at low temperatures, even at low pH (pH 4), and in protein-rich environments is important, as these are conditions encountered during storage of meat, meat products, intestines, etc. During the commercial production of pork and pork products, the time and temperature of storage seldom allow the pH to fall below 5.7 (Farez and Morley, 1997) and therefore provide ideal survival conditions. Survival up to 4.5 years in frozen meat has been reported (Edgar et al., 1952). The most important factors are the temperature applied during the processing and its duration (Edwards, 2000). Survival rates in processed meat products of, for example, 90 days in salami (Savi et al., 1961) and 126 days in Iberian loins (Mebus et al., 1993) have also been reported. For a more extensive review of the survival rates in processed meat products, see Farez and Morley (1997).

As previously mentioned, thermal inactivation is strongly influenced by the matrix in which the virus is located. However, *in vitro* studies have shown that inactivation takes less than 1 minute at 100 °C, 2 minutes at 90 °C, 3 minutes at 80 °C and 5 minutes at 70 °C. Inactivation at 56 °C was shown to be more heterogeneous and strain dependent (reviewed by Edwards, 2000). In blood inactivation at 69 °C it took at least 30 minutes. Alternative methods of inactivation have also been developed. Ultra-violet light, hydrostatic pressure or a combination of both has been shown to be able to effectively inactivate CSFV (Freitas et al., 2003). Similarly, vapour-phase hydrogen peroxide has been shown to be an efficacious way for decontaminating material (Heckert et al., 1997).

3.3. African swine fever virus

ASFV belongs to the genus *Asfivirus* and is the unique member of the *Asfarviridae* family (Pringle, 1999). It is a large icosahedral and enveloped double-stranded DNA virus. ASFV shares some characteristics with large DNA viruses (*Poxviridae*, *Iridoviridae*).

ASFV is very resistant to inactivation in environmental conditions. For example, contaminated pig pens in the tropics were shown to remain infectious to domestic pigs for 3 days (Montgomery, 1921, cited in Wardley et al., 1983). The virus can be isolated from sera or blood kept at room temperature for 18 months.

ASF virus is inactivated by heat treatment at 60 °C for 30 minutes (Mebus et al., 1997) and by many solvents that disrupt lipid bilayers and by commercial disinfectants (1 % formaldehyde in 6 days, 2 % NaOH in 1 day). Paraphenylphenolic disinfectants are very effective. ASFV can survive over long periods (months or years) when frozen or stored at 4 °C (Dixon, 2005). Infectivity is stable over a wide pH range. Some infectious virus may survive treatment at pH 4 or pH 13.

In meat products, ASFV may persist for several weeks or months in frozen or uncooked meat (Wilkinson, 1989). In products prepared by curing, such as Parma ham, viral infectivity was not demonstrated in ham 300 days after processing and curing (Farez and Morley, 1997). The virus survived for 140 days in Iberian and Serrano hams and for 112 days in loin. However, in these curing processes, virus inactivation occurs before the products are released for marketing. No infectious ASFV has been found in cooked or canned hams when processed at 70 °C.

3.4. Rinderpest virus and peste des petits ruminants virus

Although rinderpest is mentioned in the animal health attestation of the import certificates for fresh meat of the EU (Commission Regulation (EU) No 206/2010), the disease does not play any further role as it was eradicated globally in 2011. However, a close relative of the RPV, the PPRV is still present in many countries of the world and might play a role as an important pathogen in casings.

RPV and PPRV are members of the *Morbillivirus* genus within the family *Paramyxoviridae* (Kingsbury et al., 1978). The virions are pleomorphic particles with a lipid envelope enclosing a ribonucleo-protein core, which contains the genome, a single-stranded RNA of negative polarity. The RPV virions have a maximum diameter of 300 nm while those of PPRV are larger with a mean diameter of 400 500 nm (Bourdin and Laurent-Vautier, 1967; Gibbs et al., 1979).

RPV does not persist outside the host for more than few hours at normal temperature. It is heat and light sensitive. However, it is relatively resistant to cold and may survive for 1 month in blood kept under refrigeration. The RPV is stable between pH 7.2 and 8.0, relatively stable between pH 4.0 and 10.2 and is rapidly inactivated at lower and higher pH values. RPV is readily destroyed by heat, drying and most disinfectants, including lipid solvents. In general it can be stated that survival of the virus increases when the temperature is low. The half-life of RPV in spleen or lymph nodes has been calculated to be 5 minutes at 56 °C, 105 minutes at 37 °C, 6.4 hours at 25 °C and 2.3 days at 4–7 °C (Plowright, 1968). Assuming that in intestines the initial virus load would be below or about $10^{5.0}$ $TCID_{50}$ the virus would be inactivated in about 4 days if the pH was neutral and the storage temperature about 25 °C. The tenacity of PRRV is similar to RPV. Lefevre (1982) calculated a half-life of about 2 hours at 37 °C; at 50 °C infectivity was destroyed within 30 minutes.

3.5. Swine vesicular disease virus

SVD is a disease of pigs caused by a virus of the family *Picornaviridae*. All isolates are classified in a single serotype, with four distinguishable antigenic/genomic variants. SVD was included in the list of diseases notifiable to the OIE because of the similarity of its lesions to those produced by FMD; however SVD is often mild in nature and may infect pigs subclinically. Most recent SVD outbreaks have been subclinical, and SVD can rarely be diagnosed now on the basis of clinical signs, necessitating laboratory diagnosis (Bellini et al., 2007, 2010). Pigs can be infected by contact or by exposure to an infected environment (Dekker et al., 1995). Movement of subclinically infected animals is the most common means of spreading SVDV.

The virus is remarkably stable in the pH range of 2.5–12.0. It can be inactivated by sodium hydroxide combined with detergent. Direct treatment of swine waste with 1.5 % (w/v) NaOH or $Ca(OH)_2$ for 30 minutes can inactivate SVDV at either 4° C or 22° C. A mixture of didecyldimethylammonium chloride and 0.1 % NaOH for 30–60 minutes was also efficient. For personal disinfection and in the absence of gross organic matter, oxidising agents, iodophores and acids are suitable if combined with detergents. SVDV is preserved by refrigeration and freezing, but inactivated by a temperature of 56 °C for 1 hour (OIE SVD Technical Disease Card).

In cured meat products (e.g. ham and salami) derived from experimentally infected animals, the virus could still be found after several hundred days (McKercher et al., 1974; Mebus et al., 1993). While Hedger and Mann (1989) found feeding of waste food an important source of infection through the ingestion of contaminated meat (15 %), swill feeding does not appear to have played a role in recent epidemics, and thus its importance is unclear (EFSA Journal 2012;10(4):2631)

4. Studies with casings from experimentally infected animals

4.1. Foot and mouth disease virus

Böhm and Krebs (1974) not only reported FMDV titres of up to 2.5 $\log_{10}$ $TCID_{50}$/mL in casings of experimentally infected sheep even after storage for 14 days at 4 °C, but they also confirmed the efficacy of a 5-minute treatment with 0.5 % citric or lactic acid on infected sheep casings to inactivate FMDV. However, as the industry considered this treatment detrimental to the technological parameters of the casings, it was never adopted as a standard procedure.

McKercher et al. (1978, 1980) mentioned that residual infective FMDV remains in untreated processed casings for as long as 250 days. Unfortunately, they did not provide information on the processing and storage of these casings (temperature, pH, salting). From earlier reports of experiments carried out by McKercher et al. (1974) with FMDV and SVDV in casings, it appears that the casings had been stored in saturated NaCl brine at 39 °F (4 °C).

Wijnker et al . (2007) investigated whether the alkaline phosphate salt brine suggested by the industry as a new standard treatment option for casings would inactivate FMDV in a different way from the usual neutral NaCl brine. At a pH of 10, they found for cell culture virus a reduction of

3 $\log_{10}$ $TCID_{50}$/mL within 120 minutes. However, the inactivation of FMDV is biphasic, and lipids and proteins may protect the virus. Furthermore, owing to the buffer capacity of the proteins present in casings, the pH of 10 may not be consistently reached under practical conditions. Therefore, these *in vitro* data are of limited value. In order to elucidate whether FMDV in casings would survive standardised industrial procedures, Wijnker et al. (2007) used casings derived from pigs and sheep experimentally infected with FMDV to study virus survival in casings. While the highest titres found in cleaned large intestines after slaughter were similar to those reported by Böhm and Krebs in 1974 ($10^{3.0}$ PFUs/mL in ovine and $10^{2.56}$ PFUs/mL in porcine samples), in small intestines most samples were negative and only one ovine sample had a titre of $10^{2.0}$ PFUs/mL. The casings were treated with either NaCl or a mixture of phosphate salts and NaCl (86.5 % NaCl; 2.8 % sodium phosphate (Na_3PO_4, molecular weight 164) and 10.7 % sodium hydrogen phosphate (Na_2HPO_4, molecular weight 142). They were stored for 30 days at either 4 °C or 20 °C. Although after storage at 20 °C with either salt, no remaining infectivity was found, after 30 days at 4 °C, 3 out of 12 samples of casings examined per treatment group still contained virus, irrespective of the salt used. As the initial virus titres in most samples were too low, no inactivation kinetics could be generated with these samples. It was also not entirely clear whether the results obtained with porcine and ovine casting would apply to bovine casings.

Wijnker et al. (2012) carried out a further study with casings of infected bovines, using the same salts as described above. Six cattle were infected by tongue inoculation with strain A Iran 97 and on days 2, 3 and 4, two animals were slaughtered, casings produced according to standard procedures and the initial (pre-salting) virus titres determined. However, only in seven samples taken from three out of six cattle (on a total of 30 samples) could FMDV be isolated, directly after stunning, exsanguination and processing, prior to salt treatment. The infectivity titres in cleaned casings were close to the detection limit (about 10 $TCID_{50}$/mL) and neither exact quantification nor the generation of an inactivation kinetic was possible. Samples (n = 30) were taken on days 1, 3, 5, 7, 14 and 30 and examined for FMDV. No FMDV could be isolated in any bovine samples treated with phosphate-supplemented salt and stored at 4 °C and 20 °C at any time point. In NaCl salt, the virus survived longer. On day 1 after storage in NaCl salt, FMDV could be isolated in three samples stored at 20 °C (no samples were stored at 4 °C on day 1). On day 3 after storage in NaCl salt, FMDV could be isolated in two samples stored at 4 °C and one sample stored at 20 °C. At later time points, virus could be isolated only from samples stored in NaCl salt at 4 °C: FMDV was found in one sample on day 5, in four samples on day 7, in one sample on day 14 and in one sample on day 21. On day 30, none of the bovine samples stored in NaCl were found to contain any FMDV.

The experiments in sheep, pigs and cattle can be summarised by stating that only low virus titres were found in cleaned intestines derived from animal infection experiments. Intestines are embryologically of endodermal origin and are neither primary sites of infection nor secondary sites of replication (Alexandersen et al., 2003) and thus the virus found was probably derived from the small remaining volumes of viraemic blood. No virus could be found after 30 days at 20 °C.

4.2. Classical swine fever virus

Only a few studies have been conducted to determine the survivability and resistance of CSFV in casings. Most studies have involved meat and meat products without particular consideration of the casings.

From data regarding the tenacity of CSFV in general it is known that the virus is relatively stable within a pH range of 5–10 and at room temperature (~ 20 °C). A study conducted on the thermal and pH stability of cultured CSFV strains revealed a mean half-life of 50 hours at neutral pH and 20 °C and a mean half-life of 260 hours at pH 4 and 4 °C. If these findings are extrapolated to CSFV-infected casings, it can be concluded that the virus will survive a storage period of 30 days at 4 °C but will be inactivated at 20 °C. Theoretically, if in casings the initial virus load were below or about $10^{5.3}$ $TCID_{50}$ the virus would be inactivated in about 30 days if the pH were neutral and the storage temperature about 20 °C (standard preservation treatment with NaCl for casings). Unfortunately, no

studies have been performed with infected casings under exactly these temperature and pH conditions. Depner at al. (1998) conducted a study in which it was shown that CSFV can survive in casings when stored in saturated brine (neutral pH) for a period of 30 days at 4 °C. This study highlights that at 4 °C the stability of CSFV is prolonged. So it can be concluded that storage of CSFV-infected casings below 20 °C for 30 days at a neutral pH does not necessarily lead to a complete inactivation of the virus.

Wijnker et al. (2008) treated CSFV-infected casings with phosphate-supplemented or citrate-supplemented NaCl to increase or decrease the pH respectively. Such treated casings were stored for 30 days at either 4° C or 20 °C. After 15 days of storage CSFV could be isolated in all samples. After 30 days of storage the casings were fed to susceptible pigs and CSF infection was confirmed only in the animals that received casings treated with citrate-supplemented salt and stored at 4 °C.

4.3. Swine vesicular disease virus

McKercher et al. (1974) reported that SVDV survived for at least 200 days in processed intestinal casings derived from SVDV-infected animals and stored in saturated NaCl brine at 39 °F (4 °C). Mebus et al. (1993), apparently referring to the same set of samples, reported a survival time of 780 days.

5. Experimental studies with casings that had been spiked with virus after slaughter

Casings derived from experimentally infected animals always had too low virus titres to generate proper inactivation kinetics. In order to generate such inactivation kinetics for FMDV in casings, Wijnker et al. (2012) "spiked" samples of casings with high-titre FMDV. Although these spiking experiments cannot completely mimic the situation in animal casings, as the virus is contained in cell debris, not in a tissue matrix, the conditions for virus inactivation (e.g. pH, protein and lipid concentrations) were certainly closer to those in casings than in experiments with cell culture-derived virus suspended in brine. Two experiments using high-titre vesicular material derived from cattle infected with FMDV strain A Iran 97 were conducted. The first experiment was conducted over a 15-day period. As in the experiments with infected animals, the casings were treated with either NaCl or the mixture of phosphate salts and NaCl and stored at either 4 °C or 20 °C. A temperature-dependent virus inactivation was found in oesophagus, jejunum, colon and bladder samples.

Treatment with NaCl and storage at 4 °C resulted in a decline in virus titre of around 2 $\log_{10}$ in 15 days, and no FMDV titre could be determined after day 13. Treatment with NaCl and storage at 20 °C resulted in a steeper decline in virus titre, with no FMDV titre determined after day 9.

Treatment with phosphate salt and storage at 20 °C resulted in rapid inactivation, with no virus found at any time point; storage at 4 °C resulted in a similar reduction with no FMDV titre determined after day 9.

As the last time points at which virus could be isolated were close to the end of the experiment, the authors repeated the experiment with spiked jejunum and caecum samples that were then stored for 55 days, attempting to elucidate whether the apparently linear inactivation kinetics would also apply to longer storage times. Again, samples were stored at 20 °C and 4 °C in either NaCl or phosphate salt. This time, samples were taken every 5 days. Treatment with NaCl and storage at 4 °C resulted in an inactivation of about 3 $\log_{10}$ in 55 days, whereas storage at 20° C resulted in complete inactivation within 15 days, the last virus being detected on day 10, indicating a reduction of around 6 $\log_{10}$.

Treatment with phosphate-supplemented NaCl and storage at 4 °C or 20 °C resulted in complete inactivation of the virus by day 5.

In order to elucidate whether the inactivation kinetic observed with strain A Iran 97 applies also to other FMDV strains, similar experiments were carried out with strains A Turkey 06, C Oberbayern

and O Manisa. Colon and caecum samples were stored over a period of 35 days. For strains A Turkey 06 and C Oberbayern, an inactivation kinetic could be determined for storage in NaCl brine at 4 °C.

Treatment with NaCl and storage at 4 °C resulted in virus inactivation of around 2 $\log_{10}$. After 30 days, FMDV could still be determined.

Treatment with NaCl and storage at 20 °C resulted in rapid inactivation, with no virus found on day 3 or beyond.

Treatment with phosphate-supplemented NaCl and storage at either temperature resulted in rapid inactivation with no FMDV found on day 3 or beyond.

FMDV of strain O1 Manisa could not be isolated on day 3 or beyond, irrespective of treatment or storage, and no kinetic could be generated. It is unclear whether this difference actually reflects a property of the strain.

6. Experimental studies with a 3D collagen matrix model for animal casings

Owing to the limitations of the simple "spiking" model additional experiments with a 3D collagen matrix model were carried out (Wieringa-Jelsma et al. 2011). In this one study a 3D collagen matrix model for animal casings was used to determine the inactivation of four viruses causing highly contagious diseases in livestock: FMDV, CSFV, SVDV and ASFV.

Cells infected with the four viruses mentioned above were embedded in a bovine collagen type I gel matrix in order to investigate the effect of storage without salt (untreated), storage in NaCl and storage in phosphate-supplemented NaCl at four different temperatures (4, 12, 20 and 25 °C) over a period of 30 days. As expected, the results showed that all four viruses were inactivated faster at higher temperatures. At lower temperatures the inactivation curves of the four viruses differed, and often the influence of the salt could also be observed.

6.1. Foot and mouth disease virus

For FMDV the influence of temperature was clearly seen in the untreated FMDV samples, with increasing inactivation rates at increasing temperatures. The detection limit of 1.8 $\log_{10}$ $TCID_{50}$/mL was reached after 21, 7, 3 and 2 days of incubation at 4, 12, 20 and 25 °C, respectively, equivalent to a reduction of 3.2 $\log_{10}$ $TCID_{50}$. Incubation with NaCl at the different temperatures resulted in curves that were similar to those for the untreated FMDV samples.

In contrast to the NaCl and untreated samples, the phosphate salt-treated samples showed biphasic inactivation curves. At all temperatures, a period of rapid inactivation by about 2.5 $\log_{10}$ $TCID_{50}$ within 24 hours was followed by a period in which FMDV titres remained constant. The reduction of FMDV titres by phosphate salt treatment was significantly higher than the control treatment during the first 7, 3, 2 and 1 days of incubation at 4, 12, 20 and 25 °C, respectively. At storage temperatures of 20 °C and 25 °C, FMDV titres were higher in the phosphate salt samples than in the untreated and salt-treated samples for 5 and 4 days, respectively, although these differences were not significant. Because of the biphasic inactivation kinetics, no linear regression analysis was performed and no *D*-values calculated.

6.2. Classical swine fever virus

Phosphate-supplemented salt treatment increased the effect that temperature had on inactivation of CSFV. The salt treatment only increased CSFV inactivation at the higher temperatures (20 °C and 25 °C). The influence of treatment temperature on CSFV titres was also observed with the untreated CSFV samples, with increasing inactivation rates at increasing temperatures. However, the detection limit of 1.4 $\log_{10}$ $TCID_{50}$/mL was only reached on day 21 at 25 °C after a reduction of 5.8 $\log_{10}$ $TCID_{50}$. At temperatures of 4 °C and 12 °C no significant additional inactivation effect of the salt treatment over the control was observed. The results of the salt treatment were comparable with the untreated samples, as also indicated by the *D*-values. However, at 20 °C and 25 °C, treatment with

salt resulted in significantly greater virus reduction. Highly significant virus inactivation by phosphate-supplemented salt was observed at all temperatures for the entire period of incubation (30 days), except on days 21 and 30 at 25 °C, when the untreated samples had reached the detection limit. The phosphate-supplemented salt showed the lowest *D*-values, confirming this inactivation method as the most effective for inactivating CSFV.

6.3. African swine fever virus

ASFV was the most stable virus, even at higher temperatures. However, both NaCl and phosphate salt were able to inactivate ASFV. At 4 °C, low titres were still observed after NaCl treatment up to day 15, while phosphate salt treatment resulted in titres reaching the detection limit of 1.4 $\log_{10}$ $TCID_{50}$/mL by day 2 at all temperatures, which means a reduction in AFSV titre of at least 2.2 $\log_{10}$ $TCID_{50}$/mL.

6.4. Swine vesicular disease virus

SVDV showed a temperature-dependent inactivation, but, even at 25 °C, some virus survived until day 30 in the untreated samples. While NaCl treatment resulted only in a limited additional inactivation of SVDV, phosphate salt treatment had a significant additional inactivation effect at all temperatures compared with control samples. However, even with phosphate salt treatment, the detection limit was only reached after 15 days of incubation at 25 °C.

Table 3: *D*-values of four viruses embedded in a 3D collagen matrix after 30 days of treatment by various inactivation methods

		***D*-value (days/r^2) of various treatments**		
Virus	**Temperature (°C)**	**Untreated**	**Salt (NaCl)**	**Phosphate salt[a]**
FMDV	4	NA[b]	NA	NA
	12	NA	NA	NA
	20	NA	NA	NA
	25	NA	NA	NA
CSFV	4	35/0.88	36/0.67	8/0.92
	12	16/0.97	10/0.90	6/0.66
	20	7/0.97	5/0.90	3/0.72
	25	4/0.97	3/0.89	1/NA[c]
SVDV	4	76/0.45	26/0.87	9/0.75
	12	9/0.97	7/0.98	9/0.58
	20	5/0.95	5/0.86	4/0.69
	25	4/0.87	3/0.86	1/0.82
ASFV	4	44/0.34	10/0.37	1/NA
	12	54/0.53	1/NA	1/NA
	20	29/0.50	1/NA	1/NA
	25	25/0.84	1/NA	1/NA

D-value is the time required in days to reduce the viral population by a factor of 10^1.
r^2 is the correlation coefficient of the regression analysis for calculating the *D*-value.
ASFV, African swine fever virus; CSFV, classical swine fever virus; FMDV, foot and mouth disease virus; SVDV, swine vesicular disease virus.
[a]Phosphate salt is phosphate-supplemented salt.
[b]Not applicable because *D*-values could not be determined.
[c]Input data for regression analysis did not include the ones reaching the detection limit. An exception was made for data reaching the detection limit immediately after day 0.

CONCLUSIONS

GENERAL CONCLUSIONS

- The survival time of FMDV depends on a number of factors including the matrix (e.g. buffer, media, tissue), temperature and pH value.

- In experimentally infected sheep, pigs and cattle only low FMDV titres were found in cleaned intestines prior to salting.

- CSFV is relatively stable at a pH range of 5–10 at temperatures up to 20 °C. Raising the temperature dramatically reduces the survival of the virus, as does changing the pH to below pH 5 or above pH 10. Increased stability is favoured by protein-rich environments.

- ASFV can be regarded as relatively stable. It can survive over long periods (months or years) when stored at 4 °C. Infectivity is stable over a wide pH range, and it may survive treatment at pH 4 or pH 13. In meat products, ASFV may persist for several weeks or months in frozen or uncooked meat

- The tenacity of PPRV and RPV are similar. While they are relatively stable at a neutral pH, both viruses are rapidly inactivated at low and high pH values. The survival of the virus increases when the temperature is low.

- It appears likely that SVDV will survive for prolonged periods of time if kept below room temperature.

CONCLUSIONS FOR TOR1

- The efficacy of salting with NaCl for a period of 30 days at room temperature (20 ºC) is well documented for inactivation of FMDV in casings derived from experimentally infected animals. However, after 30 days at 4 °C, some FMDV survived.

- The experiments with FMDV "spiked" casings revealed a linear temperature-dependent inactivation of virus during the observation period. Inactivation occurred more rapidly at 20 °C, and no virus could be found after storage in saturated NaCl for 30 days at this temperature. However, at 4 °C infective virus was still present.

- In a 3D matrix model the detection limit of FMDV in untreated samples was reached after 21, 7, 3 and 2 days of incubation at 4, 12, 20 and 25 °C, respectively, equivalent to a reduction of 3.2 $\log_{10}$ $TCID_{50}$. Treatment with NaCl at the same temperatures resulted in similar inactivation kinetics, which emphasises the role of temperature in inactivation.

- Salting of casings derived from experimentally infected animals with NaCl for a period of 30 days at room temperature (~20 °C) inactivated CSFV. However, the same treatment at low temperature (4º C) was not fully efficacious in terms of inactivating the virus.

- In a 3D matrix model the detection limit of CSFV of 1.4 $\log_{10}$ $TCID_{50}$/mL was first reached on day 21 at 25 °C and on day 30 at 20 °C. An additional effect of NaCl salting on virus inactivation compared with the untreated controls was evident at 20 °C and 25 °C but not at 4 °C and 12 °C.

- There are no data available on inactivation of ASFV from experimentally infected or spiked casings.

- In a 3D model ASFV was readily inactivated by NaCl salting at all temperatures (4, 12, 20 and 25 °C) after 21 days. However, from its general characteristics, ASFV can be regarded as more stable than CSFV. Therefore these results should be interpreted with caution.

- There are no data available on inactivation of PPRV and RPV on casings from experimentally infected animals, in spiked casings or in a 3D matrix model. However, from the general characteristics of these viruses it could be assumed that they will be inactivated within 30 days at room temperature.

- For SVDV there have been no studies of inactivation with NaCl salting at room temperature. Survival of the virus for at least 200 days at 4 °C has been reported in casings, preserved with NaCl, derived from experimentally infected pigs. In a 3D matrix model SVDV showed temperature-dependent inactivation, but even at 20 °C with NaCl salting or 25 °C in untreated controls, some virus survived until day 30.

- Based on the general characteristics of SVDV, it is possible that NaCl salting for 30 days at room temperature will not completely inactivate the virus.

- *Brucella* spp. are readily inactivated by NaCl salting, but *M. avium* may survive well beyond 30 days in intestines in conditions similar to those used for the salting of casings. It is probable that *M. bovis* and other mycobacteria may show similar resistance to the salting treatment in casings, but this needs to be investigated.

- For most of the infectious agents investigated, NaCl salting showed greater efficacy at the upper range of temperature (20 ºC and above) than at 4 ºC.

CONCLUSIONS FOR TOR2

- Several treatments have been applied to casings with the aim of inactivating infectious agents, but none of them has been extensively investigated with viruses relevant to animal health.

- Acid treatments with either lactic (0.5 %) or citric (2 %) acid on casings previously salted for 14 days were enough for full inactivation of FMDV after only 5 minutes. A similar treatment with citrate-supplemented salt solution was successful in removing CSFV infectivity only after 30 days, and only when casings were preserved at 20 ºC. However, these treatments led to a significant loss in quality of the product. Studies with other viruses were not found.

- Ozone in water has been shown to be only partially effective against bacterial contamination in casings. No studies have been performed with viruses.

- Gamma irradiation has been tested with natural pork and lamb casings for the reduction or elimination of bacterial contamination. This treatment may be useful for improving the microbial quality of casings, without having adverse effects on quality characteristics, but it has not been tested in casings derived from animals experimentally infected with the relevant viruses.

- There are many reports on the use of other substances for treatment of animal casings, with the purpose of improving the quality of the casings, but they do not address the issue of pathogen inactivation.

CONCLUSIONS FOR TOR3

- In casings derived from experimentally infected animals, as well as in spiked casings, the treatment with phosphate-supplemented NaCl often led to faster inactivation of FMDV than treatment with NaCl salt alone. Also, with the phosphate treatment, a temperature of 20 ºC was more effective than lower temperatures for inactivation.

- In contrast to the studies with actual casings, with phosphate-supplemented salt, a biphasic virus (FMDV) inactivation was seen in the 3D matrix model, with low levels of virus surviving at 12 °C until day 21.

- Treatment with phosphate-supplemented salt resulted in effective inactivation of CSFV in casings derived from experimentally infected animals within 30 days.

- For most of the infectious agents investigated, phosphate-supplemented salt treatment showed greater efficacy at the upper range of temperature (20 ºC or above) than at 4 ºC.

RECOMMENDATIONS

ToR1

Salting with NaCl for 30 days should be done at 20 ºC or above to achieve effective inactivation of infectious agents.

Further data on the influence of salting on mycobacteria should be sought in order to be able to assess the risk of their introduction by casings.

ToR2

Owing to a lack of data no alternative procedures can be recommended as substitutes for current salting treatments based on NaCl and phosphate-supplemented salt.

Owing to a lack of specific scientific studies on the efficacy of bleaching and drying for pathogen inactivation, these treatments cannot be recommended as stand-alone treatments, i.e. without prior salting for 30 days.

ToR3

Salting with phosphate-supplemented salt for 30 days should be done at 20 ºC or above to achieve effective inactivation of infectious agents.

The 3D collagen matrix should be evaluated as a suitable model for assessing the efficacy of treatments for the inactivation of infectious agents, as a replacement for animal experiments.

REFERENCES

Alexandersen S, Oleksiewicz MB and Donaldson AI, 2001. The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time-course study using TaqMan RT–PCR. Journal of General Virology, 82, 747–755.

Alexandersen S, Zhang Z, Donaldson AI and Garland AJM, 2003. The pathogenesis and diagnosis of foot-and-mouth disease. Journal of Comparative Pathology, 129, 1–36.

Ameni G, Aseffa A, Engers H, Young D, Hewinson G and Vordermeier M, 2006. Cattle husbandry in Ethiopia is a predominant factor affecting the pathology of bovine tuberculosis and gamma interferon responses to mycobacterial antigens. Clinical and Vaccine Immunology, 13, 1030–1036.

Bachrach HL, Breese SS, Callis JJ, Hess WR and Patty RE, 1957. Inactivation of foot-and-mouth disease virus by pH and temperature changes and by formaldehyde. Proceedings of the Society for Experimental Biology and Medicine, 95, 147–152.

Bakker WAM, Houben JH, Koolmees PA, Bindrich U and 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 difference temperatures. Meat Science, 51, 163–174.

Bartenschlager-Blässing E, 1979. Technical, organoleptic and bacteriological properties and histological characteristics of sausages in natural casings (sheep small-intestine) and in synthetic casings (bovine corium). 1. Fleischwirtschaft, 59, 293.

Becher P, Orlich M, Kosmidou A, König M, Baroth M and Thiel H-J, 1999. Genetic diversity of pestiviruses: identification of novel groups and implications for classification. Virology, 262, 64–71.

Bellini S, Alborali L, Zanardi G, Bonazza V and Brocchi E, 2010. Swine vesicular disease in northern Italy: diffusion through densely populated pig areas. Revue Scientifique et Technique de l'Office International Des Epizooties, 29, 639–648.

Bellini S, Santucci U, Zanardi G, Brocchi E and Marabelli R, 2007. Swine vesicular disease surveillance and eradication activities in Italy. Revue Scientifique et Technique de l'Office International Des Epizooties, 26, 585–593.

Benli H, Hafley BS, Keeton JT, Lucia LM, Cabrera-Diaz E and Acuff GR, 2008. Biomechanical and microbiological changes in natural hog casings treated with ozone. Meat Science, 79, 155–162.

Bohm HO and Krebs H, 1974. [Isolation of foot-and-mouth-disease virus from organs of infected, slaughtered sheep]. Berliner und Munchener tierarztliche Wochenschrift, 87, 410–412.

Botka-Petrak K, Hraste A and Petrak T, 2001. Influence of different treatment procedures on morphological structure of beef intestines. Veterinarski Arhiv, 71, 1–9.

Bourdin P and Laurent-Vautier A, 1967. [Note on the structure of the small ruminant pest virus]. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux, 20, 383–385.

Burrows R, 1968. The persistence of foot-and mouth disease virus in sheep. The Journal of Hygiene, 66, 633–640.

Byun MW, Lee JW, Jo C and Yook HS, 2001. Quality properties of sausage made with gamma-irradiated natural pork and lamb casing. Meat Science, 59, 223–228.

Chawla SP, Chander R and Sharma A, 2006. Safe and shelf-stable natural casing using hurdle technology. Food Control, 17, 127–131.

Cottral GE, 1969. Persistence of foot-and-mouth disease virus in animals, their products and the environment. Bulletin de l'Office International des Epizooties, 71, 549–68.

Dekker A, Moonen P, Deboerluijtze E and Terpstra C, 1995. Pathogenesis of swine vesicular disease after exposure of pigs to an infected environment. Veterinary Microbiology, 45, 243–250.

Depner K, Bauer T and Liess B, 1992. Thermal and pH stability of pestiviruses. Revue Scientifique et Technique de l'Office International Des Epizooties, 11, 885–93.

Dixon LK, 2005. Evasion of host defence systems by African swine fever virus. In: Digard P, Nash AA, Randall RE (Eds.), Molecular Pathogenesis of Virus Infections. pp. 291–318.

Edgar G, Hart L and Hayston JT, 1952. Studies on the viability of the virus of swine fever. Report from the 14th International Veterinary Congress, 2, 387–391.

Edwards S, 2000. Survival and inactivation of classical swine fever virus. Veterinary Microbiology, 73, 175–181.

ENSCA, 2012. Community Guide to Good Practice for hygiene and the application of the HACCP principles in the production of natural sausage casings. European Natural Sausage Casings Association, Brussels.

Farez S and Morley RS, 1997. Potential animal health hazards of pork and pork products. Revue Scientifique et Technique de l'Office International Des Epizooties, 16, 65–78.

Fischer A and Schweflinghaus M, 1988. Natural casings. I. Anatomy and production. Fleischerei, 39, 10–14.

Gabis DA and Silliker JH, 1974. Salmonella in natural animal casings. Applied Microbiology, 27, 66–71.

Gibbs E, Taylor W, Lawman M and Bryant J, 1979. Classification of peste des petits ruminants virus as the 4th member of the genus morbillivirus. Intervirology, 11, 268–274.

Heckert RA, Best M, Jordan LT, Dulac GC, Eddington DL and Sterritt WG, 1997. Efficacy of vaporized hydrogen peroxide against exotic animal viruses. Applied and Environmental Microbiology, 63, 3916–3918.

Hedger RS and Mann JA, 1989. Swine vesicular disease virus. In: Pensaert M.B. (ed.), Virus Infections of Porcines. Elsevier Science Publishers BV, Amsterdam, pp. 241–250.

Helwig DM and Keast JC, 1966. Viability of virulent swine fever virus in cooked and uncooked ham and sausage casings. Australian Veterinary Journal, 42, 131–135.

Houben JH, 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.

Jo CR, Lee JW, Cho KH, Yook HS and Byun MW, 2002. Quality properties of sausage made with gamma irradiated natural casing from intestine of pork or lamb. Radiation Physics and Chemistry, 63, 365–367.

Kingsbury D, Bratt M, Choppin P, Hanson R, Hosaka Y, Termeulen V, Norrby E, Plowright W, Rott R and Wunner W, 1978. Paramyxovirus. Intervirology, 10, 137–152.

Koolmees PA, 1998. Histology of bovine natural casings. VVDO report 9806, Utrecht University.

Koolmees PA and Houben JH, 1997. Inventory part: histology and microbiology of hog and sheep casings. Color print leaflet EU – CRAFT project BRE 2. CT 94. 1495. Improved treatment of natural sausage casings for quality improvement in automated stuffing processes. Deutches Institut für Lebensmitteltechnik eV, Quackenbrück, Germany.

Koolmees PA, Tersteeg MHG, Keizer G, van den Broek J and 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.

Lefevre PC, 1982. Viral pest of small ruminants and rinderpest virus infection in sheep and goats.

McKercher, P.D., Graves, J.H., Callis, J.J., Carmichael, F., 1974. Swine vesicular disease: virus survival in pork products., in: Proceedings, Annual Meeting of the United States Animal Health Association, 78, p. 213a–213g.

McKercher P, Hess W and Hamdy F, 1978. Residual viruses in pork products. Applied and Environmental Microbiology, 35 142–145.

McKercher PD, Morgan DO, McVicar JW and Shuot NJ, 1980. Thermal processing to inactivate viruses in meat products. Proceedings, Annual Meeting of the United States Animal Health Association, 84, 320–328.

Mebus C, Arias M, Pineda JM, Tapiador J, House C and Sánchez-Vizcaíno JM, 1997. Survival of several porcine viruses in different Spanish dry-cured meat products. Food Chemistry 59, 555–559.

Mebus C, House C, Gonzalvo F, Pineda J, Tapiador J, Pire J, Bergada J, Yedloutschnig R and Sánchez-Vizcaíno J, 1993. Survival of swine vesicular disease virus in Spanish Serrano cured hams and Iberian cured hams, shoulders and loins. Food Microbiology, 10, 263–268.

Mitscherlich E and Marth EH, 1984. Microbial Survival in the Environment. Bacteria and Rickettsiae Important in Human and Animal Health. Springer-Verlag, Berlin.

Nishiumi T and Sakata R, 1999. Histological and biochemical evaluation of connective tissue of natural hog and sheep casings. Proceedings of the 45th International Congress of Meat Science and Technology, pp. 174–175.

Nishiumi T, Nojiri T, Yoshihara T, Ichinoseki S, Suzuki A, Tanabe M and Sakata R, 2005. High pressure with or withour organic acids for tenderizing of Chinese hog casings. Proceedings of the 51st International Congress of Meat Science and Technology, pp. 1138–1141.

Ockerman HW and Hansen CL, 2000. Animal By-product Processing and Utilization. Technomic Publishing Co., Lancaster, PA.

Palasek J, Pavlas M and Kubu I, 1991. Survival of salmonellae and mycobacteria in salted and unsalted swine guts used as sausage casing and sausage emulsion of a hard salami. Acta Veterinaria Brno, 60, 375–381.

Plowright W, 1968. Rinderpest virus. Virology Monographs, 3, 25–110.

Pringle CR, 1999. Virus taxonomy – 1999. Archives of Virology, 144, 421–429.

Ryan E, Mackay D and Donaldson A, 2008. Foot-and-mouth disease virus concentrations in products of animal origin. Transboundary and Emerging Diseases, 55, 89–98.

Sakata R, Nakae S, Oshida T, Nishiumi T and Yoon H, 2008. Inspection of stability and breaking strain. Fleischwirtschaft, 88, 59.

Sakata R, Oshida T, Nishiumi T, Yoon H and Waga M, 2011. Utilization of beer and brewer's grains to tenderize a sausage casing. Presented at the 57th International Congress of Meat Science and Technology, Ghent, Belgium, p. 350.

Sakata R, Segawa S, Morita H and Nagata Y, 1998. Tenderization of hog casings: application of organic acids and proteases. Fleischwirtschaft, 78, 703–704.

Savi P, Baldelli B and Morozzi A, 1961. Tissue culture test for foot and mouth disease virus in meat and meat products. I. In tissues and organs of pigs. Atti della Società Italiana delle Scienze Veterinarie, 15, 736–741.

Scherer CFC, O'Donnell V, Golde WT, Gregg D, Estes DM and Rodriguez LL, 2007. Vesicular stomatitis New Jersey virus (VSNJV) infects keratinocytes and is restricted to lesion sites and local lymph nodes in the bovine, a natural host. Veterinary Research, 38, 375–390.

Schwanz S and Schnackel W, 2007. Assays for resistance of natural casings. 2. Scalded sausage – improvement of the attributes of texture. Fleischwirtschaft, 87, 99–103.

Schweigmann A and Seeger H, 1988. Tests to determine the standard quality of natural casings. Fleischwirtschaft, 68, 970–&.

Sellers RF, 1971. Quantitative aspects of the spread of foot and mouth disease. Veterinary Bulletin, 41, 431–440.

Trigo MJ, Andrade ME, Veloso MG and Barreto A, 2003. Irradiation of raw Portuguese sausages and meat based ready-to-eat meals. International Atomic Energy Agency Technical Documents (IAEA-TECDOCs) 198–206.

USAHA Grey Book, 2008. Foreign Animal Diseases, 7th edn. Committee on Foreign and Emerging Diseases of the United States Animal Health Association, St Joseph, MO.

Wardley RC, Andrade C de M, Black DN, de Castro Portugal FL, Enjuanes L, Hess WR, Mebus C, Ordas A, Rutili D, Sánchez-Vizcaíno J, Vigario JD, Wilkinson PJ, Moura Nunes JF and Thomson G, 1983. African swine fever virus. Archives of Virology, 76, 73–90.

Wieringa-Jelsma T, Wijnker JJ, Zijlstra-Willems EM, Dekker A, Stockhofe-Zurwieden N, Maas R and Wisselink HJ, 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 JJ, 2009. Salts also inactivate viruses. Aspects of quality assurance during manufacture of natural casings – Review Part 2. Fleischwirtschaft, 89, 32–34.

Wijnker JJ, Depner KR and Berends BR, 2008. Inactivation of classical swine fever virus in porcine casing preserved in salt. International Journal of Food Microbiology, 128, 411–413.

Wijnker JJ, Haas B and Berends BR, 2007. Removal of foot-and-mouth disease virus infectivity in salted natural casings by minor adaptation of standardized industrial procedures. International Journal of Food Microbiology, 115, 214–219.

Wijnker JJ, Haas B and Berends BR, 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 JJ, Koop G and Lipman LJA, 2006. Antimicrobial properties of salt (NaCl) used for the preservation of natural casings. Food Microbiology, 23, 657–662.

Wilkinson PJ, 1989. African swine fever virus. In: Pensaert MB (ed.), Virus Infections of Porcines. Elsevier Science Publishers BV, Amsterdam, pp. 17–35.

APPENDIX 1—PRODUCTION OF CASINGS

DESCRIPTION OF SOURCE SUBSTANCE

All domestic ungulates farmed for food purposes can be used as a source for animal casings. However, they are predominantly produced from pigs ("hog casings"[15]), sheep and goats ("sheep casings") and cattle ("beef casings").

From pigs the entire intestinal tract is used for the production of casings, specifically the small intestines including the *duodenum, jejunum, ileum, caecum* (known as "bung"), large intestines including the *colon ascendens, colon transversum* ("chitterling"), *colon descendens* ("after end") and *rectum* ("fat end"). From sheep, the small intestines are used, particularly the *duodenum* and *jejunum* and sometimes also the *ileum* and *caecum.*

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 small intestines including the *duodenum* and *jejunum* ("beef rounds") and the *caecum* and from the large intestines, including the entire *colon* ("beef middles"). Beef casings are also produced from the urinary bladder (Ockerman and Hansen, 2000) and the *oesophagus* ("weasand"). However, that part is outwith the scope of the current mandate.

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

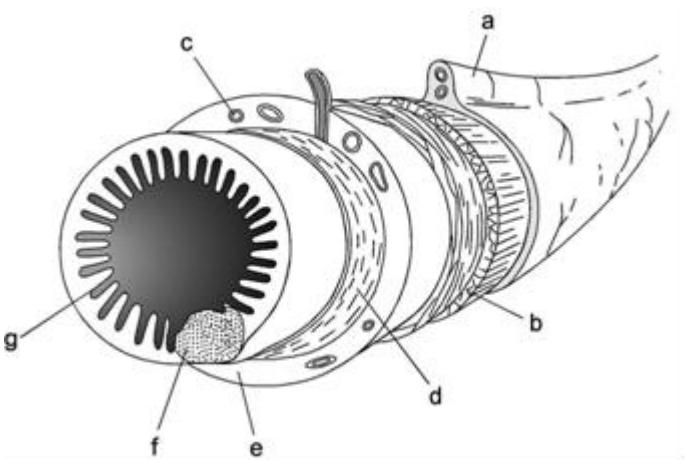

Figure 1: Schematic diagram of sheep small intestine showing mesentery and serosa (a), inner and outer muscle layers (b), submucosal blood vessels (c), muscularis mucosae (d), submucosa (e), lymphoid nodule (Peyer's patch) (f), and tunica mucosa (villus and crypt layers) (g). The tunica mucosa, the muscularis, the serosa and Peyer's patches are removed during processing, so the animal casing consists of only the submucosa (e) (From Koolmees et al., 2004).

[15] The terms in the chapter are broadly recognised and used by the casings industry and indicated in the ENSCA Guide to Good Practice available on the website of DG SANCO (http://ec.europa.eu/food/food/biosafety/hygienelegislation/good_practice_en.htm).

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 I), elastin and blood vessels of different sizes (Nishiumi and Sakata, 1999). For hog and sheep casings, this *submucosa* is the remaining layer of the intestine after processing and forms the natural sausage casing (Figure 2).

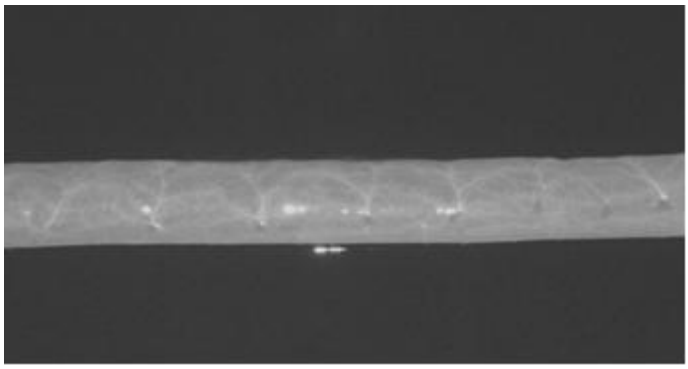

Figure 2: Fully processed sheep casing. Photograph courtesy of Dr J.J. Wijnker.

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 tends 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 (Wijnker, 2009).

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).

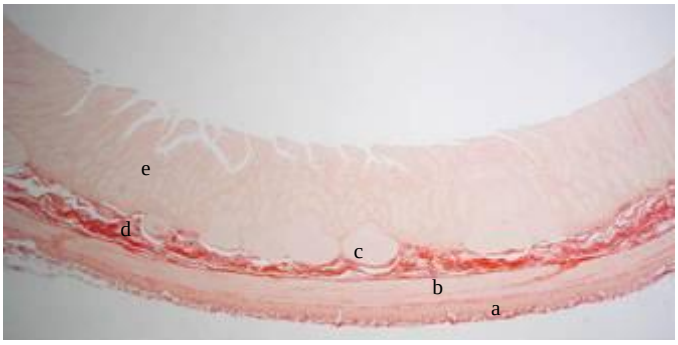

Figure 3: Sheep casing, before cleaning showing mesentery and serosa (a), inner and outer muscle layers (b), lymphoid nodule (Peyer's patch) (c), submucosa (d) and mucosa (e) (picrosirius red staining).

Figure 4: Sheep casing, after cleaning showing submucosa (picrosirius red staining). From Koolmees et al., 2004.

PROCESSING

As described by Wijnker (2009), the subsequent cleaning process of porcine and ovine (caprine) small intestines can vary between species and the 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. Contrary to hog casings, sheep casings are usually fermented prior to the cleaning process. After the faeces have been removed from the intestines, sheep casings are placed overnight in cooled storage during which the *mucosa* will degrade for easier removal.

In general for hog and sheep casings, the faeces are removed from the small intestines, the *mucosa* is crushed and removed in various steps and the outer layers (the *tunica muscularis* and *tunica serosa*) are scraped off, leaving the *tunica submucosa* as the actual animal casing (Fischer and Schweflinghaus, 1988; Ockerman and Hansen, 2000). However, during the cleaning process the actual collagen/elastin structure of the animal casing is not altered, nor during the subsequent curing and final preservation using salt as preservative agent (either dry salted or in saturated brine).

Large-scale manual cleaning of hog casings is no longer used around the world, as it has become more economical to use only mechanical processing. Koolmees et al. (2004) used sheep casings to determine whether there was a difference in the efficacy of cleaning between manual and mechanical processing techniques. Results showed, however, that no significant differences existed between the techniques and that no lymphatic tissue (Peyer's patches) remained after cleaning. Apart from the apparent absence of differences in cleaning efficacy, subsequent selection and grading of the cleaned casings would not allow any tissue residues to remain in the final product. Therefore, making a distinction on whether animal casings were manually or mechanically cleaned is not relevant with respect to quality or product safety parameters.

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).

Equine small intestines are not used for casing production at all; only the large intestines are processed into horse casings, with the exclusion of the caecum. Similar to the porcine large intestines, processing is mostly done by hand and all layers remain identifiable (personal communication, ENSCA 2012).

In contrast to the extensive processing of hog and sheep small intestines into casings, beef casings retain all original layers after cleaning (Figure 5). Similar to sheep and hog casings produced from the small intestines, beef casings are processed mechanically too. Several studies (Koolmees, 1998; Botka-Petrak et al., 2001; Wijnker et al., 2008) on the histology of beef casings have shown that, although most of the *tunica mucosa* is removed from the small intestines, the *tunica muscularis* and *serosa* can be clearly identified and the Peyer's patches also remain present.

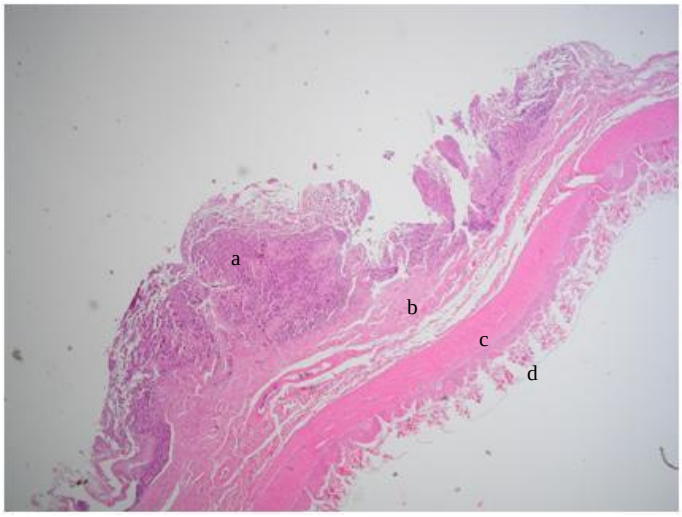

Figure 5: Beef casing, after cleaning (haematoxylin–eosin staining) with remains of a lymphoid nodule (Peyer's patch) (a) embedded in mucosa, submucosa (b), muscular layers (c) and serosa (d). From Wijnker et al., 2008.

MARKET DESCRIPTION OF CASINGS

In Europe, the species available for the production of animal casings are limited to porcines, ovines, caprines and equines, as cattle intestines are listed as SRM in accordance with the TSE Regulation (EC) No 999/2001. Therefore, any animal casings of bovine origin are imported into the EU only from countries with a negligible risk status for BSE, as defined by OIE .

In 2011, approximately 252 million pigs and 52 million sheep and goats were slaughtered in the EU Member states (EU Commission, DG AGRI). It is estimated that per pig 100 g of cleaned animal casings is produced and per sheep (goat) 75 g. This amounts to approximately 25 000 tonnes of hog casings and 4 000 tonnes of sheep and goat casings, assuming that all intestines derived from slaughtered animals are processed into animal casings.

Part of this production (including intestines as well as bladders) is sent out of the EU for further processing and then re-imported as animal casings.

According to the ENSCA Community Guide to Good Practice for hygiene and the application of the HACCP principles in the production of natural sausage casings, natural casings are defined as follows: "Natural casings are used in sausage production (and similar products referred to in section 1601 of the TARIC code), are derived from the intestinal tract or bladders of farm animals, have been scraped and cleaned and have been treated with salt (NaCl) or dried after cleaning."

The import of animal casings into the EU is covered by Council Directive 92/118/EEC and Commission Decision 2003/779/EC, laying down animal health requirements and the required veterinary certification for the import of animal casings from third countries.

All third country establishments from where shipments originate have an implemented HACCP system, are subject to FVO inspection and are approved for the import of animal casings into the EU.

In 2011 the following quantities of animal casings were imported into the EU from in total 29[16] different countries (TRACES):

- bovine casings (23 928 tonnes)
- porcine casings (EU and non-EU origin) (43 434 tonnes)
- ovine and caprine casings (EU and non-EU origin) (19 157 tonnes)
- equine casings (1 152 tonnes).

Some of the countries from which significant amounts of animal casings are imported into the EU are endemically infected with FMD with a high prevalence of disease, e.g. China, Colombia, Egypt, India, Iran and Pakistan.

COMMON PRACTICES AND TREATMENTS

In 2011, ENSCA presented their Community Guide to Good Practice for hygiene and the application of the HACCP principles in the production of natural sausage casings. This document was prepared in accordance with article 9 of Regulation (EC) No 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs, and it was subsequently endorsed by the Standing Committee on the Food Chain and Animal Health (SCOFCAH). It is available on the website of DG SANCO (link). Although this document is mainly intended for the European natural sausage casing industry, it is also used extensively by the international casing industry. Its aim is to assist in the implementation of the European Hygiene Package and HACCP principles according to the Codex Alimentarius

[16] Albania, Argentina, Australia, Brazil, Canada, Chile, China, Colombia, Croatia, Egypt, Iceland, India, Iran, Lebanon, Mongolia, Morocco, New Zealand, Pakistan, Paraguay, Peru, Russian Federation, Switzerland, Syria, Thailand, Turkey, Ukraine, United States of America, Uruguay, Uzbekistan.