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Short Communication

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

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ABSTRACT

Bovine intestines, bladders and oesophagus are used for the production of natural casings ("beef casings") as edible sausage containers. Derived from cattle experimentally infected with FMDV (initial dosage 10^4 $TCID_{50}$/mL, strain A Iran 97), these beef casings were treated with sodium chloride (NaCl) or phosphate supplemented salt (P-salt). In addition, different *in-vitro* experiments using beef casings were done on a small scale with other FMDV strains (A Turkey 06, C-Oberbayern and O_1 Manisa) as "proof of principle".
Based on the combined results of the *in-vivo* and *in-vitro* experiments, it can be concluded that the storage period of 30 days at 20 °C in NaCl is sufficiently effective to inactivate a possible contamination with FMDV in beef casings and that the usage of P-salt does not clearly enhance the inactivation of FMDV infectivity.
Storage of salted beef casings at about 20 °C for 30 days is already part of the Standard Operating Procedures (included in HACCP) of the international casing industry and can therefore be considered as a protective measure for the international trade in natural casings.

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1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals (Alexandersen et al., 2003) and continuing outbreaks incur significant social and economic costs on a global scale (World Animal Health Information Database, accessed August 2011). Affected countries are limited in their ability to trade, with subsequent reductions in the value of their meat commodities. As a result, various measures are put in place on (inter-) national levels (e.g. European Council Directive 2003/85/EC on community measures for the control of foot-and-mouth disease) to prevent the spread of this disease either in live animals or its products.

Different bovine tissues can be used for the production of edible sausage containers (bovine natural sausage casings) and are derived from the intestinal tract (duodenum & jejunum, colon and caecum), the bladder or oesophagus and are generally called "beef casings" (Ockerman and Hansen, 2000; Wijnker et al., 2008a).

Limited information on the survival of FMD in bovine intestines was available, whereas specific studies on the removal of FMD infectivity in natural casings focussed on natural casings of ovine and porcine origin.

Savi et al. (1962) reported the presence of FMD in uncleaned and unsalted intestines directly after slaughter or after 120 days when stored at −30 °C. In contrast, FMD could no longer be detected after 48 h when stored at temperatures between 2 and 4 °C. Cottral (1969) also reported a relatively short survival period of only 6 days when the intestines were stored at 1–7 °C. Böhm and Krebs (1974) not only reported different FMD virus titres in specific tissues, including intestines of experimentally infected sheep, but they also confirmed the efficacy of a 5-minute treatment with 0.5% citric acid on infected sheep casings to inactivate the FMD virus. Wijnker et al. (2007) showed how FMD infectivity could be removed by using either sodium chloride (NaCl) or phosphate supplemented salt from casings derived from experimentally infected sheep and pigs, after storage for 30 days at temperatures around 20 °C. The treatment recommendations based on this study were subsequently included in the OIE's Terrestrial Animal Health Code (article 8.5.41, 2011).

Due to the limited scientific information previously available on the specific import risk of natural casings, subsequent assessments tended to adopt a "precautionary principle" approach. This would explain the high risk classification of natural casings in several risk assessments (e.g. EFSA, 2006; AQIS, 1999). In contrast to this previous situation a more recent import risk assessment (MAF Biosecurity New Zealand, 2010) on European sheep casings reached an entirely different conclusion. It concluded that the existing Standard Operating Procedure (SOP) of salting and 30-day storage put in place by the natural casing industry are sufficient to define the risk as negligible and requiring no additional risk management measures.

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However, no specific information was available on the inactivation of FMD virus in beef casings using the above-mentioned SOP. Therefore, the scope of this study is to determine the efficacy of either NaCl or phosphate supplemented salt on FMDV inactivation as a treatment during storage for 30 days at 4 and 20 °C. The results of this study will indicate whether the SOP can also be applied to prevent the spread of FMD via beef casings produced from the bovine intestinal tract, bladder and oesophagus.

2. Materials and methods

2.1. Animals

Six German Holstein Friesian cattle (approximately 300 kg, 6–-7 months old) were purchased locally, housed in the bio-security facilities at the Friedrich Loeffler Institute (Insel Riems, Germany) and used for this study. All animals were not vaccinated for FMD, came from disease-free locations and were in perfect health.

2.2. Virus strains and initial dosage

The cattle-passaged FMD virus strain A Iran 97 was chosen for the experiment as it is known for its relevance in previous FMD outbreaks and was available at the Friedrich Loeffler Institute. All six cattle were infected intralingodermally and received an initial dosage of 10^4 $TCID_{50}$/mL. The initial virus titre dosage was determined according to the method described in Section 2.4.

In order to ensure that results were not limited to the FMDV strain A Iran 97 only, *in-vitro* experiments were done on a small scale with other FMDV strains (A Turkey 06, C-Oberbayern and O_1 Manisa) as "proof of principle", which were also available at the Friedrich Loeffler Institute.

2.3. Infection experiments and processing of infectious material

Starting on the second day after infection (p.i.), every day two animals were slaughtered in order to recover organ samples during viremia (i.e. day 2, 3 and 4 p.i.).

The animals were stunned using a captive bolt and after exsanguination, the abdominal and chest cavity were opened and the intestinal tract, bladder and oesophagus were removed. These were processed manually by a technical expert provided by the natural casing industry or the institute's butcher according to standard operating procedures. The bladder and oesophagus were flushed with water to remove any content.

After pulling the intestines from the mesentery the small and large intestines were separated for further processing. The manure was squeezed out and the intestines were then scraped manually, removing most of the mucosa, but leaving the outer serosa, musculary layers and submucosa intact (Wijnker et al., 2008a).

Representative samples from the jejunum, colon, caecum, bladder and oesophagus were taken and assigned to one of four treatment groups, which were treated with sodium chloride (NaCl) or phosphate supplemented salt at 4 °C or room temperature of approximately 20 °C. The phosphate supplemented salt (P-salt) contained 86.5% NaCl, 10.7%, Na_2HPO_4 and 2.8%, Na_3PO_4 (wt/wt/wt).

Samples were dry-salted and stored for 30 days according to the respective treatment group.

When it became apparent that due to the low titres observed, no inactivation kinetics could be prepared for the dry-salted samples, additional experiments with spiked organ samples were performed. In order to obtain high titre cattle passaged FMDV, vesicles of FMD-infected animals were homogenized 1:10 in MEM with 5% fetal calf serum (virus suspension). Samples taken from the jejunum, colon, caecum, bladder and oesophagus were homogenized 1:10 in saturated brine made from sodium chloride (NaCl) or phosphate supplemented salt. The homogenate was spiked with 10% (vol/vol) of virus suspension. These samples were subsequently stored at 4 °C or room temperature of approximately 20 °C.

2.4. Analysis of intestinal samples

Prior to virus titration, the samples from infected animals were rinsed in water to remove any attached salt or phosphate supplemented salt. After homogenization in cell culture medium, the samples were treated with chloroform to inactivate bacteria and enveloped viruses and centrifuged to remove cell debris. Also the pre-homogenized samples spiked with virus were treated with chloroform and centrifuged. For virus isolation, the supernatants were put onto a monolayer of ZZ R 127 foetal goat cells (Brehm et al., 2009) following FLI diagnostic standard procedures. The specificity of positive results (cytopathogenic effect) was checked by an antigen-ELISA according to the O.I.E. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (5th edition, 2006).

Over 700 different samples were tested on the presence or absence of FMDV on day 1, 3, 5, 7, 14, 21 and 30 post-infection, divided over six animals, five sample types (jejunum, colon, caecum, bladder, oesophagus) and 4 treatment groups (NaCl at 4 °C and 20 °C, P-salt at 4 °C and 20 °C).

Starting on day 5, pooled samples were tested per animal and respective treatment group. In case FMD virus could be isolated in the pooled samples, the individual sample types were retested to confirm the actual location of the infection.

The *in-vitro* experiments using samples spiked with pooled high titre vesicular material of the infected cattle used in this experiment were tested daily over a 15-day period ($n=1$) on the presence or absence of FMDV or every 5 days over a 55-day period ($n=3$).

The *in-vitro* experiments using samples spiked with the other FMDV strains (A Turkey 06, C-Oberbayern and O_1 Manisa) were tested every 3 days over a 30-day period ($n=2$).

3. Results

3.1. Samples from infected animals

All six cattle showed typical clinical signs of FMD infection and on days 2 and 3 post-infection during viremia, virus titres of about 10^3 $TCID_{50}$/mL of serum were recorded.

However, only in 7 samples taken from three out of six cattle (on a total of 30 samples) could FMD virus be isolated, directly after stunning, exsanguination and processing of the different organs, prior to treatment. The infectivity titres were close to the detection limit of around 10^0–10^1 $TCID_{50}$/mL and no exact quantification was possible.

No FMD virus could be isolated in any samples treated with phosphate supplemented salt and stored at 4 °C and 20 °C for a period of 30 days.

On day 1 after storage in salt, FMD virus could be isolated in three out of 30 samples stored at 20 °C (no samples stored at 4 °C for day 1). On day 3 after storage in salt, FMD virus could be isolated in two out of 30 samples stored at 4 °C and 1 out of 30 samples stored at 20 °C.

In only 7 pooled samples on a total of 30 pooled samples, all stored in salt at 4 °C, could FMD virus be isolated, one on day 5, four on day 7, one on day 14 and one on day 21. From these 7 pooled samples could FMD virus be isolated in the individual samples of 2 animals on day 7. All other individual samples were negative when retested.

None of the 30 pooled samples stored in salt at 20 °C were found to contain FMD virus.

3.2. Samples spiked with virus

The different experiments using spiked samples with pooled high titre vesicular material of the infected cattle using FMD virus strain A

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