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Factors affecting the infectivity of tissues from pigs with classical swine fever: Thermal inactivation rates and oral infectious dose

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ABSTRACT

Outbreaks of classical swine fever are often associated with ingestion of pig meat or products derived from infected pigs. Assessment of the disease risks associated with material of porcine origin requires knowledge on the likely amount of virus in the original material, how long the virus may remain viable within the resulting product and how much of that product would need to be ingested to result in infection. Using material from pigs infected with CSFV, we determined the viable virus concentrations in tissues that comprise the majority of pork products. Decimal reduction values (*D* values), the time required to reduce the viable virus load by 90% (or 1 $\log_{10}$), were determined at temperatures of relevance for chilling, cooking, composting and ambient storage. The rate of CSFV inactivation varied in different tissues. At lower temperatures, virus remained viable for substantially longer in muscle and serum compared to lymphoid and fat tissues. To enable estimation of the temperature dependence of inactivation, the temperature change required to change the *D* values by 90% (*Z* values) were determined as 13 °C, 14 °C, 12 °C and 10 °C for lymph node, fat, muscle and serum, respectively. The amount of virus required to infect 50% of pigs by ingestion was determined by feeding groups of animals with moderately and highly virulent CSFV. Interestingly, the virulent virus did not initiate infection at a lower dose than the moderately virulent strain. Although higher than for intranasal inoculation, the amount of virus required for infection via ingestion is present in only a few grams of tissue from infected animals.

1. Introduction

Incursions of classical swine fever (CSF) into disease-free regions has significant consequences, resulting in ban of international trade and costly control measures (Moennig, 2000). Spread of CSF virus (CSFV) occurs via direct contact between pigs, via indirect contact with virus-contaminated fomites or via ingestion of products from infected pigs. The oral route, via swill feeding, has been responsible for primary disease introductions (Fritzemeier et al., 2000; Paton and Greiser-Wilke, 2003) and although currently banned in the EU, the practice continues in many parts of the world, both with and without mandatory cooking and the possibility of illegal activities cannot be ruled out.

Factors that affect whether or not a pig becomes infected upon ingestion of infected pork-derived material are important for assessing the risks that pork products

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may have for introduction and spread of CSFV (Farez and Morley, 1997). These factors include; the likely level of viral contamination within a product, how long the pathogen may survive within the product under the conditions to which it is exposed, what is the likelihood that a susceptible animal will ingest a contaminated product and, finally, how much product needs to be ingested to instigate an infection (Wooldridge et al., 2006).

The level of viable virus in CSFV infected animals, and hence the level of contamination of a product derived from an infected animal, varies according to the virulence of the infecting virus and the stage and course of disease (Weesendorp et al., 2009b). The tissue tropism of the virus will also affect the viral load found in different pork products. The main target cells for CSFV are leukocytes, myeloid, epithelial and endothelial cells and high concentrations of CSFV are found in lymphoid tissue, spleen and blood (Belak et al., 2008; Liu et al., 2011; Weesendorp et al., 2010). Few studies have quantified viable CSFV in muscle and fat which constitute the major proportion of pork products. Available data indicates the levels in muscle and fat are low, and are often either undetectable or at the limit of detection (Mebus et al., 1997; Thur and Hofmann, 1998), although levels as high as $10^{4.9}$ $TCID_{50}$/g have been reported (Wood et al., 1988).

After slaughter, pork tissues are subjected to various processes such as chilling of carcases, butchery, transport and cooled storage. Products, which may or may not be cured and/or cooked prior to generation of waste, will then be subjected to varying conditions depending on whatever route leads to subsequent ingestion by a susceptible animal, such as landfill, composting or storage of scraps prior to illegal swill feeding (Gale, 2004; Wooldridge et al., 2006). Temperature, pH, the tissue matrix and processing will affect the rate at which virus viability decays (Depner et al., 1992; Edwards, 2000; Farez and Morley, 1997). Studies have demonstrated the times at which virus can no longer be detected in tissues held at different temperatures (Edwards, 2000) and survival kinetics in media (Depner et al., 1992), slurry (Botner and Belsham, 2012), faeces, urine (Weesendorp et al., 2008) and diagnostic tissues (Weesendorp et al., 2010). However, there is a paucity of data on the rate of thermal inactivation of CSFV in tissues that comprise pork products.

Assessment of the risks associated with processes which may result in the ingestion of pork products by pigs requires knowledge of the oral infective dose. A review by Farez and Morley (1997) highlighted that experimental inoculation of pigs with less than 10 $TCID_{50}$ CSFV could initiate infection in a proportion of pigs (Dahle and Liess, 1995; Farez and Morley, 1997). These studies used intranasal inoculation and thus the virus was likely to have had direct contact with the tonsils, the primary site of CSFV replication. The situation is very different when a pig ingests a potentially CSFV – contaminated pork product, where the degree of mastication and rapidity of swallowing will influence if the virus can gain entry by contact with the tonsils or via oral abrasions (Farez and Morley, 1997). It is therefore expected that the oral pig infective dose via ingestion will be higher than 10 $TCID_{50}$.

To improve assessment of the risk of pork products for the introduction and spread of CSF we have determined the rate of CSFV inactivation in tissues found in pork products and gained a more accurate estimate of the pig oral infective dose.

2. Materials and methods

2.1. Viruses and cells

PK15 cells were propagated in EMEM with 10% v/v Foetal Bovine Serum (FBS) and penicillin, streptomycin and Nystatin supplement. CSFV strain UK2000/7.1 (Genotype 2.1) was isolated in the UK, the CBR/93 strain (Genotype 3.3) was kindly provided by Dr. S. Parchariyanon, National Institute of Animal Health, Bangkok and the Brescia strain (Genotype 1.1) was kindly provided by Dr. Alexandra Meindl-Böhmer, University of Veterinary Medicine, Hannover.

2.2. Tissues

Viral loads and rates of CSFV inactivation were determined in muscle (longissimus dorsi or bicep femoris), lymph node (mandibular, Ileocecal, retropharyngeal, ventral superficial cervical), fat and serum samples which had been stored at −80 °C after harvest from animals experimentally infected with CSFV CBR/93, UK2000/7.1 or Brescia, for other purposes, as described below or previously (Everett et al., 2010; Graham et al., 2012). Material from pigs exhibiting the acute form of CSF, between 7 and 21 days post infection (dpi), were used for the study.

2.3. Methods of quantitation of virus

Viable virus in tissues and oral dose inocula was quantified by titration on PK15 cells. Samples were serially diluted (1 in 2, 1 in 3, 1 in 5 or 1 in 10 depending on the viral load present in the sample) in EMEM with 10% (v/v) FBS and antibiotics and 50 μl of each dilution added to 8 wells of a 96 well plate. Plates were incubated for 4–5 days at 37 °C with 5% CO_2 prior to fixing with 4% (v/v) paraformaldehyde and detection of virus by a modified immunoperoxidase staining method (Anonymous, 2012). Briefly after washing with PBS cells were incubated with 3% (v/v) H_2O_2 in methanol for 10 min and permabilised with 1% (v/v) Tween 20, 10% (v/v) normal goat serum in PBS. Virus was detected using CSF-specific Mab WH303 or WH304 (AHVLA), rabbit anti-mouse HRP-conjugated secondary antibody (DAKO) and 3,3′-diaminobenzidine tetrahydrochloride (DAB) chromogenic substrate (Sigma).

2.4. Decimal reduction values of CSFV in porcine tissues, serum and cell cultures

Thawed tissues were cut into approximately 2 mm^3 sections and sections mixed to ensure homogeneity. Aliquots of serum, cell culture medium, or sectioned tissue (250 mg) were incubated in micro-centrifuge tubes, at 25 °C, 56 °C or 68 °C in a circulating water bath or at 4 °C