

## **Qualitative assessment of the commodity risk factor for spread of foot-and-mouth disease associated with international trade in deboned beef**

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### **Abstract**

The risk that imported livestock and their products may introduce foot-and-mouth disease virus (FMDV) restricts trade in these commodities from parts of the world where FMDV has not been eradicated. This reduces investment and development of the livestock sector in many developing countries as well as export trade opportunities and global food supply. This review focuses on the risks associated with trade in deboned beef (DB) from foot-and-mouth disease infected cattle, countries or zones. A definition of DB is provided along with a description of the procedures required for its preparation within abattoirs. A review of the available evidence is presented for circumstances under which DB can be contaminated with FMDV and some figures are provided for the amount of this commodity that has been traded from FMDV-infected regions. Additional mitigating measures to reduce the risk of FMDV contamination of DB are discussed, particularly pre-slaughter measures, such as surveillance, quarantine and vaccination. It is clear that a combination of pre-slaughter and slaughterhouse measures has resulted in a commodity (DB) with a negligible risk of transmitting FMD. Nevertheless, it is concluded that the current evidence does not provide absolute assurance that abattoir procedures for producing deboned beef can on their own result in a commodity with a negligible risk of transmitting FMDV without complementary measures to reduce the likelihood of slaughtering infected cattle. The main areas of uncertainty are the amounts of residual FMDV-harboring tissues within DB, and our understanding of what constitutes a safe level of contamination. More detailed guidance should be developed to specify what mitigating measures are needed in support of the export of DB from regions that are not officially FMD-free. Generic or ambiguous guidance that leads to differences in interpretation can give rise to obstacles to trade and should be avoided. Further data to evaluate the safety of DB might be provided by a study of the amounts of residual lymph node and bone marrow tissues within DB.

### **Introduction**

International Trade Standards set by the World Organisation for Animal Health (also known as the Office International des Epizooties, OIE) aim to prevent the spread of animal diseases that can have devastating health and economic consequences, thus facilitating safe trade in animals and animal products. As specified within the World Trade Organisation (WTO) Sanitary and Phytosanitary (SPS) Agreement, OIE standards must balance safety considerations against the need to promote trade and avoid discriminatory measures that are not based on scientific evidence. The role of livestock products as trade commodities and the importance of fair market access for developing countries have been discussed by Perry, et al. (2005). Scoones and Woolmer (2008) have considered different approaches to achieving market access for beef from southern Africa.

The global demand for meat currently outstrips supply and over the past two decades beef exports have become significant sources of export revenue and jobs for developing countries. In 2006, more than 70% of global meat was produced in developing countries (DC). However, Least Developed Countries (LDC) with the potential to produce quality meat still have impediments to access regional and international markets (Perry et al., 2005). This is despite tariff barriers that are frequently low or non-existent for LDCs. Barriers to trade, imposed by importing countries, are sometimes based on perceived rather than actual risk and in such cases may not reflect internationally agreed upon trade standards. Such barriers can limit the capacity of Developing and LDC to export high value livestock and animal products. This has a knock-on effect in reducing investment in the livestock industry and the availability of products for both internal and external consumption. Impacts of animal diseases on

global livestock and meat markets as well as challenges for livestock producers, industries and policy-makers in a context of rising demand for locally produced and imported livestock products have been reviewed by Morgan and Prakash (2006). Global risks of infectious animal diseases and factors affecting emergence or spread of livestock diseases have been reviewed by CAST (2005). This report has pointed out that despite enormous progress in scientific knowledge and improvements in sanitary standards in livestock production, several FMD outbreaks caused by international spread of the disease have resulted in major economic losses in recent years.

Foot-and-mouth disease (FMD) has been considered a sufficiently serious infectious animal health problem for most developed countries to have expended a great deal of effort on its eradication. These countries, or zones within them, have the status of FMD-free, with or without vaccination, approved by the OIE. If they do not vaccinate their livestock against FMD prophylactically, their animals are highly susceptible to infection from any introduced form of the disease and their own exports can then readily pass on newly acquired infection to third countries. In contrast, many DCs and LDCs lack the resources to eradicate the disease, have endemic or sporadic occurrence of the disease and do not have the OIE FMD-free status. FMD-free countries attempt to protect their livestock industries against introduction of FMD virus (FMDV) by regulating imports of FMD susceptible animals and their products, since it is known that infected animals as well as some of their infected products can introduce FMDV and give rise to outbreaks of the disease. As different types and/or strains of FMDV occur in different parts of the world, there is also logic in preventing the spread of infection between countries from different regions that are not FMD-free. Consequently, FMD is a significant barrier to trade in both live animals and many of their products and even for animal products that do not pose a direct risk of spreading disease. Facilitating access to international markets will assist with poverty alleviation by increasing revenue, jobs and food security in LDCs but should be brought about with safeguards against increasing the risk of spreading disease.

The risk of instigating an outbreak of FMD in an importing country through a traded animal product is a combination of the likelihood of (1) the animals from which the product is derived being infected with FMDV at the time of slaughter, (2) the likelihood of FMDV surviving during preparation, storage and transportation of the commodity, (3) the probability of FMDV infected product reaching susceptible animals in sufficient quantity and causing an outbreak of FMD, and (4) the volume of trade. A variety of measures can be used to mitigate the first three of these risks and the OIE Code provides guidelines on what measures are appropriate for trade in different commodities between countries at different stages of FMD control and eradication. Where scientific evidence demonstrates that it is safe to trade specific animal products that have been processed in a manner which precludes the presence, or removes or inactivates the disease agent of concern then international regulations should be adapted to enable these products to be traded. Alternatively, a combination of measures to reduce both the likelihood of slaughtering infected animals and FMDV survival thereafter may be appropriate.

In the UK, some outbreaks of FMD were attributed to imports of frozen bone-in meat from FMD infected countries in South America, notably the large outbreak in 1967/8. However, research suggested that the risk of this recurring could be greatly reduced by restricting imports to deboned beef from areas with a systematic vaccination regime. This was the basis for the UK to permit imports of beef from Argentina in 1969, and since that time, very large quantities of this product have been imported without any evidence that this has given rise to outbreaks of FMD (Astudillo, et al., 1997b; de las Carreras, 1993). The OIE has set up recommendations for safe trading of beef as will be described later on in this review, The EU, probably the largest importer of DB has also developed stringent rules to allow safe importation of this commodity (EU Council Directive 2002/99/EC).

This review focuses on the risk of international trade in deboned beef (DB), and the extensive evidence base, historical experience and past and current processing technologies to assess the risk of spreading FMDV by trading this product from FMD affected areas. The consistency of current international trade standards of OIE to the scientific evidence is also assessed.

### **Foot-and-mouth disease**

FMDV infects cattle, buffalo, pigs, sheep, goats and various wildlife species and is a major cause of productivity loss. It exists as seven serotypes that do not engender cross-protective immunity, as well as many intra-serotypic strains that may also incompletely cross-protect (Anonymous, 1937). The virus spreads rapidly by multiple routes and is difficult and expensive to control. Hence, its occurrence

correlates inversely with economic development and it is most common in Africa, the Middle East and parts of Asia and South America. Countries with a livestock surplus have a strong incentive to control the disease in order to facilitate exports of animals and their products. However, for many there are major obstacles to be overcome in meeting the FMD criteria which would enable such exports. For example, the FMDV strains circulating in Africa, Asia and South America are almost entirely distinct and consequently vaccines must be tailored regionally. The greatest diversity of serotypes and strains occur in Africa, but control efforts are least developed in many countries of the continent. There are competing priorities and limited resources and in countries with poor prospects for FMD control there is little incentive to conduct surveillance in order to determine the variety and predominance of different FMDV strains affecting livestock and wildlife populations. Thus, the range of tailored vaccines is probably inadequate as well as the quantities available and the resources and political will required to organise and sustain effective control campaigns. This is in contrast to the successful control and eradication schemes carried out in continental Europe and South America which have often relied upon mass vaccination, requiring a high proportion of the cattle population to be repeatedly immunised for many years.

The pathogenesis of FMDV has been reviewed (Alexanderson et al., 2003). Susceptible livestock are most commonly infected by FMDV through the oropharynx, although the virus can also enter through abrasions in the skin. After replication at the portal of entry, the virus drains to the local lymph nodes and then the bloodstream leading to viraemia, widespread dissemination throughout the body and viral shedding in many bodily secretions. The virus reaches high titres in the stratified epithelia of the mouth, feet and udder associated with the development of painful vesicles that rupture and release large amounts of virus into the surrounding environment. Virus replication in heart muscle can occur in young animals; evidence for replication in skeletal muscle is less convincing. The incubation period between infection and the onset of clinical signs may be from 2-14 days depending upon dose, but most commonly is 3-5 days. Virus may be present in a variety of tissues and bodily fluids and excretions prior to the onset of clinical signs. Whereas cattle and pigs usually develop obvious clinical signs of FMD, the disease is often much less easily recognised in small ruminants. Systemic antibodies appear rapidly, from 5 days after infection, and are associated with clearance of virus from the circulation. Virus persists longer at the site of lesions and in a high proportion of cattle, low levels of the virus can be detected in the oropharynx beyond 28 days after infection and up to three and a half years post infection. These persistently infected cattle are known as FMDV carriers. Carrier cattle do not readily transmit infection to other susceptible animals but the risk that they pose in this regard has not been quantified with certainty.

### **OIE Recommendations on Trade in Beef**

One way to facilitate beef exports from countries that are not FMD-free is to establish one or more FMD-free zones in which animals are completely segregated from those in adjoining infected zones. The current OIE requirements for trading beef from FMD-free zones have been harmonised with those for countrywide freedom and no longer require deboning of meat from cattle. Compartments, in which animals are separated by management, rather than mainly geographical barriers have also been proposed (Scott et al., 2006), but their implementation by OIE for FMD is still under review. Alternatively, the OIE recommends that beef can be exported as a safe commodity from countries or zones that are not FMD-free, subject to certain precautions to reduce the likelihood of infected animals being slaughtered and providing that certain procedures are followed during preparation of the commodity. The requirements are given in Article 8.5.23 of the OIE Terrestrial Animal Health Code (TAHC) and include the general need for an official FMD control programme, involving compulsory systematic vaccination of cattle and the following specific conditions:

For [fresh meat](#) of cattle (*Bos taurus* and *Bos indicus*) and buffaloes (*Bubalus bubalis*) (excluding feet, head and viscera) [Veterinary Authorities](#) should require the presentation of an [international veterinary certificate](#) attesting that the entire consignment of meat:

1. comes from animals which:
  - a. have remained in the [exporting country](#) for at least 3 months prior to [slaughter](#);
  - b. have remained, during this period, in a part of the country where cattle are regularly vaccinated against FMD and where official controls are in operation;

- c. have been vaccinated at least twice with the last vaccination not more than 12 months and not less than one month prior to [slaughter](#);
  - d. were kept for the past 30 days in an [establishment](#), and that FMD has not occurred within a ten-kilometre radius of the [establishment](#) during that period;
  - e. have been transported, in a [vehicle](#) which was cleansed and disinfected before the cattle were loaded, directly from the [establishment](#) of origin to the approved [abattoir](#) without coming into contact with other animals which do not fulfil the required conditions for export;
  - f. have been slaughtered in an approved [abattoir](#):
    - i. which is officially designated for export;
    - ii. in which no FMD has been detected during the period between the last [disinfection](#) carried out before [slaughter](#) and the shipment for export has been dispatched;
  - g. have been subjected to ante-mortem and post-mortem inspections for FMD with favourable results within 24 hours before and after [slaughter](#);
2. comes from deboned carcasses:
- a. from which the major lymphatic nodes have been removed;
  - b. which, prior to deboning, have been submitted to maturation at a temperature above + 2°C for a minimum period of 24 hours following [slaughter](#) and in which the pH value was below 6.0 when tested in the middle of both the *Longissimus dorsi*.

Otherwise, in the case of [meat products](#) of domestic ruminants and pigs for importation from FMD infected countries or zones, it is recommended that the meat is processed to ensure the destruction of the FMD virus – i.e. fresh meat cannot be traded. In this context, fresh meat means all edible parts of an animal (apart from the head, feet and viscera) that has not been subjected to any treatment irreversibly modifying its organoleptic and physicochemical characteristics. This includes frozen meat, chilled meat, minced (ground) meat and mechanically recovered (deboned) meat.

### **Abattoir Procedures and Post Mortem Changes**

For the purpose of this review DB comes from veterinary inspected cattle transported and slaughtered as prescribed in Article 8.5.23 of OIE TAHC. Carcasses have been aged (matured) at refrigeration temperatures until ultimate pH has been reached and have been fabricated (by meat cutting) to obtain a prescribed fresh (not processed) refrigerated or frozen beef item. A processed product refers to one that has been subjected to a food preservation treatment other than chilling and freezing (e.g. curing, heating, dehydration, ionising irradiation, etc). DB mainly corresponds to muscle tissue, after deboning, including fat cover, connective tissue, small vessels and nerves as well as all tissues which were not removed during slaughtering and fabrication procedures. A brief description of common, correct slaughtering and fabrication procedures is pertinent to this review.

The process of slaughtering involves transport of animals to an abattoir, holding and ante-mortem inspection with no evidence of clinical disease in a lairage, stunning, bleeding out, hide removal, eviscerating, halving (splitting the beef into sides), post mortem inspection with no macroscopical evidence of disease, chilling of the carcasses, fabrication (final deboning) and packaging. The extent to which these processes lead to removal of infection with FMDV have an important impact on the risk of final product contamination. Since inspection of the final product does not reveal how procedures have been followed during pre-harvest and post-harvest stages and its preparation, adequate Food Safety and Quality Assurance Schemes (FS&QAS), including traceability and auditing of the process are vital (CODEX, 2005; Dagg, et al., 2006; Caporale et al., 2001; McKean, 2001).

After death, anaerobic glycolysis takes place in muscle tissues and stored glycogen is converted to pyruvate, which is then reduced to lactic acid resulting in a fall in pH, ultimately to a value of 5.6 - 5.7 (Foegeding, et al., 1996). Puolanne et al. (2002) have calculated that a decline in pH from 7.0 to 5.5 (ultimate pH) requires the formation of 60 to 80 mmol lactic acid per kg muscle tissue depending on the muscle tissue and the animal species. This has an important impact on FMDV survival because the virus is inactivated by acid conditions; as well as an extremely important influence on food safety and

quality of the final product (deboned meat). The accompanying depletion of ATP is responsible for *rigor mortis* (stiffening of the muscle) which normally takes 6 – 12 hour for beef muscle. Glycogen can be depleted by several pre-slaughter stress conditions including exercise, fasting, hot and cold temperatures and fear (Lister, et al. 1981), resulting in reduced muscle tissue acidification and improved survival conditions for FMDV. Good transportation conditions, handling and animal welfare practices are crucial to obtain DB with an ultimate pH value of 5.8 or lower after ageing or maturation (EU, 2002). There is approximately 1% glycogen in the muscle tissue and this will generate 1.0 to 1.1% lactic acid. For each 1% lactic acid formed the pH will be lowered by approximately 1.8 pH units. Nonetheless, both the rate of pH fall and the ultimate pH achieved are influenced by factors such as, species, type of muscle in an animal, genetic variability between animals, administration of drugs which affect metabolism, environment prior to slaughter (feeding, stress), post-mortem temperature - increased temperature increases rate of pH decline - and electrical stimulation of excised muscle increases rate of pH decline (Ockerman, 1996).

Bachrach et al., (1957) studied rates of inactivation of tissue culture derived type A FMDV at various pH levels. At 4°C, and pH 6.0, infectivity was lost at a rate of about 90% per minute. Bachrach et al (1957) found that inactivation rates were biphasic resulting in a very low level of residual virus that is rather pH stable. However, it is generally accepted that FMDV is totally inactivated at pH 6.0 or below after 48 hr at a temperature of 4°C (Pharo, 2002). pH changes may occur at different rates in different muscles, a measurement of pH 5.8 in the *Longissimus dorsi* (LD) muscle has been used as a proxy to indicate non-survival of FMDV in the carcass (CEC, 1986). Extensive LD muscle pH data showed that 66,220 out of 694,719 beef carcasses had a pH equal to or greater than 6.0 at 24 hr post slaughter (USDA, 2002).

General requirements for safe preparation of meat are described in the Codex Alimentarius Code of Hygienic Practice for Meat (CODEX, 2005). These guidelines emphasise the importance of a risk-based approach tailored to food safety issues, local threats and the needs of importers. The Codex is therefore not prescriptive concerning specific mitigations for FMD, such as the precise nature of ante-mortem and post-mortem inspection procedures. Beef carcasses and beef cuts, from exporting, slaughtering and fabrication commercial facilities, have been extensively studied and characterized from the hygienic, keeping quality and food safety stand-points (Lasta, et al., 1992; Rodríguez, et al., 2000). Furthermore, best practices for handling vacuum-packed beef cuts have been developed (AMI 2003). Meanwhile, some markets apply specific hygiene rules for food of animal origin to ensure a high level of food safety and health protection (EU, 2004).

Commercial beef slaughtering operations for exporting markets are fully mechanized and procedures are carried out at different stations on an assembly line. This enshrines the principle of moving the carcass always forward aiming to avoid cross contamination. Each carcass bears an identification or bar code which helps to retrieve information on slaughter date, origin of animal, type of animal, carcass characteristics, and other production and quality attributes. On the slaughter floor, the feet, head, and hide (all of which can harbour FMD infectivity) are removed at the very beginning of the process. This first stage of the slaughtering line is known in the meat industry as the “dirty zone”. After evisceration (at the “intermediate zone”), beef carcasses are split into right and left sides at the “clean zone” to ensure rapid cooling in the chilling room. It is crucial to run all slaughtering procedures under proper FS&QAS (i.e. SSOP’s, GMP’s and HACCP). Adequate traceability procedures must ensure that each particular carcass, head and viscera (the three items are moved separately along the slaughter floor in different conveyor lines) bear the same identification tag at the slaughter floor level to facilitate Veterinary Inspection as well as further FS&QAS carried out by the industry.

Immediately after leaving the killing/slaughter floor, beef carcasses are kept in the chilling rooms at appropriate refrigeration temperatures (carcasses will begin chilling within one hour from bleed-out). The chilling room should be designed according to the number of animals that are slaughtered so as to provide not only enough room for storage but also adequate conditions of air movement and temperature transfer among beef carcasses. Carcass chilling is crucial for safety and quality. Refrigeration temperatures will reduce carcass surface moisture to produce unfavourable conditions for microbial growth as well as slow down microbial growth rate. Moreover, chilled beef will be easier to handle for cutting and will preserve quality characteristics as well. The beef muscle ageing (maturation) process crucial for FMDV inactivation via pH drop is also temperature dependant. Early ageing (24 – 36 hr post slaughter) also starts muscle protein denaturation improving tenderness and eating quality. Under most common commercial practices, ageing will continue after beef cuts have

been prepared and packaged and kept under refrigeration as will be briefly described below. For the purpose of this review, DB has been considered adequately aged after muscle tissue has achieved its ultimate pH (5.8 or below). pH is measured in each LD immediately before the carcass is broken in quarters and consequently before entering the deboning room at a pH control station. pH measurement is carried out according to a specified protocol (i.e. electronically measured, with daily calibration of instruments, proper registration chart/notebook, etc) and under the audit of the Veterinary Inspection Service (VIS).

The process of carcass fabrication starts immediately after carcasses leave the chilling room and takes place in the deboning room where beef cuts are obtained under environmental refrigeration temperatures (usually <10°C). Carcass temperature (usually between 4 and 7°C) and pH (5.8 or below) are controlled before entering into the deboning room to ensure compliance with Veterinary Service Inspection Authorities and specifications of importing countries. Each carcass side or half is divided into quarters. The forequarter, composed of specified wholesale cuts, is usually the heavier quarter. The hindquarter, also composed of specified wholesale cuts is the most valuable quarter based on market prices. There are several methods used to break specific wholesale units down into smaller retail market units. Local or international preferences, industry market capabilities, merchandising trends, as well as many other factors may determine the optimal cutting procedure to produce any particular fresh beef item. Cutting beef quarters for exporting markets is an area in which a variety of options are utilised by meat cutters at the industry level. Photographs, diagrams, anatomical references as well as a summary of the main cutting descriptions are commonly utilized in explaining cuts of beef for trading purpose (IPCVA, 2008). For DB preparation, as it was defined above, all bones as well as major blood vessels, visually identifiable lymph nodes, blood clots and specified amounts of fat tissues are removed according to market or commercial specifications before final packaging. From the stand-point of FMD risk mitigation procedures, specific VIS stations eliminate – as far as is practically possible - lymph glands, fragments of bones and any other suspected tissue at the deboning room level. Adequate traceability procedures at deboning and packaging rooms ensure that each particular beef item corresponds to a particular carcass. All deboning, packaging, chilling or frozen storage as well as labelling and shipping procedures should be under Veterinary Inspection as well as further FS&QAS carried out by the meat industry. There are protocols that cover non-compliance with a specified product (i.e. carcasses with a pH reading equal or higher than 6.0 should be properly identified, separated in a different cooling facility, and not exported. These carcasses are diverted to local/domestic markets). If FMD is eventually detected in a herd at the slaughterhouse level it is excluded for export markets. Depending on the amount, localization and extension of lesions in organs or carcasses they are diverted to local market or condemned if necessary. After slaughter of a FMD herd proper cleaning and sanitation procedures (facilities, personnel) are carried out with approved FMDV inactivation agents. The whole process should be under VIS rules and audit.

### **Literature review on FMDV survival in fresh meat**

There is a considerable body of literature on the amount of FMDV detectable in the tissues, secretions and excretions of different species of animals during infection. However, many variables may affect these values, including differences in host species and breeds, types and strains of FMDV, stage of infection and methodology used to make measurements. Virus persistence in animal products after slaughter depends upon many of the same variables and especially on changes in pH that take place in different organs and tissues under different conditions. Although the subject has been reviewed on numerous occasions, the number of publications that provide actual data on virus survival in cattle carcasses, collected and stored so as to mimic beef abattoir slaughtering processes, is relatively few and much of the literature is not easy to access. Table 1 summarises the most important of these where detailed methodologies are available. None of the in-depth studies has involved serotypes Asia 1 or any of the South African Territory (SAT) serotypes (in general, the thermal stability of the Asia 1 FMDV serotype is relatively high and that of SAT serotypes is relatively low (Doel & Baccharini, 1981)). In most cases, cattle were slaughtered a few days after direct inoculation with FMDV and mostly when showing clinical signs of disease. Such studies may represent a worst case scenario for peak FMDV contamination. The majority of these studies involved cattle that had not been vaccinated against FMD. In one major study, however, large numbers of vaccinated cattle were used (NASNRC, 1966). Since the cattle had received at least six vaccinations with a vaccine strain homologous to that used for subsequent challenge, but were killed at peak viraemia this study provides a best case scenario for the likely reduction in levels of viral contamination associated with FMDV infection in vaccinated animals. Reviews of FMDV survival in meat are summarised in Table 2. FMDV survival in various

beef processed items as well as virus behaviour and stability under different thermal and non-thermal processes have been extensively studied notably in North and South America in the '80s and '90s (Blackwell, et al., 1982; Blackwell, et al., 1988; García Vidal, et al., 1988; Lasta, et al., 1992; Vermeulen, et al., 1993; Masana, et al. 1995a; Masana, et al. 1995b; Pagliaro, et al. 1996). These studies provide relevant experimental data on FMDV thermal stability, further insight on pH effect, as well as additional FMD-safe processing treatments for international trade for various edible beef items.

Usually, the methods used to detect FMDV survival in meat products have been by inoculation of test material, either into cattle, guinea-pigs, mice or cell cultures. These cannot be considered as natural routes of infection or ones that mimic the most likely form of risky exposure following importation of deboned beef, which is ingestion, especially by pigs. The titres of virus reported in different studies are not directly comparable due to differences in the sensitivity of the test systems used. A minority of the studies also fed animal products to small numbers of pigs. The results are also not directly comparable in that the studies involved a wide variety of serotypes and strains of virus but the individual and/or comparative characteristics of these viruses in respect of thermal and pH sensitivity are unknown or unstated.

The conclusions of these studies are that the acidification of skeletal muscle that takes place during maturation of the carcass is normally sufficient to inactivate all FMDV in this tissue, even when cattle are killed at the height of viraemia. Since it is known that the required level of acidification cannot be guaranteed under all circumstances, measuring of the pH of the carcass can be used to ensure that it has occurred. This is the basis for the current requirements concerning maturation and pH assessment of beef carcasses (EEC, 1986; OIE, 2008).

In contrast, other tissues and organs that may harbour FMDV do not undergo acidification and in these tissues the virus can survive the maturation process and subsequent low temperature carcass storage. These include blood, heads, feet, viscera, bones and major lymph nodes, all of which can be removed during the processing of the carcass. Under commercial beef operation conditions FS&QAS are in place in order to control and to eliminate these defined non-muscle tissues. However, residual blood, fragments of bones and small lymph nodes are likely to remain in the cuts. FMDV in bone tissues would most likely be found in the bone marrow rather than the bone itself. There are no available data to quantify amounts of fragments of bones or lymph node tissues that remain in a specified beef cut (USDA, 2002).

Immunisation of cattle by repeated vaccination using vaccines closely matched to the challenge strain of FMDV confers a high degree of protection upon infection. It has been shown to greatly reduce the level of virus present in lymph nodes (NASNRC 1968) and presumably also in other parts of the animal and its products.

### **Risk associated with FMDV survival in animal products**

Table 3 lists and comments upon the risk assessments reviewed in this study.

The risk associated with FMDV survival in animal products depends not only on the quantity of surviving virus but on the likelihood and route of exposure to susceptible animals, the species of susceptible animal, the amount of the material inoculated, inhaled or ingested and the number of animals that are actually exposed (Sellers, 1971; Suttmoller and Vose, 1997). This makes it difficult to establish a threshold level of FMDV contamination of a commodity, below which it could be considered as representing a negligible risk.

Ingestion of contaminated animal products by pigs is one of the most likely routes by which an imported, contaminated meat product could start an outbreak of FMD, although other means are possible and some infections of cattle in the UK in 1967 have been attributed to their exposure to personnel who had been handling contaminated, imported meat (Sellers, personal communication). Due to their higher susceptibility to inhalation of FMDV, Sellers (1971) suggested that ruminants might be infected by sniffing contaminated materials rather than by eating them. From feeding FMDV contaminated materials to relatively small numbers of pigs, the minimum oral dose of FMDV to infect pigs has been estimated at around  $10^5$  tissue culture infectious doses (Sellers, 1971; Donaldson, 1997). This was deduced from a small number of rather disparate studies, mostly not involving titration of the challenge dose, and in which results were not always consistent (Table 4). Actual data on how readily

pigs become infected by ingestion of FMDV contaminated carcass material is very scarce (Table 4) and there appear to have been no studies in which material equating to DB as a commodity has been fed to pigs.

Considering the daily feed intake of a pig, Sellers (1971) concluded that at a virus concentration of less than  $10 \text{ ID}_{50} \text{ g}^{-1}$ , the amount of product needed to be ingested by a pig to establish infection would exceed its daily feed intake. This assumes that an effective dose can be acquired cumulatively, whereas the relationship between concentration, volume and total effective dose is poorly understood. An added complication arises due to non-homogeneous commodity contamination. For example, if a small fragment of bone within a large amount of meat had a virus concentration above  $10 \text{ ID}_{50} \text{ g}^{-1}$  within the bone fragment, there might still be insufficient virus in total to infect pigs through ingestion. The physical nature of the food may also be important since it has been shown that infected bone marrow was infectious to pigs only if crushed bones were incorporated into the feed. It was presumed that crushed bones facilitated infection through causing oral abrasions (Anonymous, 1927) which suggests that animals with pre-existing oral lesions might also be more susceptible to infection by FMDV. Finally, Suttmoller and Vose (1997) were of the opinion that doses below those normally considered the minimum for establishing infection still have a certain low probability to cause infection and begin an outbreak. However, this argument assumes that the material would be fed to a very large number of pigs some of which would have greater susceptibility than average. In the case of a small fragment of contaminated bone within a large consignment of meat, there would be insufficient material to be eaten by many pigs.

Practices within an importing country such as vaccination against FMD or prohibitions on swill feeding of pigs also militate against, but not necessarily negate, the risk from contaminated, imported animal products.

#### **Information on trade in beef in relation to FMD dissemination**

Beynon (1968) reported that between 1954 and 1967, 54% of primary outbreaks of FMD that occurred in England were attributed to imported meat, bones and meat wrappers. Similar figures are provided by a Wellcome Trust Witness Seminar on the 1967/8 UK FMD outbreaks recorded in 2001. However, these outbreaks predate the introduction of requirements for deboning and maturation of carcasses imported from South America as well as introduction of the ban on all swill feeding to UK pigs. Valarcher et al. (2008) in their review of the origins of FMD outbreaks within Europe in the last 20 years, record only a single case attributed to beef importation and this concerned the outbreaks in Albania in 1996. In this case, the import permit on the beef consignment stated that it was deboned, but in fact it was bone-in.

Blajan and Callis (1991) noted that more than 100,000 tons of boneless beef were imported into the European Community in 1989 from South America and Southern Africa. Furthermore, between 1968 and 1990, 500,000 tons of boneless meat had been imported into the UK from Argentina. There is no evidence that this led to any outbreaks of FMD. FMD risk mitigation procedures that have been in use in South America for more than 30 years have contributed to the development of a safe and highly technical and specialized beef exporting industry.

Total DB exports from Argentina were more than 9,271,850 tons (product/shipping weight) between 1965 and 2008; while exports of DB to FMD-free countries such as the UK, in the same period, were 913,608 tons; 1,230,207 tons to Germany and 769,973 tons to Chile (Otaño, 2009). These trade figures are summarised in Figures 1-5. Deboned beef exports expressed as equivalent carcass weight for Brazil were 21,325,000 tons, while Uruguay exported 4,247,000 tons expressed as equivalent carcass weight.

It is interesting to analyze exports from Argentina to the UK between 1963 and 1995, since vaccination was not applied in the UK. It is possible to estimate the number of steers slaughtered in order to obtain one ton of DB shipped to the UK. For type A cuts (special cuts from hindquarter) it is assumed that one animal provides between 18 and 20 kg. Hence, it will be necessary to slaughter between 50,000 and 55,000 steers to obtain 1,000 tons of DB (SENASA, 1994). For the year 1991, 42,837 tons were exported, from approximately 214,000 beef cattle corresponding to 7,130 troops of cattle – one troop is approximately 30 steers. Steers slaughtered for the export trade historically came from the “Pampa region”, particularly from its central fattening areas. Notably, this Pampa region, described as a Secondary Endemic zone, from the FMD standpoint, used to have the largest number of annual FMD



outbreaks in Argentina (Dillon, 2009). Therefore, it is very likely that some DB was shipped to the UK after being obtained from FMDV infected animals. If such has been the case, no evidence has been found that an outbreak of FMD had occurred in the UK due to this commodity trade. Reported FMD outbreaks in Argentina are summarized in Figure 6.

Figure 7 provides figures for DB imports into the EU from countries that do not have OIE FMD-free country status. However, there is no differentiation between imports from FMD-free regions and regions that are not free. As well as large-scale imports from South America, smaller scale imports also took place from southern Africa (Botswana, Namibia, Zimbabwe and South Africa). Further information on this trade is available from a recent workshop on transboundary animal disease and market access, see: <http://www.steps-centre.org/ourresearch/vetscience.html>. These southern African countries used similar principles of separating FMD-endemic from free areas by fencing and movement restrictions, biannual vaccination of cattle in proximity to infected African buffalo and active clinical surveillance (Thomson, 2008). However, their exports to Europe were mostly (apart from Zimbabwe) only permitted from OIE recognized FMD-free zones. A further precaution was and is that DB from southern Africa could/can not be imported into Europe until three weeks after the source animals were slaughtered, allowing time for recognition of any recent outbreaks that could affect the safety of the commodity. Since the time to ship to Europe exceeds three weeks, the precaution fits well with the export process.

According to a study by PANAFTOSA and Tuskegee University (PANAFTOSA, 1995; Table 3), the risk of DB spreading FMD internationally following a reintroduction of FMDV into Uruguay or Argentina during the 1990's was exceedingly small, providing that outbreaks would have been limited in number and rapidly brought under control.

### **Risk Assessment**

A risk assessment strictly adhering to OIE guidelines will focus on conditions in the exporting as well as the importing country. Information needed to conduct a risk assessment of this nature will include information on the exporting country's Veterinary Service, disease surveillance, eradication and control programmes, zoning systems, incidence and/or prevalence of disease, existence of disease-free areas and areas of low disease prevalence, animal demographics, farming and husbandry practices, geographical and environmental characteristics including rainfall and temperature, etc (OIE, 2004).

The above-mentioned information (inherently specific to a particular country) is not available due to the broad scope of this project that includes all infected countries, zones and compartments globally. Furthermore, this information need not be applicable when focussing on a specific commodity and therefore it was decided to use a commodity risk factor approach. Following this approach, each commodity that is handled in exactly the same manner would have the same commodity risk regardless of the status of the country or zone or compartment of origin. For this purpose, it is presumed that the animal producing the commodity is infected (worst case scenario) and every step in the slaughtering and storing process is evaluated in order to determine how much the infection is reduced by each process (Metcalf et al., 1996).

It should, however, be noted that all the factors mentioned above, especially disease prevalence, are key factors that need to be taken in account when determining the risk of infected animals actually arriving at the slaughter plant. Also, most of these factors are critical control points that have the potential to decrease the probability of infected animals being presented for slaughter and thus reducing the risk associated with the final product.

### Methodology:

The commodity risk factor approach described by Metcalf *et al* (1996) was used to determine the risk associated with trade in deboned beef from FMD infected animals, countries and zones.

A scenario tree was used to identify the risk pathways, to ensure a logical chain of events and to identify information requirements (Figure 8).

To adhere to the principles of the SPS Agreement, which states that risk must be assessed according to the SPS measures which might be applied, the standards set by the OIE TAHC were used where

applicable, for example maturation standards as described in Article 8.5.23 was used in the risk evaluation.

The risk of each of the six events in the scenario tree and ultimately the risk associated with the final product were qualified using data obtained through an extensive literature review process.

The following terms were used to describe the risk / likelihood estimates (OIE, 2004):

<b>Term:</b>	<b>Oxford Dictionary Definition:</b>
Average	The usual amount, extent, rate
Extremely	Outermost, furthest from the centre; situated at either end; utmost; the highest or most extreme degree of anything
High	Extending above the normal of average level
Highly	In a high degree
Insignificant	Unimportant; trifling
Low	Less than average, coming below the normal level
Negligible	Not worth considering; insignificant
Significant	Noteworthy; important; consequential
Remote	Slight, faint

Possible risk mitigations (quarantine and vaccination) and their influence on the commodity risk factor were evaluated from data acquired through the literature review.

Assessment of the commodity risk factor:

Event 1 (Disease not detected during ante-mortem inspection):

Key points at the ante-mortem inspection stage:

- Cattle from Veterinary Inspected farms arrive at slaughter facility pens.
- Traceability registers as well as sanitary documentation are analyzed for each cattle group (usually one truck carries a group of approximately 30 animals).
- Cattle should be transported and handled according to Veterinary Inspection Service (VIS) rules. Cattle should be allowed to rest, and be provided with *ad libitum* water as well as feed when appropriate.
- A systematic procedure should be followed to consistently inspect animals involving a thorough visual examination as part of an official VIS scheme. Veterinarians should be able to walk around the animal holding facility to check for any abnormal movement or symptoms.
- When necessary (i.e. VIS detect any abnormality) animals should be individually examined and rectal temperature, mouth and feet are checked for visible lesions by VIS.
- Trucks, floors and pens should be properly cleaned and sanitized under VIS procedures.

Fact:

- During a study by Cox *et al* (1961), high virus titres were found in lymph nodes, 24 hours post-inoculation and before any clinical signs were observed.
- A study by McVicar and Suttmoller (1976) showed that viraemia was detected before the onset of clinical signs in some animals.
- Different studies indicated that considerable amounts of virus were recovered from the mucosae and lymphoid tissues of the pharyngeal region of cattle for periods of up to 3-9 days before the detection of viraemia and/or clinical signs (Burrows, 1968; Burrows *et al.*, 1981; Sellers *et al.*, 1968).

- Alexandersen et al. (2003) reported that FMDV could be detected in serum, pharyngeal fluid, saliva, nasal swabs and milk prior to the first appearance of macroscopical lesions. Viral titres in serum averaged  $10^{3.2}$  TCID<sub>50</sub>/ml the day before onset of clinical signs, peaking at  $10^{4.9}$  and  $10^{5.3}$  on the day of and day after first clinical signs respectively.
- Infection of Chinese yellow cattle with O PanAsia FMDV failed to cause clinical disease although these cattle were able to transmit infection to susceptible in-contact animals (Kitching, 2002; Huang et al., 2000).

Opinion:

- Pyrexia of 40°C for 1-2 days precedes vesicle development (Kitching, 2002). During this time, cattle show only non-specific signs of malaise. Vesicle development is often accompanied by other visible signs such as drooling of saliva, grinding of teeth and lameness.
- In endemic regions in cattle that have partial natural or vaccinal immunity, clinical signs may be mild and may be missed (Kitching, 2002).
- In clinically sick cattle, the likelihood that lesions will be missed is low (Astudillo et al., 1997a). However infected animals in the incubation period present a high risk (Sutmoller 2001).

The available evidence suggests that there is a low risk that infected cattle, showing pathognomonic clinical signs will be missed during ante-mortem inspection, however, the risk should be considered as high when animals presented for slaughter do not show detectable clinical signs (for example cattle in the incubation period partially immune animals and cattle infected with a mild strain of the virus). The levels of virus present in animals peak at around the time of onset of clinical signs, but significant levels of virus may be present before this time.

Event 2 (Disease not detected during post-mortem inspection):

Key points at the post-mortem inspection stage:

- After killing the animals, each carcass is subjected to a macroscopic examination of all organs and tissues and when necessary microscopic and lab analysis are carried out. Thorough inspection of the feet and mouth including the tongue and buccal surfaces is essential.
- Post-mortem inspection is carried out by properly trained professionals of the VIS.
- Head, viscera and carcass must carry the same identification tag until the VIS is finished at slaughter level.
- Post-mortem inspection includes visual observation, palpation and excision of lymphoid glands and organs.
- Feet, hoofs, tongue, gums are carefully examined. Feet and hoofs are rapidly removed from the killing floor.
- Carcasses are released for either fresh or processing markets according to the VIS decision. When necessary, carcasses or their parts are subjected to condemnation.
- Immediately after finishing slaughtering, carcasses are sent to appropriate chilling rooms to allow ageing and muscle pH drop.

Fact:

- Considerable amounts of virus were recovered from the mucosae and lymphoid tissues of the pharyngeal region of 21 of 23 cattle killed before the onset of viraemia and in many of these and other animals for periods of up to 3 days before slaughter or the detection of viraemia and/or clinical signs (Burrows et al., 1981).

Opinion:

- Astudillo et al. (1997a) estimated that the post-mortem inspection process would be at least five times more sensitive than the ante-mortem inspection on account of the thorough individual inspection of each carcass.

- Carrier cattle are unlikely to have scars on the tongue or foot epithelium and will escape detection at the farm of origin and at ante-mortem or post-mortem inspection (Sutmoller, 2001).

Compared to ante-mortem inspection, post-mortem inspection has an enhanced probability of detecting macroscopic lesions but a reduced probability of detecting non-specific signs of illness such as lameness and depression that are the main, albeit poor, indicators at the onset of illness due to FMD. A systematic procedure should be followed to consistently inspect high risk tissues.

Event 3 (Infected tissue not removed during slaughter):

Key points at removal of specified organs:

- The head, as well as viscera, are handled in different lines (pulley systems) from corresponding beef carcass. The three elements though bear the same tag identification.
- Tonsils are eliminated (not used for any food purpose).
- Pharynx and throat are longitudinally excised, visually inspected and by palpation, the presence of lesions are investigated. They can be used for pet food.
- When necessary samples (pharyngeal or lymph nodes) are taken for lab studies.

Fact:

- The pharynx is a major site of primary and secondary FMDV replication during acute infection and along with pharyngeal and other lymph nodes is the major site of FMDV persistence in cattle (reviewed by Alexandersen et al., 2003; Juleff et al., 2008)
- The dorsal surface of the soft palate and the pharynx was indicated as the main sites for virus persistence and multiplication. Virus was recovered from these sites, from 41 out of 54 cattle killed 14-196 days after infection (Burrows, 1966).
- The quantity of virus present in the pharynx of acutely infected animals is high (up to  $10^{7.4}$  TCID<sub>50</sub>/ml), but in convalescent animals the amounts of virus are much less (~10-100 TCID<sub>50</sub>/ml) (Alexandersen, 2003) and therefore carrier animals do not readily infect other susceptible animals through contact (Tenzin et al., 2008).

Opinion:

- Superficial mechanical contamination of beef by virus present in the throat is a risk to be considered. With proper slaughtering techniques and destruction of the pharyngeal area, mechanical contamination poses a negligible risk for the international beef trade (Sutmoller, 2001).

The successful removal of potentially infected tissue (e.g. head, feet, pharynx, etc), will reduce the risk of contamination of the final product. The risk that these tissues will not be removed completely depends entirely on the meticulous execution of this step of the slaughtering process. An adequate FS&QAS with proper documentation, and critical control point management, should be put in place to ensure removal of potentially infected tissues.

Event 4 (Virus survives maturation at temp above 2°C, minimum 24 hours with pH below 6):

Key points at ageing stage including pH measurement:

- Beef carcasses are allowed to start ageing at refrigeration temperatures (usually for a 24/48hr period). Refrigeration temperatures (cooling room and carcasses) and time frame are usually Critical Control Points in a commercial facility that follows a FS&QAS.
- Before entering into the deboning room each carcass is subjected to a standardised pH measurement. Ultimate pH (5.8 or lower) is tested for the *Longissimus Dorsi* (LD) muscle of each beef carcass. Good correlation has been found between the pH level of LD muscles and many other beef muscles of the same carcass. LD is a standard comparator muscle of beef with regard to pH.

- pH measurements are usually carried out by industry QA specialists and audited by the VIS. Instruments for pH measurement should be inspected and calibrated daily. Adequate forms and documentation are kept as evidence of proper procedure and product attribute.

Fact:

- Virus in muscles may be accounted for by either the direct infection of the tissues or by its presence in the capillary beds and vessels because of viraemia (Cottral et al., 1960).
- A study by Cottral *et al* (1960) included muscle pH curves that showed that the virus population in muscles was probably greatly reduced within eight hours, but infectious virus may have persisted in the superficial portions of the muscles for nearly 48 hours, since the pH was still 6.0 or higher in some areas.
- Prolonged survival of the virus in muscle tissue is only likely if the pH is above 6.2 (Henderson & Brooksby, 1948).
- The pH level reached by the meat of normal animals depends on at least two factors: the glycogen content of muscle at point of death and the buffering capacity of the muscle. In animals where the activity before slaughter was prolonged and severe, the pH may be very high. Exercise, stress and certain disease conditions may also inhibit lactic acid formation (Bate-Smith, 1948).
- FMDV was found in both fresh and ripened (72 hours at 4C) haemal nodes (Cox et al., 1961).
- In samples of bone marrow taken from cattle during the acute stage of infection (48 hours post infection), FMDV survived at 1-4C for as long as 210 days. In similar samples of lymph nodes and haemal nodes, virus persisted for 120 days (Cottral, 1969).
- Lymph nodes that were examined by Cottral *et al* (1960) maintained pH readings between 6.4 and 6.9, a favourable range for virus survival (at 4C for 72 hours).
- Lymph nodes and blood clots in large vessels, even though in close proximity to the muscles, do not develop the degree of acidity that is present in the muscle tissue and the pH of a lymph node does not become sufficiently acid to inactivate the virus (Henderson & Brooksby, 1948).
- Liver, kidney, rumen, lymph node and blood from disease cattle have all been shown to be highly infective and to remain so if stored frozen (Henderson & Brooksby, 1948).
- Virus was detected in the ripened lymph nodes (from carcasses hung for 72 hours at 3-6 C) from all animals infected 32 hours prior to slaughter (NASNRC. 1966).
- A study by Garcia-Vidal *et al* (1983) showed that virus was not detected in muscle at pH 6.0 or below. The minimum pH value in which the virus was present was pH 6.4.

Opinion:

- Further research has been advised to investigate the effect of pre-slaughter stress on the depletion of glycogen stores and subsequent reduced pH drop in FMD-infected sheep (Ryan et al., 2008), but there is also little information on how this may impact upon FMDV survival in cattle carcasses. However, this potential problem is controlled by pH checks on beef during DB preparation.
- No data are available on the kinetics of virus inactivation in meat at pH 6.0 (Astudillo et al., 1997a).

The most important factor for post-slaughter inactivation of the virus in the carcass is pH. Virus might be present in muscle tissue at slaughter as a result of viraemia or direct infection and a variety of conditions exist where the desired pH to inactivate virus might not be reached. The risk of virus surviving maturation at temperatures above 2°C, for minimum 24 hours can thus be considered significant, unless a pH of less than 6.0 is reached during that time. Measuring the pH in the middle of both *Longissimus dorsi* muscles, as described in the OIE Code, will ensure that the muscle pH has decreased sufficiently to inactivate FMDV. However, it was shown that the pH in lymph nodes, bone marrow and haemal nodes often does not reach the required value to inactivate virus. Therefore, the risk of virus surviving in a carcass at this point of the slaughter process is still significant.

Event 5 (Virus not eliminated during deboning and removal of lymph nodes):

Key points at deboning stage:

- Carcass fabrication, deboning and beef cut preparation should be carried out by professionals and skilful meat cutters at commercial facilities.
- Beef cuts and specified fresh beef items are prepared according to market specifications.
- Meat cutting is carried out under FS&QAS and VIS schemes.

Fact:

- Human error cannot be completely ruled out during the deboning process. Blood clots, bone chips and pieces of large vessels or parts of lymph nodes might not be removed completely (Astudillo et al., 1997a; Cottral et al., 1960; Suttmoller 2001).
- Muscles taken from the vertebrae may be particularly contaminated with bone, because they are near the point where the carcass is split (Cottral et al., 1960).
- FMD virus can survive 120 days at 1-4°C in lymph nodes and 210 days at 1-4°C in bone marrow (Cottral, 1969).
- The amount of surviving FMDV in bone marrow has been found to be sufficient to infect pigs by the oral route when fragments of bone were included in the material fed to the pigs (Table 4 and Cox et al., 1961).
- The virus may survive and be demonstrable in commercially boned cured or uncured meat, if the meat were obtained from an area where foot-and-mouth disease is present. It was concluded by Cottral *et al* (1960) that meat derived from animals infected with foot-and-mouth disease was not rendered free of the virus by the usual commercial procedures of ripening, boning, salting and storage.
- FMDV was found in both fresh and ripened (72 hours at 4C) haemal nodes, which are difficult to remove from meat during trimming (Cox et al., 1961).

Opinion:

- Maturation and deboning of the carcass will eliminate most of the virus, but beef from cattle slaughtered in the incubation period is likely to pose a considerable risk (Suttmoller, 2001).

The evidence shows that there is a low risk that all virus will not be removed during the process of deboning and the removal of lymph nodes. Since the amount of residual lymph node and bone tissue is unclear, and even though FMDV in bone tissues would most likely be found in the bone marrow rather than the bone itself, the risk of viable virus still being present at this point of the slaughter process is thus not demonstrably negligible. Whether DB prepared from an infected animal contains enough FMDV to infect susceptible animals by natural routes of exposure has not been directly measured.

Event 6 (Cross-contamination of clean product or packing materials):

Key points at environmental, packaging and related stages:

- Processes and operations at meat industry facilities are designed and carried out to avoid cross-contamination. A forward-moving “conveyor-belt system” ensures that clean products are not allowed to move back or have contact with a zone containing products at an earlier and potentially contaminated point of processing. Adequate cleaning procedures and sanitation schemes must be in place.
- FS&QAS and VIS schemes must be in place to avoid cross-contamination.

Fact:

- Virus can survive for at least four days in infected blood splashed on carcass surfaces (Cottral et al., 1960).
- The survival of FMDV on or within various contaminated objects would be shorter for free virus than for virus within cells from epithelial lesions. Also the amount of protective colloids

and tissue debris as found in mucous from the nasal and salivary discharges of infected animals would lengthen the survival time. According to Cottral (1969), sunlight, temperature, pH changes and relative humidity will also have an influence. Virus can for example survive on a meat cloth (at 4°C) for 6 weeks (Cottral, 1969).

- FMD virus remained infectious for approximately 33 to 398 days on meat packaging materials experimentally contaminated with infected bovine tissues. The contaminated materials were stored at 4 C with an average relative humidity of 85% (Gailiunas et al., 1969).
- Suttmoller and Vose used binomial modelling to illustrate that when sufficient numbers of susceptible animals are exposed to products which have low levels of contamination (and even if all individual animals receive less than the so-called minimum infective dose) there is still a chance of infecting one animal from the group, which is likely to start an epidemic with a highly infectious disease such as FMD (Suttmoller & Vose, 1997).

Opinion:

- The slaughter of viraemic cattle creates an additional hazard of gross environmental viral contamination of the slaughterhouse facilities. It seems reasonable to assume that contamination of personnel and products leaving the premises, including packaging material and vehicles, cannot be excluded (Suttmoller 2001).
- In their review article Suttmoller *et al* (2003) address the concern regarding mechanical contamination of a carcass with “carrier virus” from the pharyngeal area. They conclude that because of antibodies in blood and other fluids and additional measures which may be applied during slaughter and processing (e.g. for BSE) the risk is negligible.

It has been shown that when clean product or packing materials come in contact with contaminated blood, other fluids, etc., the clean product can be contaminated with virus and have the potential to transmit disease. The risk of this happening depends on the hygiene procedures of the slaughtering process and the virus concentrations on the contaminated materials.

Final commodity risk qualification:

As mentioned previously, for the purpose of the commodity risk factor approach, it was presumed that the animal producing the commodity is infected. For this reason, the initial risk started off as high, whereas under field conditions it might be very low due to other pre-slaughter risk mitigation procedures such as surveillance, vaccination, quarantine, etc. not discussed so far.

Cattle showing pathomonomic clinical signs have a high probability of being detected during ante-mortem or post-mortem inspection and therefore constitute a low risk. However, infected cattle that do not show overt clinical signs associated with FMD, e.g. partially immune cattle, cattle infected with a mild strain of the virus, breeds of cattle that do not show obvious clinical signs or animals early in the incubation period, introduce additional risk to the process. Preclinical viraemia represents the highest risk.

The removal of potentially infected tissues and organs, for example the head, feet, pharynx, etc., followed by maturation of the carcass according to the standards in the Code, will mitigate the risk, although not entirely. It was shown that the FMDV can survive maturation in the lymph nodes and bone marrow and that these tissues might not be completely removed during the mitigation processes. Therefore until more evidence on the amount of residual lymph node and bone tissue become available, the risk associated with deboned beef cannot be ascribed a negligible rating.

Cross-contamination of clean product and packing materials is an additional possibility. The related risk will depend on the likelihood of cross-contamination happening in a specific abattoir and the viral levels on the contaminated product.

Overall, the risk associated with deboned beef, when only considering OIE recommended risk mitigations applicable to the slaughtering process, although low, cannot be completely ignored based on current knowledge. Some additional measures to mitigate the risk outside the slaughtering process are discussed below. A combination of pre-slaughter and slaughterhouse measures has been shown to be very effective in reducing risk to negligible levels.

### Effect of pre-slaughter risk mitigations on commodity risk factor:

Scenarios for additional risk mitigation measures are summarised in Figure 9.

#### Surveillance:

Early detection of disease in the source herds, accompanied by appropriate control measures is extremely important and will significantly reduce the risk of selecting infected animals for slaughter. Surveillance programmes need to be designed according to the disease situation in the country of origin and should adhere to the principles of Chapter 1.4 (Animal Health Surveillance) and Articles 8.5.40 to 8.5.46 in the TAHC.

#### Vaccination:

##### Fact:

- Cattle develop an effective immune response within 3-5 days after vaccination (Doel et al., 1994).
- Vaccination can reduce the number of infected animals and the risk of slaughtering viraemic cattle in the pre- or sub-clinical stage of disease. For example, the findings of a study by Orsel *et al* (2005) indicate that single vaccination in a population of calves can reduce transmission and that it might be sufficient to eradicate the virus during an epidemic of FMD.
- During a series of experiments organized by the Argentine-United States Joint Commission on FMD it was shown that multiple vaccination markedly reduced the chance of recovering virus from lymph nodes at the time of slaughter, of cattle exposed to virus by tongue inoculation 32 hours previously (NASNRC, 1966).
- Neutralising antibodies induced in vaccinated animals are probably the best guarantee for meat, blood, lymph nodes, bone marrow and organs being free of virus (NASNRC, 1966; Suttmoller & Casas Olascoaga, 2003).
- Vaccinated ruminants will continue to carry live FMD virus in their pharynx after contact, regardless of the development of clinical or sub-clinical disease (Kitching, 1998). Doel *et al* (1994) showed that a large number of cattle (at least 11/28) given O1 Lausanne vaccine became persistently infected when challenged.
- McVicar and Suttmoller (1976) during their study concluded that the high virus titres seen in vaccinated cattle in the absence of obvious clinical signs suggest that partly immunized cattle, after exposure to virus, may become inapparent virus shedders and therefore dangerous sources of infection.
- Experiments to demonstrate transmission of FMD virus from carriers to susceptible in-contact animals have been unsuccessful (Van Bekkum et al., 1959; Suttmoller & Barteling 2004; Kitching, 1998).
- Where FMD outbreaks were controlled by consistent vaccination with a qualified vaccine the disease did not re-occur. There are also no documented cases where cattle vaccinated with a qualified vaccine caused new outbreaks. Therefore, the risks posed by vaccinated carriers must be an acceptable, “close to zero” risk (Barteling & Suttmoller, 2002).
- Emergency protective vaccination will reduce the risk of encountering recently infected animals, whereas the risk posed by carriers established prior to vaccination would not be significantly altered by vaccination (Have, 2003).
- Circulating antibodies, whether acquired passively or actively, do not prevent the establishment of FMDV infection in the pharyngeal area in cattle, but it will prevent detectable viraemia. The risk of meat from carrier animals being contaminated is thus negligible or close to zero, because there will be no virus in the bloodstream, muscles, lymph glands or other organs (Suttmoller, 2001; Suttmoller et al., 1968).
- According to Suttmoller *et al* (2003) in countries where FMD was controlled by the use of systematic vaccination of the cattle population only, transmission of disease from carrier cattle to non-vaccinated or other susceptible species has not been observed. Also, in situations in which, after a period of “freedom of FMD”, vaccination was discontinued there has been no case of FMD linked to the existence of carriers. Only circumstantial historical evidence exists



to implicate carrier animals as the source of an outbreak, however there are numerous cases in which large numbers of convalescent cattle introduced into non-protected herds did not cause new outbreaks.

- Subclinically infected vaccinated cattle can transmit infectious levels of FMDV to susceptible animals for up to seven days post-infection (Donaldson and Kitching, 1989).
- Effective vaccination requires the vaccine strain to be antigenically matched (i.e. correct serotype and strain) to the challenge strain against which protection is required in the field (Paton et al., 2005). This requires knowledge of the circulating field viruses to which vaccinated livestock may be exposed. This is feasible in some parts of the world, where effective surveillance has ensured that the range of locally circulating field viruses has been properly documented and where well-matched vaccines are available. However, it is difficult to achieve in regions where there is considerable or unknown antigenic diversity amongst circulating field viruses and use of well-matched vaccine strains cannot be guaranteed (OIE/FAO Reference Laboratory Network Report, 2008).

Opinion:

- For vaccinated animals, an antibody test of a blood sample at the time of slaughter could provide a high margin of assurance of the absence of virus from the carcass (Sutmoller & Casas Olascoaga., 2003).
- Thompson *et al* (2009) suggests that a single vaccination at three weeks prior to slaughter is sufficient, while Sutmoller & Casa Olascoaga (2003) advocate double vaccination.
- Sutmoller *et al* (2003) concludes that transmission from carrier animals must be a very rare event and it is not known whether it happens by a special set of circumstances or whether it is merely an infrequent stochastic phenomenon, or both.
- Sutmoller *et al* (2003) addresses the concern that meat, meat products and milk from vaccinated FMD carriers are a risk for FMD free regions, zones or countries and states that apart from the regular risk reduction processes that are applied to meat and meat products, the vaccinated animal offers even less risk. The neutralizing antibodies in the vaccinated animal are the best guarantee that meat, blood, lymph nodes, bone marrow, organs, etc. will be free of FMDV.
- Antibodies to vaccine viruses may not protect against infection with viruses that are not closely related antigenically to the vaccine strain of virus.
- High yielding dairy cows in the Middle East are not always protected from high level challenge with FMDV despite vaccination every ten weeks with vaccine produced under European standards containing eight strains of virus (Kitching, 2002).
- The progressive control pathway for FMD recommended by OIE/FAO under the umbrella of the Global Framework for control of Transboundary Animal Diseases (GF-TADS) requires as a first step, that countries that are not free of FMDV should identify the types and strains of circulating viruses. This requirement might be made a prerequisite for those countries wishing to export deboned-beef.

The protective effect of vaccination with an efficient vaccine, applied according to acceptable international standards will very significantly reduce the probability of animals becoming infected and thereby reduce the risk of infective animals being presented for slaughter. However, if infection of vaccinated animals occurs, virus replication can take place, albeit often at reduced levels compared to unvaccinated animals, with or without the appearance of obvious clinical signs. Vaccinated and infected animals can also become virus carriers regardless of whether they show clinical signs of infection. Neutralising antibodies in correctly vaccinated animals are likely to ensure that meat, blood, lymph nodes, bone marrow and organs are free of virus. Vaccination is therefore a very valuable mitigation measure, provided that vaccines closely matched to the challenge strain of FMDV are used and applied correctly. Serology could be used in conjunction with vaccination as an additional safe guard to ensure that protective antibody levels are indeed obtained.

Quarantine:

Fact:

- For the purpose of the OIE Terrestrial Code, the incubation period of FMD is 14 days (OIE, 2008) and a 3 week quarantine period should thus suffice.
- The incubation period depends on the species, dose, route and strain of virus. For within farm spread: the incubation period can vary from two to ten days. While for between-farm spread by the airborne route the range is four to 14 days, depending on the infecting dose (Donaldson, 1987).

Opinion:

- Thompson *et al* (2009) suggest that a 3 week quarantine period will create the opportunity for any animals in the batch of cattle destined for slaughter to manifest disease. Any suspicion of disease should result in all the animals being discarded.
- Since FMD has a short incubation period, infection of the animals either at the farm of origin or in transit would probably be visible during ante-mortem inspection, with lesions on at least a few animals (Astudillo *et al.*, 1997).

Given that the risk associated with DB described above is mainly as a result of slaughtering animals in the incubation period, a 3 week pre-slaughter quarantine will be a valuable mitigation measure providing that undetected infection of cattle does not occur during quarantine.

#### Waste product management:

The institution of a ban on the feeding of waste products (swill) to pigs is an important risk mitigation measure. This measure will ensure that any residual FMDV that might have entered through the importation of DB will not establish or spread in the importing country and will thus pose no risk. The success of this mitigation measure is however dependant on the ability of the country to enforce such a ban.

#### Previous risk assessments performed on deboned beef:

Several risk assessments, models and reviews regarding the safety of trade in DB have been published (Astudillo *et al.*, 1997; Metcalf *et al.*, 1996; Suttmoller, 2001; Suttmoller & Casas Olascoaga, 2003; Yu *et al.*, 1997 and others mentioned in Table 3). Whereas the risk assessment in this review only focused on the commodity itself for reasons already mentioned, most of the other assessments focused on specific countries and could thus include conditions in the importing as well as in the exporting country. The final risk rating of these assessments can therefore not be compared to the final rating of this review, which only took risk mitigations during the slaughter process into account. It is however noteworthy that the risk in most of these assessments was negligible when including additional mitigation measures (such as vaccination, surveillance, cattle originating from free zones, etc).

However, the paper by Suttmoller and Olascoaga (2003) reviewed previous risk assessments and concluded that the risk mitigation methods recommended in the TAHC will effectively eliminate FMDV from beef, but in viraemic cattle, this elimination may not be complete and virus in organs from these animals will not be affected by maturation and deboning (with reference to Cottral *et al.*, 1960). Furthermore, in the paper by Suttmoller (2001) the risk mitigation measures were reviewed and the author classified the risk associated with viral survival after treatment of carcasses (according to OIE recommendations) as moderate for animals in the incubation period.

Metcalf *et al* (1996) used example data to describe the application of risk assessment to international trade in animal products and thus no source was referenced for the data used. The process of estimating the source and commodity risk factors was described. In this example the commodity risk factor was calculated to be negligible, but no explanation was given on how the probability estimates (for example the probability of virus not eliminated during deboning and removal of lymph nodes) were determined and the animals presented for slaughter were assumed to be vaccinated. Although this paper is an excellent model for conducting similar risk assessments, it is difficult to evaluate the value of the quantitative results for the specific commodity risk factor in this review; since extensive supporting

evidence for the estimated probabilities is lacking and a pre-slaughter mitigation was taken into account.

Early detection of disease in the source herds is one of the most important risk reduction factors featuring in all the risk assessments.

From these assessments it can be concluded that the risk associated with DB when only applying the risk mitigations associated with the slaughtering process cannot be considered negligible, but when applying additional risk mitigation measures, such as described in Article 8.5.23 of the TAHC, the risk can be classified as negligible.

## Discussion

FMDV survives poorly in bovine muscle tissue and even in experiments where cattle were slaughtered at the peak of viraemia, FMDV did not survive the changes associated with *rigor mortis* and carcass maturation (Henderson and Brooksby (1948). Certain conditions may reduce post mortem acidification of muscle and might therefore be expected to contribute to improved FMDV survival. Studies have confirmed that not all beef carcasses reach the required level of post mortem acidification (USDA, 2002), but no data were found to validate or refute the effect of this on FMDV survival. This could be studied, although it may be considered that the testing of the pH of meat provides sufficient assurance that acidification has been adequate, even if the practice of testing the *Longissimus dorsi* muscles may not totally guarantee the pH fall of all other beef muscles. In contrast, FMDV survives in other tissues that do not become acidic, such as blood, lymph nodes and bone marrow (Henderson and Brooksby, 1948). The practice of bleeding out carcasses and removal of bones and major lymphatic glands reduces the risk of residual FMDV survival in boneless beef, but would not be expected to eliminate these tissues entirely leaving a residual but unquantified risk of FMDV survival. The risk posed by a low level of residual virus is difficult to assess because few studies have examined the susceptibility of pigs (or other susceptible species) to infection by plausible infection routes such as ingestion of contaminated carcass materials. In general, relatively high doses of virus are needed to infect pigs reliably by the oral route (Sellers, 1971) and this would suggest that risk due to deboned beef would be very low. However, without information on the amounts of non-muscle tissue present in deboned meat and also on the probability of any low level of contamination being able to initiate downstream infection through exposure to susceptible animals, it can be concluded that deboned beef is a very low risk commodity with respect to spread of FMD, but it cannot be concluded that the risk is negligible without other complementary risk reduction measures.

Alternative evidence for the safety of DB when exported from FMD infected countries is the data showing that very large quantities of this product have been shipped from South America to Europe without causing FMD outbreaks – even during periods of FMD outbreaks in South American countries (Astudillo, et al. 1997b). Furthermore, the fact that outbreaks were regularly attributed to beef imports prior to this precaution being introduced is highly suggestive of a beneficial impact from the measure. However, this does not provide categorical evidence for the absolute safety of the commodity, since other risk mitigation measures such as quarantine, surveillance and vaccination were also in operation that ensured a very low level of virus circulation in the livestock sector servicing the export industry. Smaller, but still very significant quantities of deboned beef have also been exported to Europe from Southern Africa, but in this case the exports have been mostly from FMD-free zones. The question therefore remains as to what extent virus circulation needs to be understood and controlled before DB becomes an acceptable risk.

The current OIE Terrestrial Animal Health Code requires a number of additional measures to reduce the likelihood of an infected animal being presented for slaughter at an export abattoir. Thomson et al. (2009) have recommended an alternative procedure whereby animals would be held in a quarantine facility for at least three weeks prior to slaughter and vaccinated against FMD on entry to the facility. Furthermore, they recommended that the farms from which the animals were sourced, the quarantine facility and the abattoir should be operated so as to comply with the requirements of a FMD-free compartment as defined by the OIE, operating under an integrated bio-security system. Requirements of the existing OIE Code with respect to abattoir procedures and commodity preparation and deboning were endorsed, meanwhile other measures to minimize impact of food borne hazards at both production and industry levels (i.e. veterinary drug residue programs, pathogen reduction programs, application of FS&QAS, etc) were also stressed. Perry et al. (2005) have stated that the commodity

based approach needs to be translated into practice for specific products from specific regions of developing countries in order to gain trading opportunities for those products. Moreover, they suggest further development of specific guidelines for defined livestock priority commodities from developing countries. These matters are under consideration by the OIE (OIE, 2008).

Although optimal vaccination greatly reduces the levels of FMDV in infected animals, it can be anticipated that as vaccination becomes less effective, for example due to low potency, single dose, long or very short interval before challenge or poor antigenic match to challenge strain, then the protection conferred will diminish towards that of an unvaccinated animal. Therefore, vaccination as a mitigation measure will only be effective if suitable vaccines are used and this requires both a surveillance system to ensure that the vaccine strain is tailored to the threats from locally circulating field isolates of FMDV and a system of accreditation to ensure adequate potency and correct application. This justifies the requirement in the OIE Code for vaccination to be part of an official control scheme. The alternative model of Thomson et al (2009) might be compatible with this requirement provided that some system of local surveillance and accreditation can be provided. The Progressive FMD Control Pathway recently promoted by FAO/OIE provides a possible approach to establish credible surveillance and risk management without FMD freedom (Rweyemamu et al., 2008; Paton et al., 2009). The Pathway encompasses six stages, the first four of which cover steps towards FMD freedom. Stage 0 is the starting point, stage 1 is reached when risks have been identified and a control strategy developed and stage 2 is when critical risks have been managed. Stage 1 or 2 might be considered as the minimum requirement for compatibility with exporting deboned meat to FMD-free countries.

Most beef exporting countries that maintain ongoing vaccination for FMD control (i.e. South American countries) have achieved a well mechanized and highly specialized industry through 40 years of safe trading DB mainly to the EU. However, as new beef exporting actors may enter in the international arena they will need to upgrade their operations to be able to respond to market opportunities as well as to face new challenges which in turn impact the whole international sector. For instance, recent studies on pre-slaughter management (i.e. before and during transport to slaughter, during handling at livestock markets, and at the time animals are put-up for slaughter within abattoirs) draw attention to pre-slaughter stress, food safety and quality issues affecting the final product (Gregory, 2008). Therefore, it will be helpful to understand these trends and to develop new guidelines for defined beef items intended for international safe commodity trade, as has been suggested by Perry, et al (2005).

## Conclusions

1. Several countries that have not had countrywide FMD freedom have used a combination of measures to (i) reduce the likelihood of infected animals being presented for slaughter at export abattoirs, and to (ii) minimise FMDV survival during the slaughter process and the preparation of DB. This combination of measures has proved extremely successful in eliminating risk associated with trading DB.
2. Existing best practice for preparation of DB in export abattoirs provides a high level of risk mitigation against contamination of the commodity by FMDV. Nonetheless, harmonised protocols for procedures such as ante mortem and post mortem inspection could be established as guidance for new players.
3. Deboned beef is a very low risk commodity with respect to spread of FMD. However, neither data on the safety of trade in the commodity to date nor a risk assessment of the survival of FMDV during the preparation of the commodity under currently recommended procedures provide conclusive evidence that the risk is negligible without measures that reduce the likelihood of infected cattle being presented for slaughter.
4. The current OIE Terrestrial Animal Health Code comprises both specific and general recommendations for minimising the risk of FMDV contamination of exported DB. Whereas general guidance is non-prescriptive and leaves open the possibility of utilising a range of specific measures that might be balanced and effective, it also suffers from the disadvantage of being open to different interpretations as to what is necessary and this may act as a major impediment to trade. For example, the requirement that there should be an official control programme for FMD does not give details of what is required in this regard. Other measures

than those proposed in the Code might provide a similar or sufficient level of risk reduction, but those that rely on application of the principles of compartmentalisation for FMD are weakened by the lack of detail on what this would entail.

5. Vaccination has the potential to be a very effective mitigation measure to ensure the safety of deboned beef, but it is reliant on the effective use of appropriate vaccines and this requires an adequate knowledge of the strains of FMDV that are most likely to threaten the vaccinated cattle population. Post-vaccination serology could add to the assurance that vaccination has been effective. Single or double dose vaccination can provide an effective level of immunisation, although immunisation is stronger after two rather than one vaccination.
6. The Progressive FMD Control Pathway recently promoted by FAO/OIE could be developed to provide a possible approach to establish credible surveillance and risk management without FMD freedom.
7. The competence of the National Veterinary Services will always be critical, both for surveillance and vaccine selection, and for enforcement of mitigation measures including those carried out before, during and after slaughter.
8. Food Safety and Quality Assurance Schemes (i.e. SSOPs, GPMs, HACCP, traceability, etc) at the livestock and meat industry level are crucial to provide enhanced monitoring and controlling procedures in a sector that combines many mechanized operational stations with others based on qualified human labours.
9. Actual data on virus survival in cattle carcasses, collected and stored so as to mimic beef abattoir slaughtering procedures are scarce with respect to FMDV serotypes, Asia 1 and SAT 1-3.
10. There is no agreed threshold level for safe FMDV contamination of a commodity such as deboned beef, and the minimum dose of FMDV within deboned beef that can infect pigs by ingestion is poorly understood.
11. Information was not found on the amount of residual blood clot, lymph node and bone tissue within deboned beef.
12. Information was not found on the survival of FMDV in deboned beef from carcasses where the normal acidification of skeletal muscle had not occurred nor on FMDV survival in fat tissues (other than bone marrow and infected blood splashed on beef carcass surfaces).
13. It was difficult to combine the data on safely traded deboned meat with that on FMDV occurrence within the relevant exporting and importing countries in order to estimate the proportion of this meat that had come from infected cattle. However, while very large volumes of DB have been imported into countries which have OIE freedom from FMD without vaccination there is no direct evidence that they have caused disease, even though some unknown proportion has almost certainly involved cattle infected with FMD, even if only as carrier animals.

## **Recommendations**

1. More specific guidance should be developed on mitigation measures that will provide adequate assurance that FMDV infected animals, particularly those in the early stages of infection and possibly incubating the disease, are not presented for slaughter at export abattoirs in regions that are not officially FMD-free.

The FMD Progressive Control Pathway of the Global Framework for the progressive Control of Transboundary Animal Diseases (GF TADs) could provide a useful framework to guide the implementation of the necessary measures that should encompass both procedures to be followed and measures by which their implementation can be monitored, including the circumstances of disease risk escalation under which the trade would be suspended. Guidance should include:

- a. Options for isolating animals that are three weeks or less away from slaughter so that they do not become exposed to infection and/or are not incubating FMD at the time of slaughter.
  - b. Other options for reducing the weight of challenge, such as specified measures of surveillance and vaccination to control FMD in the areas that are epidemiologically related to the source of animals (“in the vicinity”).
  - c. Procedures to survey the antigenic variants of FMDV that are circulating in the vicinity, including neighbouring regions, in order to validate the protective immunity likely to be provided by use of particular FMDV vaccines.
  - d. Vaccination between 4 and 12 weeks prior to slaughter for all cattle destined for presentation at export abattoirs, using vaccines that comply with OIE norms.
  - e. Recommendations on enforcement and accreditation procedures including the role of the Veterinary Authorities in supervising and approving the arrangements.
2. More specific guidance should be developed on mitigation measures required at export abattoirs in regions that are not FMD-free. This guidance should encompass both procedures to be followed and measures by which their implementation can be monitored. It should include:
- a. Procedures and measures to regularise ante mortem and post mortem inspection, including specific guidelines based on best practices in the beef industry.
  - b. Specific guidelines should be developed for the preparation of specified beef commodity items (beef cuts, beef trimmings, ground meat, etc) to provide adequate assurance that FMDV is not present in such commodities.
  - c. Enforcement and accreditation procedures including the role of the Veterinary Authority regarding procedures, measures and guidelines outlined in 2.a. and 2.b.
3. Further research and investigation are recommended to better understand the following points:
- a. The behaviour and survival of FMDV in bovine fat tissues.
  - b. The amounts of residual bone marrow, lymph node and blood clot in DB.
  - c. The effective oral dose of FMDV for pigs.
  - d. The relative contribution of “pre-slaughter” versus “at-abattoir” control measures aimed at reducing the likelihood of FMDV contamination of DB exported from zones that were not OIE free. A more detailed retrospective study from one or more countries where detailed records are available might be developed to analyse the likelihood that DB from infected animals were actually exported.
  - e. The survival in carcasses of a wider range of serotypes and strains, including especially Asia1 and SAT viruses.
  - f. Gaps in availability of suitable vaccine strains for some regions.

Removal of uncertainty over some of these issues, particularly items (c) and (d) above might lead to a downgrading of the FMD risk associated with DB. However, the difficulty of quantifying the levels of specified residual tissues in DB and of establishing a safe threshold for FMDV contamination of DB should not be underestimated.

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**Table 1. Studies of FMDV survival in meat and other tissues<sup>1</sup>**

Number of infected animals slaughtered and FMDV serotype	Stage of infection at slaughter	pH measurements	Tissues examined	Period of storage of tissues	FMDV assay system	Outcome	Conclusions	Study authors and date
Unknown number of infected cattle or method of infection. Also examined FMDV survival in carcasses of pigs and guinea-pigs	Height of pyrexia	Not reported	Long bone marrow, juice from pressed muscle and heart blood. Also examined FMDV survival on carcass surfaces following contamination by fluids containing FMDV.	Trade freezing (10 to 15°F or -12 to -9°C) or chilling (28-30°F or -2 to -1°C)	Inoculation of cattle, pigs or guinea-pigs or feeding to pigs	Marrow and heart blood were infective to cattle after 42 d storage. Juice from pressed muscle was not infective after 11 d storage. 4 pigs fed marrow were not infected. In a separate experiment with bones of infected pigs, marrow plus crushed bone but not marrow alone were infective for pigs orally.	FMDV survives in bone marrow but not muscle. Bone spicules enhance infectivity of marrow for pigs by the oral route.	Stockman et al., 1927 (Second Progress Report of the FMD Research Committee)
10 cattle infected by contact in 3 experiments involving different "strains" of FMDV. Some cattle found to have insufficient levels of viraemia for further study	At expected peak of infectivity based initially on rising temperature, but predictions not always correct. Some cattle had early macroscopic lesions.	Not reported	Tail, kidney, liver, tongue, cheeks, heart, skirt, gall, sweetbread, brain, muscle, tendon, fat, fascia, hide, carcass "drip", carcass "wrappings"	Carcasses dressed, quartered and cooked at -1°C, then stored at -2°C. Conditions made to mimic "as closely as possible those of the very large trade in imported beef"	Mainly by intramuscular inoculation of pigs with a 20 ml tissue emulsion	Various tissues as well as carcass wrapping materials soaked in blood from one of the infected cattle were infective for pigs on one or more occasions after intramuscular inoculation at up to 40 d after slaughter of the cattle. Crushed bones fed to pigs after 40 d storage also transmitted disease to pigs by the oral route.	Variable results obtained with same materials from different cattle. Sometimes muscle and carcass drip was infective for pigs by inoculation even after storage. Sometimes stored bone marrow with crushed bones was infective for pigs orally. No maturation of carcasses above freezing point.	Andrews et al., 1931 (Fourth Progress Report of the FMD Research Committee)

<sup>1</sup> The studies cited were carried out on cattle, except for one, particularly representative of commercial husbandry and slaughter conditions, that involved lambs (Gomes, et al., 1994)

6 cattle infected with serotypes "O" or "A"	2 d after tongue inoculation when fever and unruptured vesicles present	pH $\leq$ 6.2 considered critical to virus inactivation. pH maintained above this in all tissues other than muscle	Beef, defibrinated blood, liver, kidney, rumen pillars, lymph node	From fresh to 2 mths at 4°C and up to 6 mths at -10°C to -20°C	Titration in cattle by tongue inoculation (mainly) or feeding to pigs (liver and lymph node)	Virus only recovered from meat within 24 hrs after slaughter or from quick frozen meat thawed in buffer. Defibrinated blood was virus positive after 6 wks at 4°C. When liver and lymph nodes with low virus titre fed to 30 pigs, a small number became infected	Virus survives in lymph nodes and possibly blood in otherwise "safe" carcasses rendered non-infective by acidity of rigor. Results based on cattle killed at height of infection when clinical signs apparent.	Henderson & Brooksby, 1948
7 cattle FMDV infected with serotype "A"	30-35 hrs after inoculation (?tongue) when showing typical clinical signs of FMD	Peak acidity of muscle attained at 72 hrs after death and greater in deep than superficial musculature. Lymph nodes have pH 6.4-6.9 after 72 hrs at 4°C.	Citrated blood, muscle ( <i>supraspinatus</i> and <i>semitendinosus</i> ), blood clots, bone marrow, lymph nodes,	Salted fresh meat stored at 33 d at 4°C. Deboned quarters ripened at 20°C for 1 hr and then stored at 4°C for 24 hrs. Uncured and salt cured meat stored in barrels for 16-50 d at 1°C. Forequarters stored up to 73 d at 4°C.	Titration in cattle by tongue inoculation	Boned meat contains lymph nodes and large blood vessels. Occasionally, large blood clots are present and also fragments of bone, especially in muscles taken from the vertebrae near the point where the carcass is split.	Meat derived from FMDV infected animals was not rendered free of FMDV by ripening, boning, salting and storage	Cottral et al., 1960

2 donor steers were inoculated with FMDV Vallee A type, strain 119. 10 swine for feeding experiment	20 hours and 9 d post inoculation respectively	Not reported	Lymph nodes, haemal nodes, muscle tissue and bone marrow	Fresh and ripened (72 hours at 4°C and 194 d at 1°C)	Inoculation of cattle and feeding to pigs	Virus was detected in the lymph nodes and haemal nodes of the steers (both 20hrs and 9 d post inoculation), while the animals showed no signs of infection.  Pigs fed marrow supernatant with bone fragments developed FMD within 5-6 d. Pigs fed the same material without the bone fragments did not have signs of infection during 15 d of observation.	Meat from animals in the stages just preceding and shortly after the regression of signs of FMD would be hazardous to export from countries where FMD is present.  Bone fragments increased infectivity to pigs.	Cox et al., 1961
2 cattle infected by intradermal inoculation with type "A" or "C"	31 or 38 hrs after inoculation when showing typical clinical signs of FMD	Details not reported	Blood, kidney, spleen, liver, lung, brain, bone marrow, lymph nodes, heart, stomach, intestine, rumen, tongue, muscle, parotid salivary gland, testicle, uterus	Chilling for 2 or 8 d and freezing for 60, 120 or 210 d	Calf kidney cell culture inoculation	Muscle was initially FMDV positive in one animal but not after storage. Blood, bone marrow, lymph nodes and a variety of other tissues were frequently positive after storage up to 210 d	FMDV survived in blood, bone marrow and lymph nodes but not muscle. Other tissues in which FMDV survived are normally removed from deboned meat.	Savi et al., 1961
12 cattle infected with serotype "O" Method of infection unknown	Unknown. 10 carcasses said to be derived from cattle slaughtered at the time of general development of sickness	The pH averaged 01.-02 higher in tissues of diseased cattle compared to normal animals	Blood, skeletal muscle, cardiac muscle, liver, spleen, kidneys, lymph nodes, medulla, rumen, bone marrow, fat	Held at 10-12°C for 24 hrs, then at 2-7°C. Tissues examined 2, 24, 48, 72 hrs and 9 d after slaughter. Two carcasses stored for 81 d at -20°C and lymph nodes stored for 687 d at -30°C.	Inoculation of guinea-pigs, tissue cultures and in one case, cattle. Guinea-pig inoculation the least sensitive.	Non-muscle tissues not acidified during post mortem change and retained infectivity despite inactivation in muscle (one animal positive at 2 hrs after slaughter only)	The main sites for FMDV survival in carcasses are blood, lymph nodes bone marrow and fat. FMDV could not be detected in muscle tissue after 2 d storage. Lymph nodes became non-infective at 9 d post-slaughter when carcasses chilled, but retained infectivity if carcasses frozen	Wisniewski, 1963



57 cattle infected with serotypes "O", "A" and "C". 14 repeatedly ( $\geq 6x$ ) vaccinated and 5 unvaccinated cattle per serotype.	32 hrs after tongue inoculation (the time of peak viraemia in unvaccinated cattle). No secondary lesions at slaughter.	No Ph differences between vaccinated and unvaccinated cattle at time of slaughter. After cask curing, meat Ph was 5.3-6.7	Fresh and "ripened" lymph nodes were collected. Meat was boned and cut for curing in casks, by salting with sodium chloride, sodium nitrite and sodium nitrate mixture at 4.5 kg per 100 kg meat.	Carcasses hung at 4°C for 72 hours. Curing was at 4°C for approximately one month	Inoculation of cattle, mice and tissue cultures. Feeding to pigs.	By cattle inoculation, fresh, ripened and cured lymph nodes from unvaccinated cattle were FMDV positive; 1/42 vaccinated cattle had a fresh lymph node with detectable FMDV. Cured lymph nodes fed to 30 pigs – 10 per serotype. None of the pigs developed FMD.	Multiple vaccination markedly reduced the chances of FMDV infection of lymph nodes. One month's storage also reduced virus survival in lymph nodes from unvaccinated cattle. The level of immunity developed by the vaccinated cattle in this experiment would be hard to guarantee under field conditions.	NASNRC, Argentine-US Joint Commission on FMD, 1966
54 Cattle were infected by tongue inoculation with either serotypes A, SAT1 or SAT3	Cattle killed 14-196 d post infection.	Not measured	Saliva, oesophageal/ pharyngeal fluid and various post mortem specimens (turbinates and posterior part of the nasal septum, tongue, pharynx, soft palate, oesophagus, trachea and bladder)	All samples were held at room temp and assayed for infectivity within 2-3 hours after collection or of the slaughter of the animal	Plaque assay, mouse inoculation and serum neutralization tests	Virus was recovered from 41 / 54 cattle killed, 14-196 d after infection. The chief sites of virus multiplication based on the frequency of virus recovery and infectivity titres were the dorsal surface of the soft palate and the pharynx.	The mucosae of the pharynx and the soft palate are the main sites of virus multiplication in the bovine carrier animal.	Burrows, 1966
12 cattle (milking cows), 9 sheep and 10 pigs were exposed (in isolation) to cattle infected by inoculation with serotype O strain	Samples taken daily from 1 – 13 d post exposure	Not measured	Samples taken from blood, milk, pharynx, rectum and prepuce or vagina	No storage	Inoculation into tissue cultures	Virus was recovered from pharyngeal samples from the majority of animals for several d before clinical disease was evident. Virus was also recovered from the blood, milk, rectal and preputial or vaginal swabs before clinical lesions were apparent.	Some animals were possible sources of infection for periods up to five d (cattle and sheep) and up to 10 d (pigs) before disease was diagnosed in the animals concerned.	Burrows, 1968

4 susceptible bulls placed in isolation with 4 steers inoculated on the tongue with serotype O, strain	Samples were collected daily	Not measured	Samples were collected from the pharynx, saliva, blood, rectum and prepuce	No storage	Inoculation in tissue culture	In three bulls virus was recovered from one or more sites before the appearance of lesions.	Virus was found in the pharynx in bulls up to 9 d before any clinical signs were noted. Ante- and post-mortem infection will thus not identify these potential sources of virus.	Sellers et al., 1968
15 cattle infected with serotypes "A", "O", "C".	Inoculated by intralingual or intramuscular routes and killed at peak of viraemia	Not reported	Blood, prescapular lymph nodes, internal iliac lymph nodes, vesicular epithelium and tallow collected	Tissue smears applied to packaging materials. After drying, specimens stored at 4°C and 82-88% relative humidity	Inoculation of ground smears into tissue cultures	FMDV survived for at least 5 weeks in all smears. Smears of ground lymphoid tissues harboured 2 log units of virus after 7 weeks.	FMDV survives on meat packaging materials longer than the durability of chilled beef or the time needed to transport animal products between continents	Gailunas et al., 1969
56 cattle were exposed to serotypes A and O using different methods (direct contact, indirect contact, feeding, intranasal spray and lung inoculation).	2 to 6 d post exposure	Not reported	Extensive samples including lymph nodes, serum, soft palate, pharynx, trachea, tonsils, nasal cavities, bronchi, lung, tongue, oesophagus, heart muscle, etc.	Most samples were assayed for virus within 6 hours of collection, but some were held at -70°C for several d	Inoculation into different tissue cultures	45 cattle were sampled after slaughter, 6 were apparently not infected, 23 were infected and examined before the onset of viraemia, 12 were viraemic and 4 were exhibiting early signs of disease. Virus was recovered most regularly and in the greatest amounts from the dorsal surface of the soft palate, the retropharyngeal lymph nodes, the pharynx and the tonsils, and least frequently from the lungs, bronchial lymph nodes and the nasal mucosae.	The distribution and amounts of virus in the tissues of 23 cattle killed before the onset of viraemia indicated that the pharyngeal area was the most likely site of initial infection and virus growth.	Burrows et al., 1981

<p>9 cattle (6 -18 months old). FMD strains O<sub>1</sub> Campos and A<sub>24</sub> Cruzeiro were used.</p> <p>Animals were slaughtered at 24 and 72hs post infections.</p>	<p>Animals were infected by intradermal injection (tongue) and nasal instillation.</p> <p>Virus suspensions of 10<sup>5.67</sup> LD<sub>50</sub> (Campos) and 10<sup>4.6</sup> LD<sub>50</sub> (Cruzeiro) were used.</p>	<p>Electronic measurements of pH in <i>Longissimus dorsi</i> (LD) (infected), <i>Biceps femoris</i> (BF) and <i>Psoas major</i> (PS) (non-infected) muscles.</p> <p>Samples were analyzed at 2, 4, 8, 12, 24 and 30 hr post slaughter.</p>	<p>Blood, lymph nodes and LD muscles.</p>	<p>Samples were kept at refrigeration temperatures and analyzed between 2 and 30 hr post slaughter.</p>	<p>Inoculation into suckling mice and in vitro assay using BHK cells.</p>	<p>45 LD muscles samples from infected animals were assayed for FMDV infectivity. FMDV was not detected at a pH value of 6.0 or below. A pH reading of 6.04 was the lowest value found from FMDV LD infectivity stand-point.</p> <p>The experimental study was also designed to consider influence of electrical stimulation on pH drop of beef carcasses using a set of 20 healthy carcasses.</p>	<p>Confirmed early research findings of Henderson and Brodsky (1948) with regard to FMDV inactivation in infected beef muscles.</p> <p>Electrical stimulation produced a pH drop to a value of 6.0 at 4 hr post slaughter in BF and PS from healthy, non infected beef muscles.</p>	<p>García Vidal et al., 1982</p>
<p>8 heifers (18 -30 months old) were inoculated (intralingual &amp; intramuscular) with FMDV serotype O<sub>1</sub> (aprox 10<sup>5</sup> TCID<sub>50</sub>/ml).</p> <p>The study considered electrical stimulation (ES) effects on pH and FMDV in carcass and offals.</p>	<p>At slaughter (36 – 40 hr after the inoculation) all animals had classical symptoms of FMD.</p> <p>2 heifers were used as control (No ES treatment applied).</p>	<p>pH values measured by direct probe and iodacetate homogenates. Samples considered: different muscles, offals and bone marrow</p>	<p>Tongue, M. masseter externus, M. masseter internus, heart, pillars of diaphragm, lung, liver, kidney, LD muscle, M. semimembranosus (S), M. extensor carpi radialis (ECR), lymph nodes (cervicales superficiales), bone marrow (humerus).</p>	<p>Samples were collected at 1, 2, 3, 4, 6, 24 and 48 hr post mortem (pm) under refrigeration (2°C).</p> <p>Temperatures were measured by a digital probe thermometer in the LD, S and ECR muscles and in the offals.</p>	<p>Samples collected at 3, 24 and 48 hr were inoculated into baby mice. Virus isolation and titration were performed in pig kidney cells.</p>	<p>FMDV recovered from masseter muscles, lymph nodes and ECR muscle. No virus was demonstrated in the heart. High concentrations of FMDV in the blood at slaughter. Virus was demonstrated in a few samples of skeletal muscle at 4 hr pm. pH values of heart were remarkably low in both infected and control animals ES had no effect on pH of lymph node, bone marrow and offals.</p>	<p>Underlines risk associated with meat containing lymphatic tissues. Recommended pH measurement should take place in each carcass before deboning. Proposed LD muscle as representative of skeletal muscle. A pH value of 5.8 or below in the LD is acceptable as indicating non-survival of FMDV. pH determinations should be made after 24 hr storage.</p>	<p>CEC, 1986.</p>

<p>9 cattle raised in a free FMD free area, unvaccinated, free of specific antibodies. FMDV strains O<sub>1</sub> (Campos), A<sub>79</sub> (Argentina), and C<sub>3</sub> (Resende) originally isolated in field outbreaks in Argentina.</p>	<p>3 groups of 3 animals were inoculated with 20,000 LD<sub>50</sub> of each strain intradermally in the tongue. Animals were killed at 72 hr post inoculation, when viraemia is normally present.</p>	<p>Mean pH of 1,296 samples were measured in triplicate from samples collected from carcasses stored at 1°C for 2 and 7 d.</p>	<p>Tissues analyzed were lymph nodes, blood clots, bone marrow (ribs) and muscles (<i>Longissimus dorsi</i>, <i>Semitendinosus</i>, <i>Biceps brachii</i>, masseter).</p>	<p>Maturation/ Ageing: One-half of a carcass from each animal was stored at 1°C for 2 d, while the other half was stored for 7 d at the same temperature.</p>	<p>Intramuscular inoculation into suckling mice. In vitro assay using primary cultures of bovine fetal thyroid cells. Final assessment was made by intradermal inoculation of cattle</p>	<p>Clear cut differences were observed between muscle (pH below 6) and lymph node (LN), blood clots (BC) and bone marrow (BM) values (mean pH above 6). No significant changes of pH were observed after maturation (2-7d). LN (2d):6.3; (7d):6.4 BC (2d):6.5; (7d):6.6 BM (2d):6.8; (7d):7.0</p>	<p>The virus was most frequently detected in bone marrow samples and less frequently but with comparable numbers of positive samples, in lymph nodes tissue and blood clots.  It does not seem necessary to prolong maturation beyond 2 d, as suggested by the infectivity detected in LN, BM and BC.  Highest titre virus survival was in BM.</p>	<p>Lasta et al., 1992.</p>
<p>25 lambs (~ 3 mth old). Virus O<sub>1</sub> Campos. Animals were intradermally inoculated in the tongue (10<sup>5.6</sup> ID<sub>50</sub>). 100 healthy (non-infected) lambs were used as controls for pH measurements.</p>	<p>24 hr post infection temperature, clinical examination and blood samples were taken. Slaughter was performed at 48, 72, 96, 120hrs and 15 and 30 d post infection (PI).</p>	<p>In LD muscle. Temperature was recorded on <i>Longissimus Dorsi</i> (LD) and <i>Semimembranosus</i> (SM) muscles.</p>	<p>LD, SM. Lymph nodes (from muscle tissue areas and viscera), tonsils, heart, oesophagus, lungs, liver, spleen, kidney.</p>	<p>Samples were aged and after finishing maturation were frozen. Carcass ageing was done at 4°C for a 24 hr period. LD and SM were kept frozen for 4 mths at -20°C.</p>	<p>Titration of muscle, organs and lymph glands were performed in duplicate.</p>	<p>In animals slaughtered in febrile state at 48, 72, and 96 hrs post infection (HPI) the virus was detected before and after maturation in the LD and SM muscles, that did not reach a pH of &lt;6.0 during ageing. No virus was found before or after ageing of carcasses in those animals slaughtered at 120 hr PI, 15 or 30 d PI. Kidney had the highest virus concentration. Lymph glands and tonsils also had high virus concentrations.</p>	<p>Virus detected in LD and SM muscles after maturation as well as after frozen storage (4 mths). Virus detected in glands and organs at 48, 72, 96 and 120 hr PI. Virus not detected in organs or glands of animals slaughtered at 15 or 30 d PI. In normal, healthy, non-infected sheep, pH of carcasses reached values of 5.96 after 6 hr and 5.36 after 24 hr of ageing at refrigeration temp.</p>	<p>Gomes, et al., 1994</p>

**Table 2. Reviews which include consideration of of FMD virus survival in meat**

Authors	Year of Publication	Title	Summary
Cottral, Cox & Baldwin	1961	The survival of FMDV in cured and uncured meat	Introduction to their own work provides a valuable review of earlier literature.
Cottral	1969	Persistence of FMDV in animals, their products and the environment	Tabulates the extremes for the earliest detection of FMDV and its longest reported persistence in living animals as well as virus survival in animal tissues and fluids and on various objects.
Roberts	1970	FMD, its relation to meat and meat processing	Literature review on FMDV in animal products with focus on treatments for virus inactivation. Identified problem of no established threshold of FMDV contamination below which a product could be considered safe.
Sellers	1971	Quantitative aspects of the spread of FMDV	Collated data on FMDV production levels, survival and required doses for infection. Considered that figures for level of FMDV contamination in air or feed must be combined with amount actually breathed in or eaten to establish minimum dose for infection.
Callis & McKercher	1978	Dissemination of FMDV through animal products	Concluded that main risk from deboned beef is residual lymph nodes, blood and bone fragments and that vaccination can reduce risk.
Blackwell	1979	Internationalism and Survival of Foot-and-Mouth Disease Virus in Cattle and Food products	In depth historical and scientifically based descriptions of FMD outbreaks in USA, Canada and Mexico, related to animal products trade including food items from South American countries.
Garcia-Vidal, Lazaneo, Correa, Urrestarazu, Huertas & Heidelbaugh	1983	Review of recent progress of the meat Institute of Uruguay on the development of industrial methods to inactivate Foot-and-Mouth disease virus in meat and meat products.	This review paper showed that virus was not detected in muscle at pH 6.0 or below. The minimum pH value in which the virus was present was pH 6.4.
Blackwell	1984	Foreign animal disease agent survival in animal products: recent developments	General review of factors contributing to survival of pathogens in different products and of effects of different commodity processing treatments.
Donaldson	1987	Foot-and-Mouth Disease: the principal features.	Describes the FMD virus, distribution, mechanisms of spread, routes of infection and pathogenesis. Gives information on the incubation period, organs that have been shown to contain

			high quantities of virus during acute disease and post-mortem pH changes.
USDA	1991	FMD emergency disease guidelines	Tabulated, referenced data on FMDV survival in different materials and from different species.
US General Accounting Office	2002	Foot-and-Mouth Disease. To protect U.S. livestock, USDA must remain vigilant and resolve outstanding issues.	Describes importance of the livestock industry to the US agricultural sector and economy. Relevance of protecting US livestock from FMD and measures for preventing FMD from entering the US are evaluated. Summarized survival time of the FMD virus in selected products and by-products.
Alexandersen, Zhang, Donaldson & Garland	2003	The pathogenesis and survival of FMD	Summary data on infective doses by various routes and on kinetics of virus replication, load and clearance.
Scott Williams	2003	Persistence of Disease Agents in Carcasses and Animal Products	Summary of persistence and inactivation of FMD virus associated with different agents and environments. Described behaviour and persistence in different matrices, elements and foods (carcass and meat products, skin, hides and fibres, semen/embryos, faeces).
Ryan, Mackay, & Donaldson	2008	Foot-and-mouth-disease virus concentrations in products of animal origin	Review collected data for the concentration of FMDV in animal tissues during the viraemic stage of the disease and in animal products derived from infected animals.  The inactivation-resistant fraction of FMDV must be taken into account when estimating the efficiency of thermal or pH-dependant reduction of virus load. The significance of this is related to the initial virus load, the nature of the product and the treatment it undergoes. If the critical control points (deboning, removing lymph nodes and blood) are achieved, the risk to an exposed animal of becoming infected from beef chilled for 72 hr post-mortem is negligible (drop in pH should be monitored).

**Table 3. Commentaries and risk assessments for FMD safety of meat**

Authors	Year of Publication	Title	Summary
Van Bekkum, Frenkel, Frederiks & Frenkel	1959	Observations on the carrier state of cattle exposed to foot-and-mouth disease virus.	A rather large proportion of cattle, which have recovered from FMD, may still harbour the virus in the saliva for several months. The virus may be demonstrated in material collected from the oesophagus by inoculation into unweaned mice or susceptible cattle. After contact with clinical cases vaccinated animals may develop a similar carrier state without having shown symptoms of the disease. In these experiments susceptible oxen kept in contact with such carriers remained unaffected, even if the oral cavity was swabbed with infective saliva. No cases of FMD occurred in vaccinated or unvaccinated cattle or in unvaccinated pigs, if such animals were introduced into a herd known to contain carriers or if they were kept on the same premises with such animals.
Brooksby	1961	International trade in meat and the dissemination of FMD	Factors to be considered in assessing the risks in relation to FMDV infected meat. Concluded that an absolute prohibition should be placed on importation of meat from areas with exotic strains of FMDV.
Sutmoller, McVicar & Cottral	1968	The epizootiological importance of foot-and-mouth disease carriers.	From these experiments it was concluded that nearly all infected cattle become carriers and the carrier state in cattle is probably a normal sequel to infection. Susceptible cattle always had viraemia, usually accompanied by fever, while cattle that had received antiserum prior to virus inoculation did not. Viraemia was prevented even in cattle with a very low level of passively acquired antibody. Circulating antibodies, whether acquired passively or actively, do not prevent the establishment of FMDV infection in the pharyngeal area in cattle, but it will prevent detectable viraemia.
McVicar & Sutmoller	1976	Growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated and recovered cattle after intranasal inoculation.	Non-immunized, vaccinated and recovered cattle were inoculated intranasally with various doses of FMD virus. Samples of oesophageal pharyngeal fluid were taken periodically for up to 7 d after inoculation and virus titres of these samples were plotted as pharyngeal virus growth curves. The extremely mild clinical syndrome exhibited by some of the vaccinated cattle after virus inoculation could easily have been missed under field conditions. Virus titres in OP fluid samples taken 2-4 d after inoculation from the four vaccinated steers with a low pre-exposure serum titre were as high as those seen in the non-immunized cattle. The high virus titres seen in vaccinated cattle in the absence of obvious clinical signs suggest that partly immunized cattle, after exposure to virus, may become inapparent virus shedders and therefore dangerous sources of infection.
Blajan & Callis	1991	International trade and FMD	Data on trade in animals and their products show that large amounts of exports from infected countries have taken place without causing outbreaks in the countries of destination.
MacDiarmid	1991	The importation into New Zealand of meat and meat products:	The review examined the potential risks posed by each type of meat & meat-based product. Regarding boneless beef and FMD, the paper stressed the relevance of ensuring that only animals free from FMD should be slaughtered, originating from regions free of FMD as well as the importance

		A review of the risks to animal health	of checking on the pH of boneless beef. Comments (quoted) "There is no evidence that boneless beef has ever been the origin of a FMD outbreak. Thirty four primary outbreaks occurred in the EC during the period 1977 to 1987. Eight of these originated from outside the Community and were probably due to imports of meat which had not been deboned. Thirteen of the outbreaks were most probably due to faulty FMD vaccines or laboratory escapes and 13 remain of unknown origin".
Doel, Williams & Barnett	1994	Emergency vaccination against foot-and-mouth disease: Rate of development of immunity and its implications for the carrier state	The study was undertaken as part of a larger programme to determine the rate with which protective immunity could be expected to develop in animals given emergency vaccines and the extent to which these animals would shed virus and spread the disease by direct or indirect contact. Two experiments demonstrated that oil- or AL(OH)3/saponin-adjuvanted vaccines made from inactivated virus antigens held in the International Vaccine Bank were capable of protecting cattle 4 or more d after vaccination. A large number of cattle (at least 11/28) given O1 Lausanne vaccine became persistently infected when challenged. Animals challenged only a few d after vaccination appeared more likely to become carriers on the basis of ease of virus recovery and possibly would pose a greater risk to healthy contact animals than those from which it was more difficult to isolated virus by probang.
Pan American Foot-and-Mouth Disease Center & Tuskegee University School of Veterinary Medicine	1995	Assessment of the risk of foot-and-mouth disease introduction into the CARICOM countries through the importation of meat from Argentina and Uruguay	Cooperative effort between the Pan American FMD Centre and the School of Veterinary Medicine, Tuskegee University, US. This study examines the risk of beef importation by CARICOM (Caribbean) countries. Quantitative Risk Assessment (QtRA) model was based on rules and procedures for exporting deboned beef to the European Community (EC). The study states that this protocol has been very effective, since deboned beef coming from millions of beef cattle has been imported by EC countries, even during times of extensive FMD outbreaks in South America countries. UK imported in this period more than one million tons of deboned beef and still remained free of FMD. This QtRA study concluded that the risk of introducing FMD for CARICOM countries by exporting deboned beef from Mesopotamia region of Argentina and from Uruguay was exceedingly small.
Callis	1996	Evaluation of the presence and risk of FMDV by commodity in international trade	Summarises policy changes on importation of meat and meat products into UK and Europe after 1968 and notes that no outbreaks were associated with this trade thereafter.



Metcalf, Blackwell & Acree	1996	Application of Risk Assessment to International Trade in Animals and Animal Products	Disease risk factors associated with the trade in animals and animal products can be grouped in three categories: source risk factors, commodity risk factors and destination risk factors. Each of these broad categories can be treated separately. Commodity risk factors are often made more complex than necessary by the tendency to mix source and commodity factors together in evaluating the risk of the commodity. To determine the commodity risk factor it is necessary to begin with the premise that the commodity is infected with the disease agent of concern and examine each step of the processing, handling and storing of the commodity in order to determine how much the infection is reduced by each process.
Yu, Habtermariam, Wilson, Oryang, Nganwa, Obasa, Robnett	1997	A risk assessment model for FMDV introduction through deboned beef introduction	A basic quantitative risk assessment model is used to determine the risk of FMD introduction through beef based on the prevalence of FMD-infected cattle in herds as well as the prevalence of infected herds in the exporting country. Mitigations taken into account were farm-level inspection, ante-mortem inspection, post-mortem inspection, chilling and deboning. The model showed that the early stage (lower prevalence) of an FMD outbreak may impose a high risk of FMD virus introduction. However, this risk decreases again at higher prevalence due to higher likelihood of detection during ante- and post-mortem inspections. Small sample sizes during inspections increased risk considerably.
Vose	1997	Risk analysis in relation to the importation and exportation of animal products	Review of modelling techniques applicable to quantitative risk analysis for trade in meat. Discusses need to model variables that are not accurately quantified and problem of accuracy in dealing very low risk. Comparison of scenario pathways and simulation for quantitative risk analysis in relation to dangers associated with animal products.
Sutmoller & Vose	1997	Contamination of animal products: the minimum pathogen dose required to initiate infection	Highlights residual risk when products are contaminated with less than the minimum infective dose due to non-zero risk from any infectious dose and impact of multiple exposures. Problem of estimating lower threshold of minimum infecting dose in absence of data from experimental challenges with very large numbers of animals. A modelling approach suggested.
Astudillo, Sutmoller, Saraiva & Lopez	1997a	Risks of introducing FMD through the importation of beef from South America	Describes post 1968 mitigation measures for export of meat to Europe from S America. Quotes estimate that > 1 million tons of deboned frozen meat safely imported to UK (SENASA, 1994). Provides estimates of the likelihood of FMDV survival at each risk mitigation stage (probability values for each event in scenario pathway) and concludes a 1 in 1 million chance of getting FMDV in meat, assuming that the mitigation measures are adhered to. Considers that no data is available on the kinetics of FMD virus inactivation in meat at a pH of 6.0. Combination of low regional risk of FMDV infection with efficient risk mitigation ensures safety of products.
Astudillo, Cané, Geymonat, Sathler,	1997b	Risk assessment and risk regionalisation, based on the	Describes two examples of risk assessments for international trade, i.e. bovine embryos and beef as a way of proposing regional risk evaluation of FMD in South America. Utilize the model developed by

Garay Roman, Sutmoller, Zottele, & Gimeno		surveillance system for foot and mouth disease in South America.	PANAFTOSA and Tuskegee Univ. Sch. Vet. Med. to analyze meat trade. Authors stress that in respect to trade in beef from infected FMD countries, it is not only the deboning and maturation processes that is relevant but also the overall safety provided from an efficient animal health surveillance and information system as well as efficient procedures in selecting herds and abattoirs.
Kitching	1998	A recent history of Foot-and-Mouth disease.	A review article on the FMD outbreaks internationally from 1991 – 1997. States that there is a reluctance to use vaccination as control measure since ruminants will continue to carry live FMDV in their pharynx after contact, regardless of the development of clinical or sub-clinical disease. However, experiments to demonstrate transmission of FMD virus from carriers to susceptible in-contact animals have been unsuccessful.
Sutmoller	2001	Importation of beef from countries infected with foot-and-mouth disease: a review of risk mitigation measures	Outlines OIE Code requirements and a risk pathway to analyse risk associated with beef trade. Considers four disease stages of FMD and the hazard and mitigation for each. Concludes that not all virus is eliminated from infected animals by deboning and maturation and that animals incubating FMD without clinical signs pose the main risk. Highlights dangers of cross-contamination between carcasses and from oropharynx. Emphasises importance of antibodies in neutralising FMDV in meat and other tissues.
Barteling & Sutmoller	2002	Culling versus vaccination: challenging a dogma in veterinary (FMD) science.	Discuss the pros and cons of culling or vaccination as control methods for FMD. Maintains that where FMD outbreaks were controlled by consistent vaccination with a qualified vaccine the disease did not re-occur and there are no documented cases where cattle vaccinated with a qualified vaccine caused new outbreaks. Therefore concludes that the risk posed by vaccinated carriers is an acceptable, “close to zero” risk.
Pharo	2002	FMD: an assessment of the risks facing New Zealand	Broad review of the pathogenesis and transmission of FMD and hazards posed for international trade. Cites Bachrach et al. (1975) on rate of inactivation of FMDV by acidic conditions: 90% per min at pH 6 and 90% per sec at pH 5. Considered that oral infection of pigs is the most likely outbreak scenario.
USDA	2002	Risk assessment –Importation of fresh (chilled or frozen) beef from Uruguay. Animal Plant Health Inspection Service, APHIS. United States Department of Agriculture, USDA.	A quantitative risk assessment (RA) to evaluate the likelihood of FMD introduction through importation of beef from Uruguay. Mitigations considered in the assessment included: a) Commodity imported is deboned prime beef cuts from carcasses that are matured for 36 hr at a temperature between 2 to 10°C. b) Beef originated from animals in herds certified to have been vaccinated with oil-adjuvant vaccine. c) All animals pass both ante- and post-mortem inspections. d) All carcasses are pH tested in the LD muscle and the pH must be less or equal to 5.8. The RA found that the likelihood of importing fresh or frozen, matured, and deboned beef infected with FMD virus would not exceed $1.03 \times 10^{-4}$ or 1 in 9,700 chances (95% confidence level).

Have	2003	An assessment of guidelines for treatment of meat from a FMD vaccination zone.	A report of the Research Group of the Standing Technical Committee of the EC for the control of FMD. Concludes that the current requirements for the heat treatment of meat from FMDV vaccinated animals, although based on empirical data, can be considered to provide a high degree of safety when applied to low-level contaminated products such as meat from vaccinated animals.
Sutmoller, Barteling, Casas Olascoaga & Sumption	2003	Control and eradication of foot-and-mouth disease.	In this review article the authors address the concern regarding mechanical contamination of a carcass with "carrier virus" from the pharyngeal area. They concluded that because of antibodies in blood and other fluids and measures applied during slaughter and processing (e.g. for BSE) the risk is negligible. They also stated that in countries where FMD was controlled by the use of systematic vaccination of the cattle population, transmission of disease from carrier cattle to non-vaccinated or other susceptible species has not been observed. Also, in situations in which, after a period of "freedom of FMD", vaccination was discontinued there has been no case of FMD linked to the existence of carriers. Only circumstantial historical evidence exists to implicate carrier animals as the source of an outbreak, however there are numerous cases in which large numbers of convalescent cattle introduced into non-vaccinated herds did not cause new outbreaks.
Sutmoller & Casas Olascoaga	2003	The risks posed by the importation of animals vaccinated against foot-and-mouth disease and products derived from vaccinated animals: a review	Repeats many of the arguments from previous paper in 2001. Advocates double vaccination of cattle prior to slaughter and use of serology at abattoirs to check antibody status. Highlights danger of non-industrial processing of small ruminants leading to risk of carcass contamination from pharynx.
Thomson et al.	2004	International trade in livestock and livestock products: the need for a commodity-based approach	Proposed an alternative commodity based approach for international animal health and food safety standards based on the fact that different commodities pose different risks when it comes to the dissemination of human and animal pathogens. They concluded that this approach would improve access to international markets for all countries, especially for those LDCs.
Orsel, Dekker, Bouma, Stegeman & de Jong	2005	Vaccination against foot and mouth disease reduces virus transmission in groups of calves.	The study investigated whether single vaccination against FMDV could significantly reduce virus transmission in groups of calves compared to transmission in groups of non-vaccinated calves. The findings suggested that single vaccination in a population of calves could reduce transmission and that this might be sufficient to eradicate the virus during an epidemic of FMD.
European Food Standards Agency (EFSA)	2006	Assessing the risk of FMD introduction into the EU from developing countries	Considered that illegal imports are a greater risk than those from countries with an established and regulated trade with Europe. Recommended additional research on virus survival in tissues and animal products, specifically: (1) the effects of pre-slaughter stress upon pH drop; (2) virus strain variability in survival; (3) the effect of vaccination on amount and distribution of FMDV in animal products. Some data on meat imports are given, but insufficiently stratified for use in this review.

Hartnett and 9 others	2007	A quantitative assessment of the risks from illegally imported meat contaminated with FMDV	Even where swill feeding is banned there is a residual risk of pigs and also wild boar gaining access to imported meat products. An estimate of the future frequency of FMD infection in GB livestock was made of 0.015 cases of infected animals per year (between 0.0017 and 0.053 with 90% certainty). Imports from the region Near and Middle East account for 47% of this risk and 68% of the risk is attributed to bone-in and dried de-boned products.
Thomson, Leyland & Donaldson	2009	De-boned beef – an example of a commodity for which specific standards could be developed to ensure an appropriate level of protection for International trade	Proposals on additional risk mitigation procedures to eliminate/reduce the increase in risk that results from slaughtering animals in the incubation stage. Vaccination and a 3 week pre-slaughter quarantine period are suggested, combined with a compartmentalisation approach to biosecurity.
Sutmoller & Barteling	2004	Discussion paper on the risks posed by FMD carriers occurring amongst vaccinated cattle.	Discussed the risk posed by vaccinated carriers and reviewed historical evidence to that effect. Stated that under a variety of experimental conditions, transmission of FMD from recovered as well as vaccinated carriers has not been demonstrated.

**Table 4. Experiments describing oral infection of pigs with FMDV**

Authors	Serotype	Inoculum	Volume & route	Dose	Dose calculation	No. exposed	No. infected	Comments
Sellers, 1971 – citing Stockman et al 1927	unknown	unknown	unknown	$10^{5.4}$ (according to Sellers 1971, but not found in original report)	$\text{Log}_{10}$ infectious units or $\text{ID}_{50}$ according to Sellers 1971	7	5	Original report describes several experiments where pigs fed with carcass tissues did or did not become infected, but there is no information on the dose of virus
Sellers, 1971 – citing Andrews et al., 1931	O 39	unknown	unknown	$10^{5.2}$ (according to Sellers 1971, but not found in original report)	$\text{Log}_{10}$ infectious units or $\text{ID}_{50}$ according to Sellers 1971	5	1	Original report describes several experiments where pigs fed with carcass tissues did or did not become infected, but there is no information on the dose of virus
Sellers – citing Henderson & Brooksby, 1948	O ASJ	bovine lingual epithelium (6 pigs) & liver, kidney and lymph nodes (30 pigs)	Unknown volume smeared on feeding troughs	$10^{5.0}$ (30 pigs) & $10^{6.5}$ (6 pigs) (according to Sellers 1971, but not in original report)	$\text{Log}_{10}$ infectious units or $\text{ID}_{50}$ according to Sellers 1971	30 6	2 1	
Cox et al., 1961	A 119	Bone marrow with (n=5) or without (n=5) bone fragments	75 ml as feed	$7.5 \times 10^{5.5} \text{ID}_{50}$	Cattle tongue titration	10	5	Only those fed bone marrow including bone fragments became infected (similar to findings of Stockman et al., 1927 and Andrews et al., 1931)
Nathans*, 1965	Pig adapted C strain	unknown	Oral instillation	5 pigs given $10^{2.4} - 10^{4.6}$ 7 pigs given $10^{5.4} - 10^{6.6}$	Suckling mouse $\text{LD}_{50}$	12	2/5 and 7/7	Further details also tabulated in Sellers, 1971
Terpstra, 1972	O <sub>1</sub> Weerselo	Vesicle suspension in medium	2 ml by oral instillation	$10^{4.6} - 10^{7.5}$	Suckling mouse $\text{LD}_{50}$ (add ~1log to convert to bovine thyroid cell culture $\text{ID}_{50}$ )	4 received $10^{4.6} - 10^{5.4}$ and 7 received $10^{6.0} - 10^{7.5}$	0/4 receiving low dose and 7/7 receiving high dose	

\* Original article not seen, used citation of Terpstra, 1972

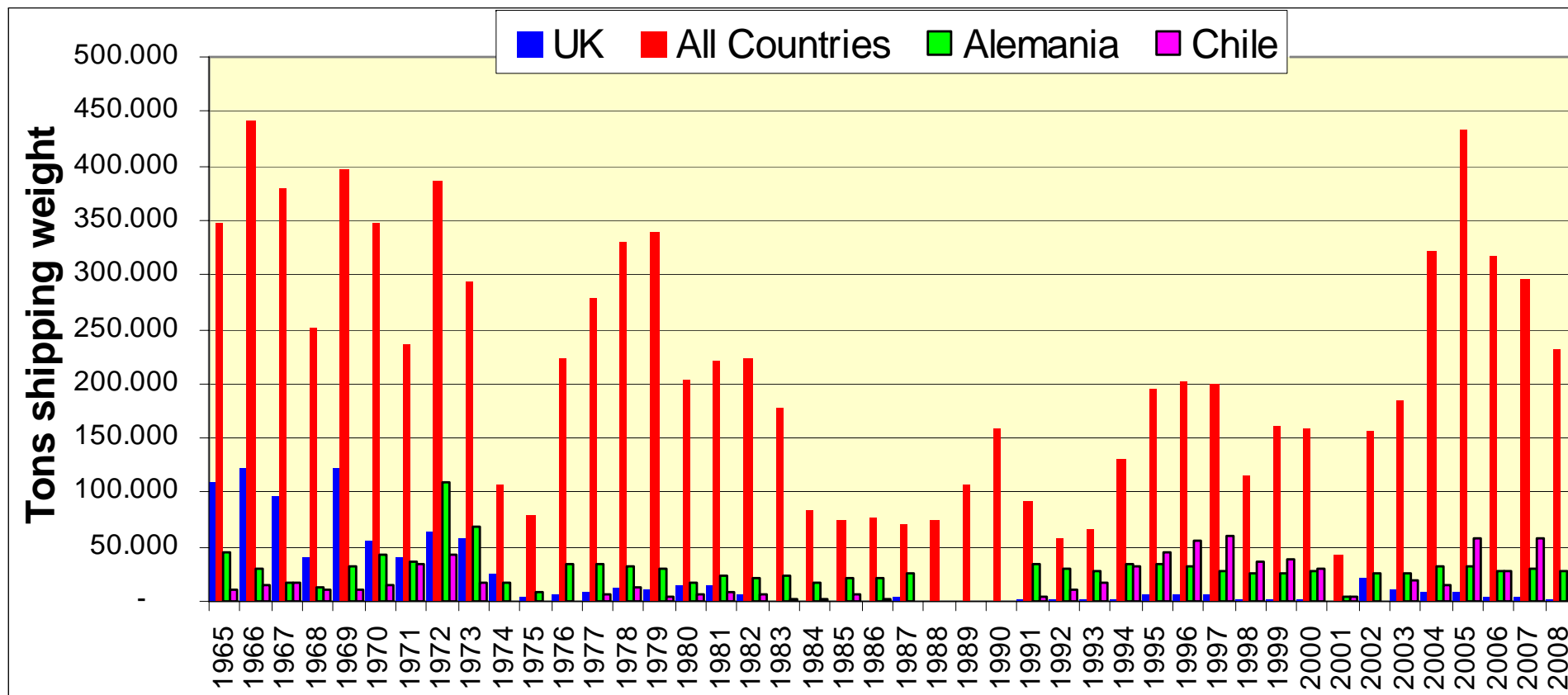


Figure 1. Deboned beef exportation from Argentina to All Countries. Years 1965 -2008. Figures are based on data found at the former Argentine National Meat Board archive and the current Argentine National Directorate of Agrifood Market, SAGPyA, statistic series. (C. Otaño, Personal Communication)

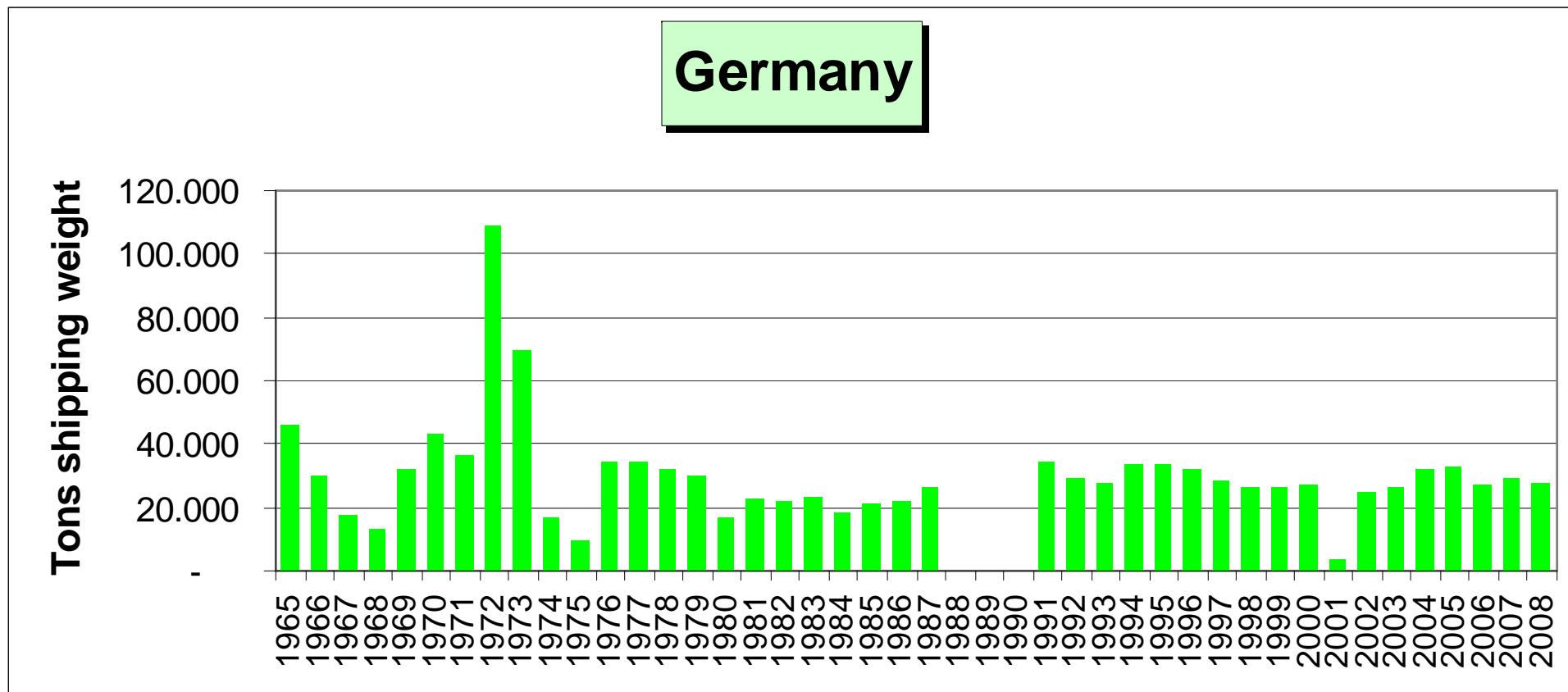


Figure 2. Deboned beef exportation from Argentina to Germany. Years 1965 -2008. Figures are based on data found at the former Argentine National Meat Board archive and the current Argentine National Directorate of Agrifood Market, SAGPyA, statistic series. (C. Otaño, Personal Communication)

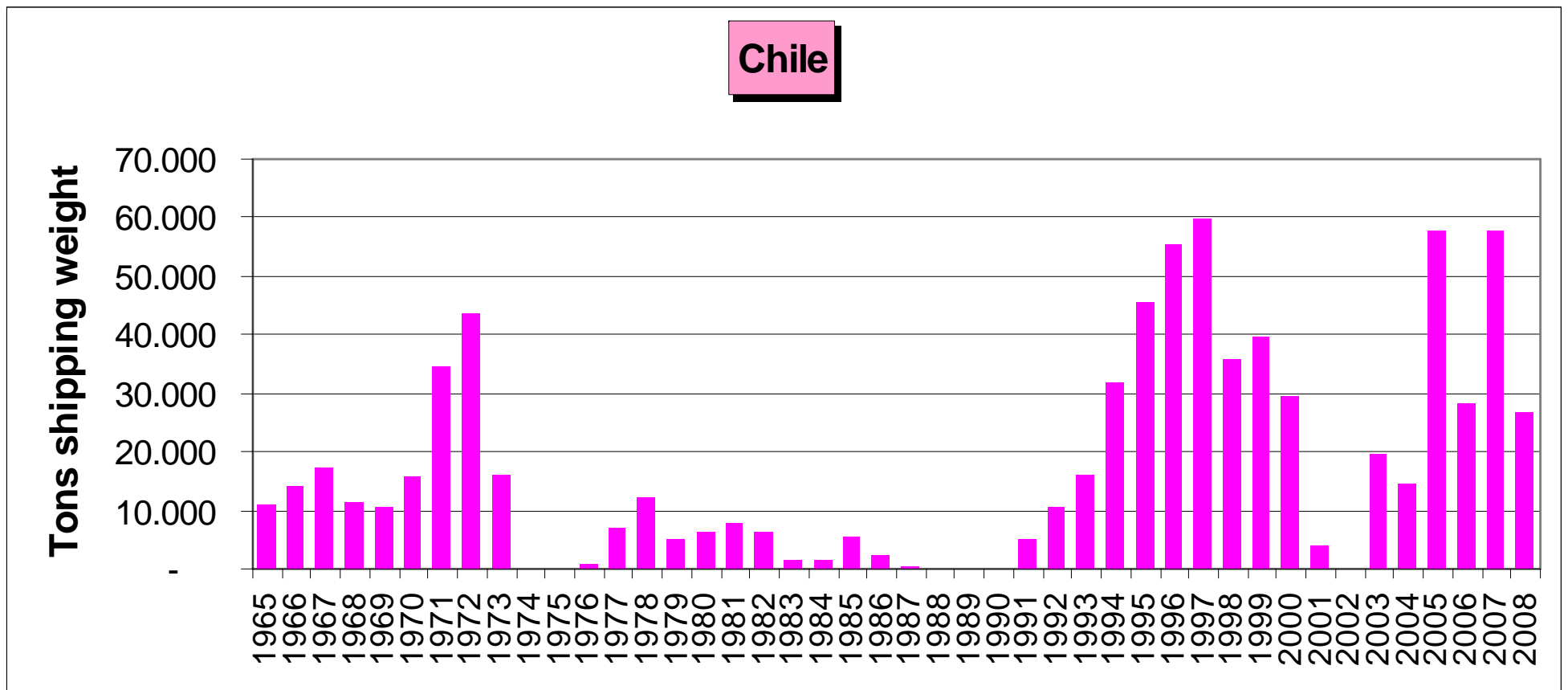


Figure 3. Deboned beef exportation from Argentina to Chile. Years 1965 -2008. Figures are based on data found at the former Argentine National Meat Board archive and the current Argentine National Directorate of Agrifood Market, SAGPyA, statistic series. (C. Otaño, Personal Communication)



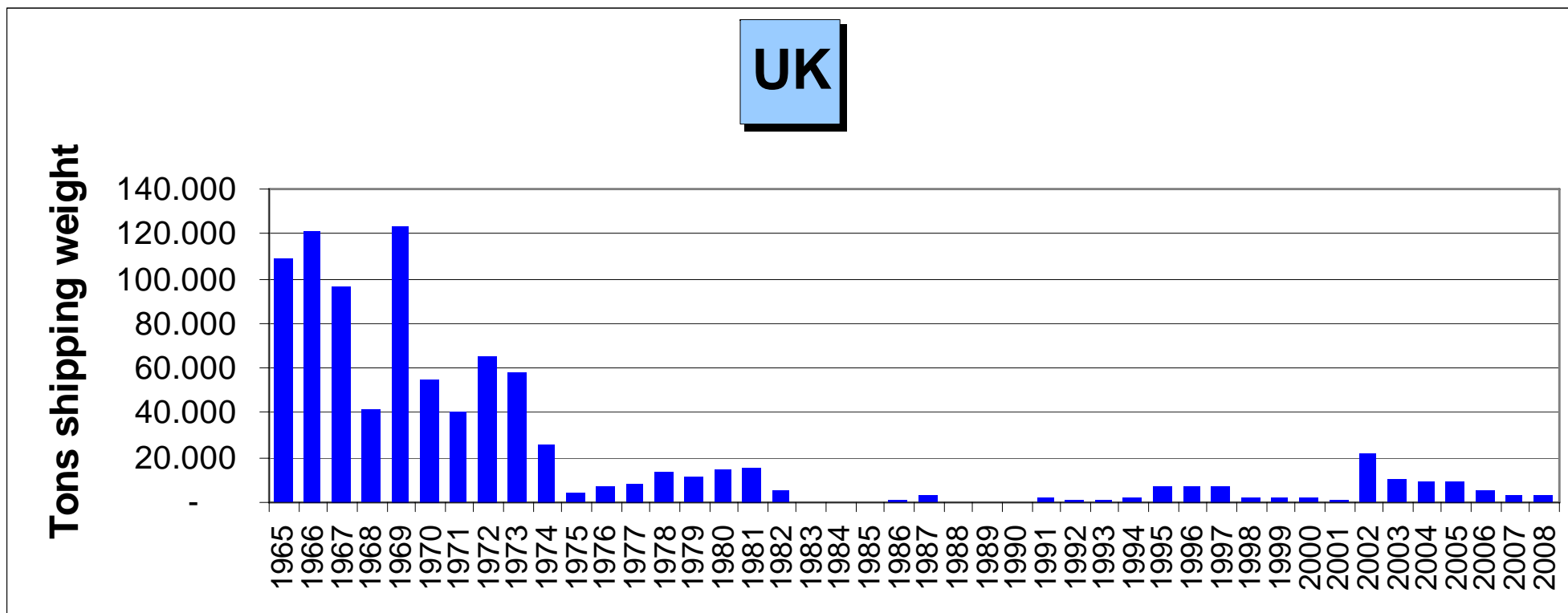


Figure 4. Deboned beef exportation from Argentina to the UK. Years 1965 -2008. Figures are based on data found at the former Argentine National Meat Board archive and the current Argentine National Directorate of Agrifood Market, SAGPyA, statistic series. (C. Otaño, Personal Communication)

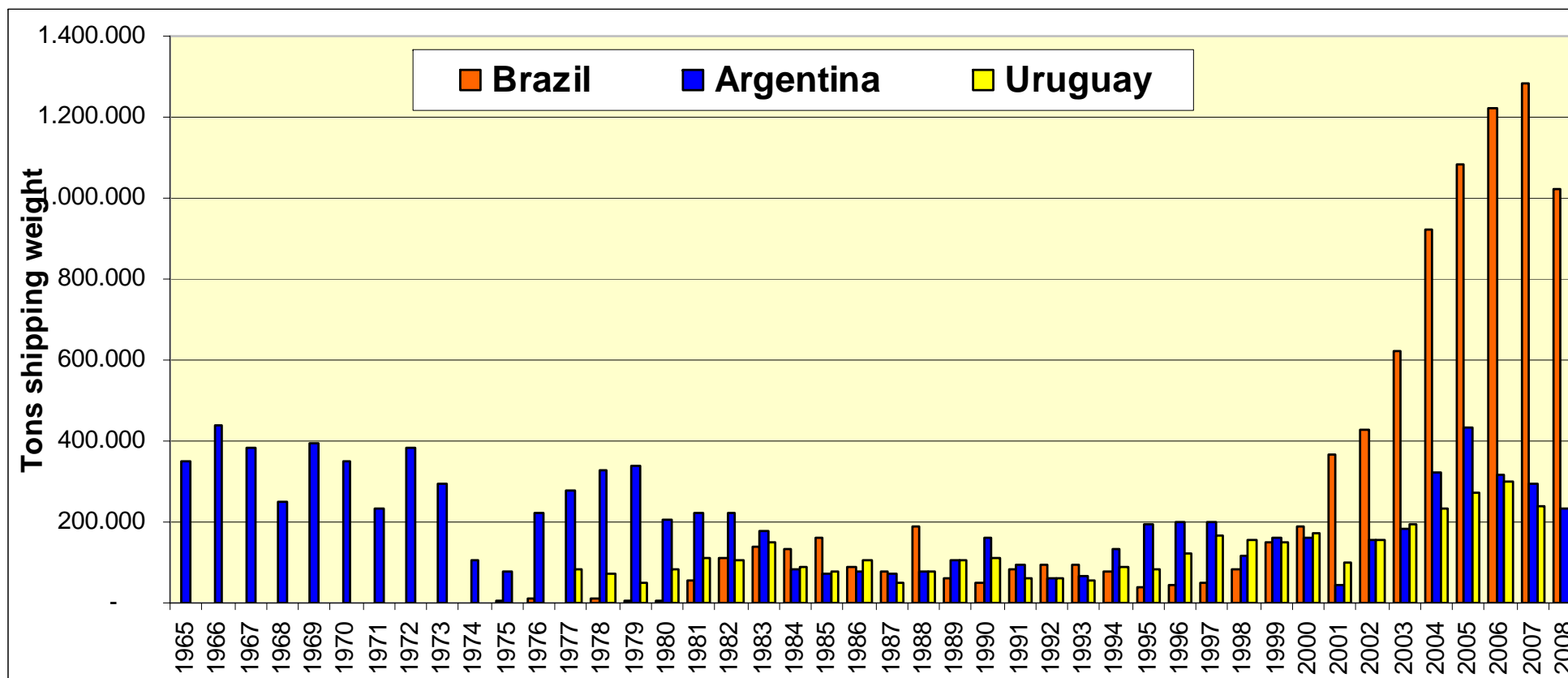


Figure 5. Deboned beef exportation from Argentina, Brazil and Uruguay. Years 1965 -2008. Figures are based on data provided by the Argentine National Directorate of Agrifood Market, SAGPyA. (C. Otaño, Personal Communication)

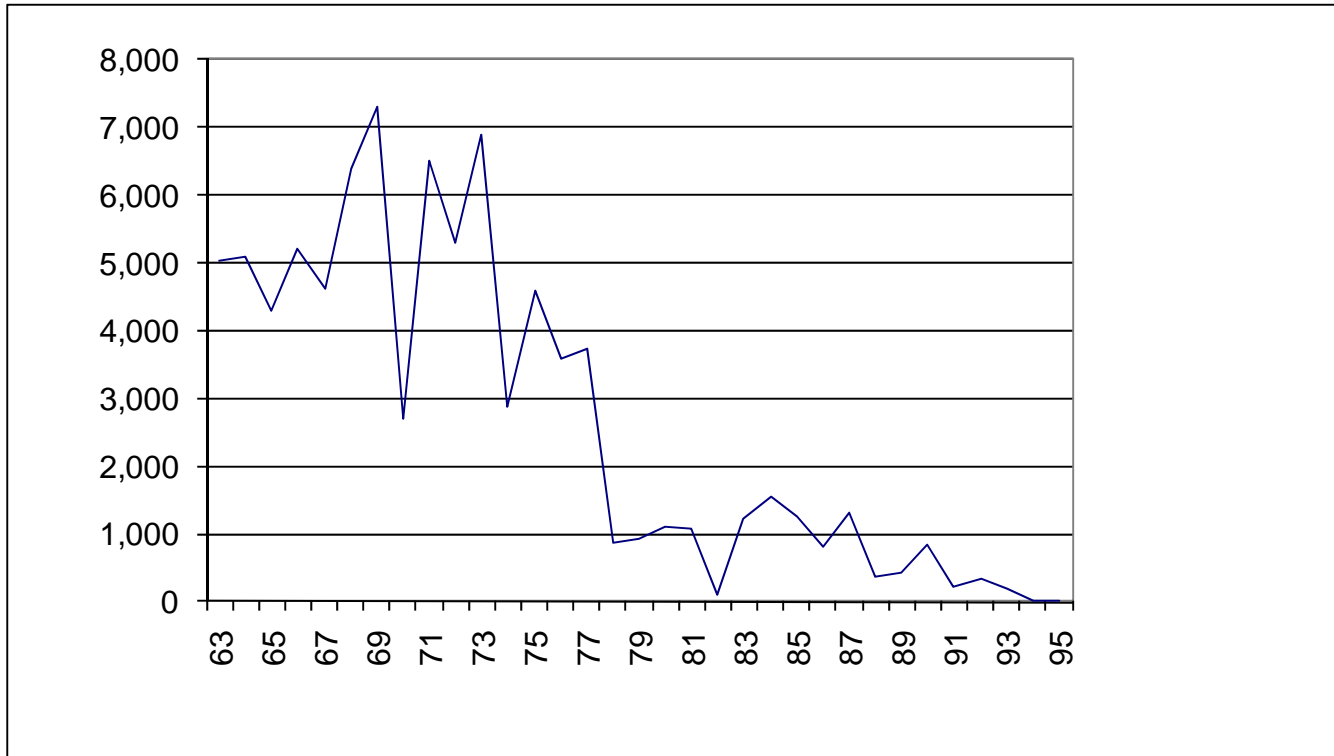


Figure 6. Argentina, FMD Reported Outbreaks. Between years 1963 and 1995  
 Figures are based on data provided by the Argentine National Directorate of Animal Health, SENASA.  
 (Dillon, J., Personal Communication)

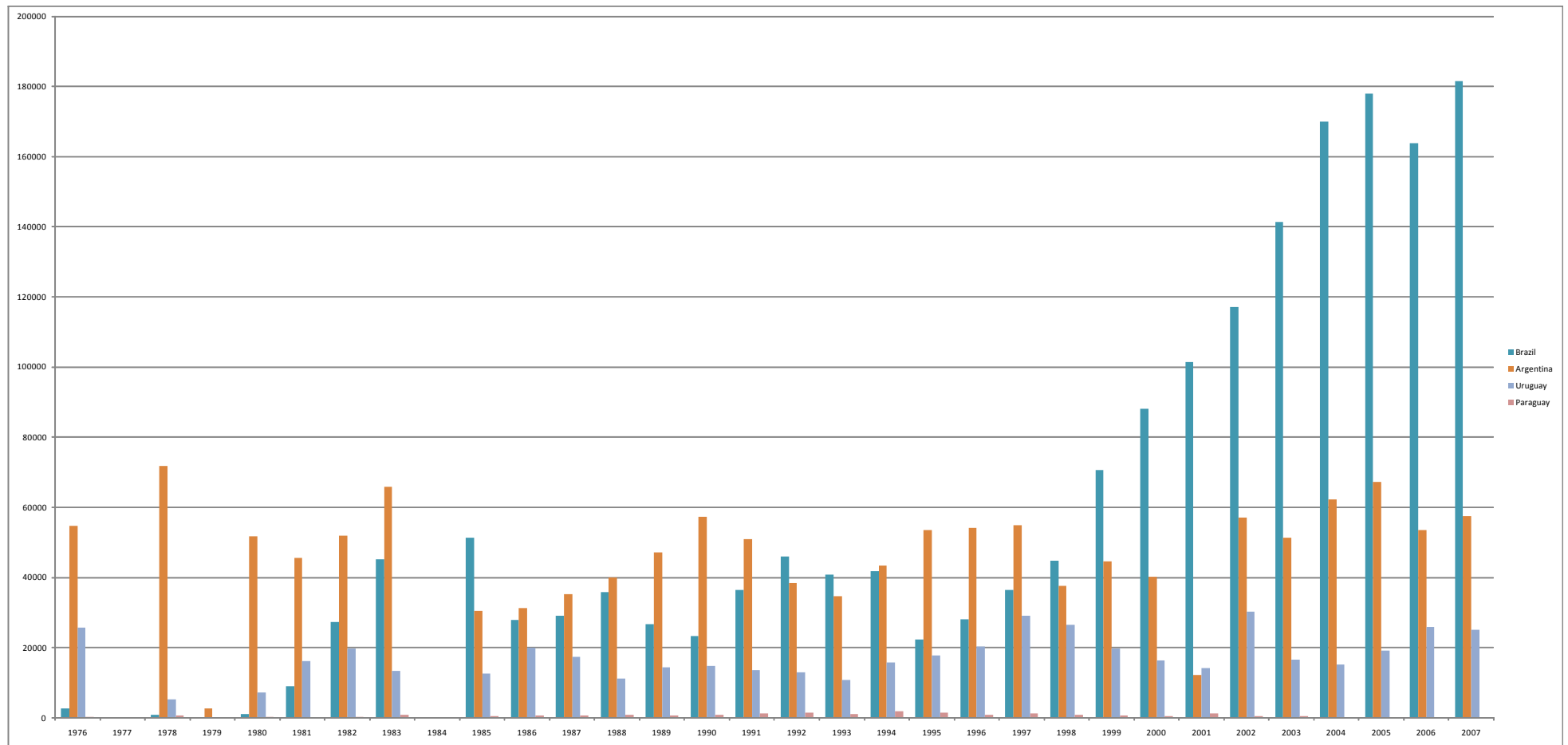
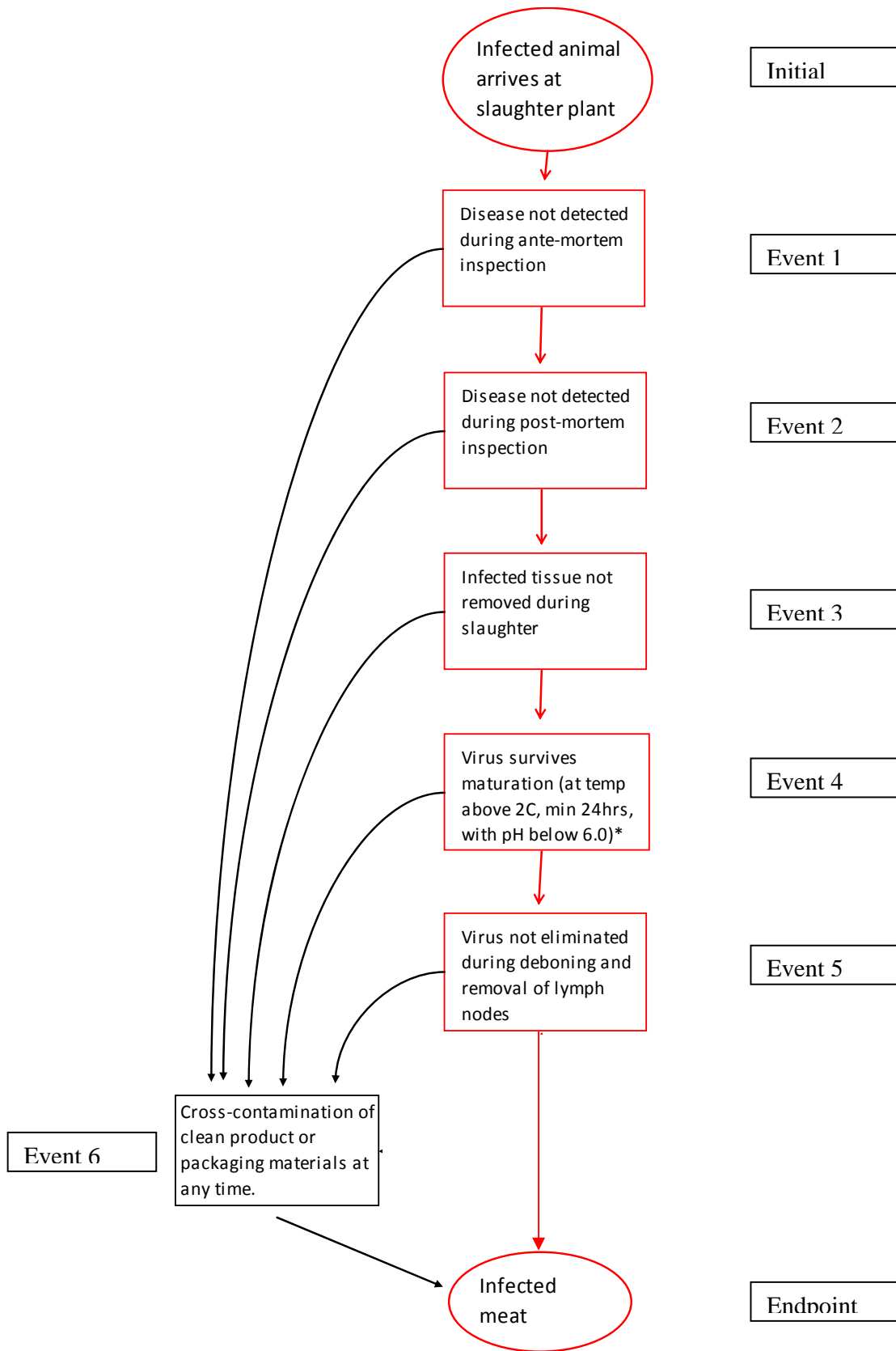


Figure 7. Fresh deboned beef imports into the European Union, 1976-2007

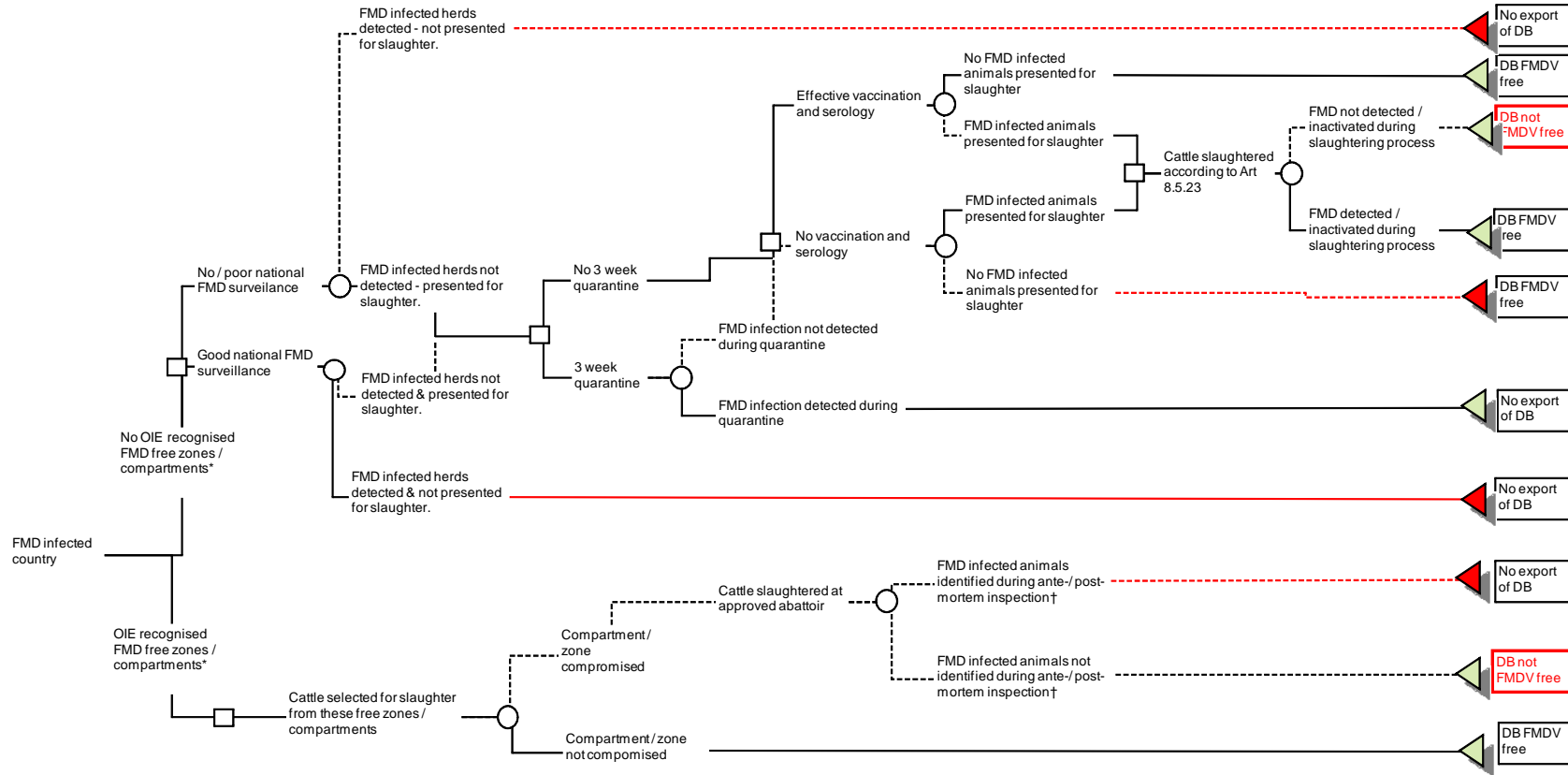
Units are tons. Data not found for 1977 and 1984. 1976 to 1987 figures are based on carcass weight equivalent figures found in the Meat and Livestock Commission (now AHDB)'s archive. They have been converted to a boneless weight to enable comparison to be made with the 1988-2008 series (Battho H, Personal Communication).

**Fig 8: Risk Assessment Scenario Tree at Slaughterhouse**



\*According to standards in Article 8.5.23 of Code

Fig 9. Scenarios for safe preparation and export of deboned beef



\*Recommendations for FMD free compartments still being developed. Assume that the same recommendations as for free zones, will apply for trade in fresh meat.

†The only recommendation for the slaughtering process for cattle from FMD free zones. Art 8.5.20 (w/without vaccination) & Art 8.5.21 (with vaccination).

- lower probability pathways
- decision node
- chance node
- ◀ outcome node
- DB deboned beef