Evaluation of the presence and risk of foot and mouth disease virus by commodity in international trade *

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Summary: Potential sources of foot and mouth disease (FMD) virus include semen from bulls, rams, goats and boars; embryos and ova from ruminants and pigs; meat and meat products and milk and milk products. The author discusses precautions to prevent the transmission of FMD via these commodities.

KEYWORDS: Aphthovirus - Embryos - Foot and mouth disease - Meat - Milk - Ova - Semen - Transmission.

SEmen

Bovine semen

It is well known that semen, along with all other body fluids of livestock infected with foot and mouth disease (FMD) virus, may contain or may be contaminated with FMD virus (FMDV). Waldman et al. and Vallée and Carré demonstrated the presence of FMDV in the reproductive organs and urine of infected and recovered cattle (32, 35). Grunnet demonstrated the presence of the virus in the semen of infected bulls for two but not three days after infection, but not in the semen of ten bulls which had been vaccinated against the infection (16). Gierloff and Jakobsen added FMDV to semen, prior to extension with egg yolk and storage at -70°C for one month (15). Virus was readily recovered from semen stored under these conditions.

In Brazil, Netto examined samples from twenty-two bulls, taken randomly from semen destined for artificial insemination, and found FMDV in seven of these twenty-two samples (26). He postulated that semen could be one of the means whereby FMDV is spread in a country where the disease is endemic. These observations have not been corroborated.

Cottral et al. studied FMDV in the semen of bulls throughout the course of experimental infection and subsequent transmission of the disease by artificial insemination (12). In this study, FMDV was shown to occur in the semen of two bulls as early as 12 h after inoculation. The virus was found in 58 of 71 semen samples from

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16 bulls for as long as 10 days after inoculation. Five of 16 heifers artificially inseminated with semen from infected bulls, and 5 of 10 heifers inseminated with FMDV added to various diluents, also developed FMD. These studies conclusively demonstrated that semen from bulls contains FMDV prior to signs of illness and that it is possible to transmit FMDV by artificial insemination. It is well known that FMD-vaccinated cattle may carry the infection after contact with FMD-infected cattle (33). To determine whether vaccinated and exposed bulls would also shed infective virus in semen, Cottral exposed groups of vaccinated and non-vaccinated bulls to FMD-infected cattle for a week (G.E. Cottral, unpublished findings). Severe signs of FMDV developed in all 7 non-vaccinated bulls and 4 of these 7 were shown to carry FMDV in the throat for 56 days. FMDV was found in the semen of 1 of the 7 non-vaccinated bulls and intermittently in another for 42 days.

Two of the nine vaccinated bulls failed to become carriers of FMDV, whereas seven of the nine remained carriers in the throat for 56 days, the length of the study. In the vaccinated bulls, FMDV was found to be present 7 days after exposure in one of the 9 animals. This animal was one of the two that developed mild signs and lesions after exposure. In this study, it was not possible to evaluate the effects of stress in inducing carriers to shed virus. This is known to occur in other diseases (5).

Rather than continue prohibition of importation of semen into the United States of America (USA) from FMD-infected countries, thus encouraging smuggling, the USA devised a system in 1964 for importing bull semen from animals in countries where the disease was endemic. This regulation provided for the following conditions (2):

- the donor animal is inspected on the farm of origin by inspectors, who determine by examination of the animal but, more importantly, by animal records that the animal was never infected with FMD and that the infection did not exist on that farm the previous year
- the donor animal is permanently identified, is not a carrier of FMDV and is free of antibodies to FMDV
- the donor animal is kept under surveillance for 30 days, and is then moved to a collection facility where it is kept for the collection period plus the following 60 days, at which time it is again examined for FMD viral antibody
- 10% of each ejaculate is retained, non-diluted, for examination for FMDV in susceptible cattle and tissue culture systems
- the entire operation must occur under the supervision of a United States Department of Agriculture (USDA) veterinarian.

Using this system, more than 1.7 million doses of semen were safely imported and used over a ten-year period. Such a method was costly and time-consuming, but was proven to be safe.

In more recent years, the system has been extended to include semen from bulls which have been vaccinated against FMD, and thus have neutralising antibody to FMDV. However, these bulls must be negative to virus-infecting antigen (VIA) antibody and may not be carriers of the infection, as evidenced by negative oesophageal-pharyngeal (OP) samples. Using this technology, several hundred thousand doses of semen have been imported and safely used (4).

Perhaps the best demonstration of the safety of bull semen from vaccinated bulls has occurred in France since 1961. In that year, the French authorities decided to institute a programme of compulsory immunisation against FMD. The decision was thus made...
to vaccinate all cattle in France, except those in the department of Finistère (approximately 250,000). These cattle were not vaccinated for several reasons, including the following:

- the need for cattle free of antibody to FMDV for control of FMD vaccines
- the official quarantine export facility for livestock is located in that department at Brest
- FMD had never occurred in Finistère
- most livestock move from Finistère, not into that area.

During the last thirty years, only a few of the cattle in that department have been immunised against FMD, and those because they were taken for exhibition in other parts of France, yet semen from bulls from all over France has been routinely used in Finistère without a single incidence of FMD in the department. This thirty-year experiment would be difficult to replicate (the relevant article by Parez covers a seven-year period, 1963-1971 [27]. The writer took the liberty of expanding this period to 1990.) The information that this experience provides, together with data from the research and other experiences noted above, allows some general conclusions to be made:

- FMD can be readily transmitted by artificial insemination if the semen is from FMD-infected bulls
- it is possible to devise safe procedures for movement of bull semen from an FMD-infected country to one which is free of the disease
- semen from bulls which have been multiply vaccinated with an efficacious product, and which are not carriers of the virus, has been shown to be safe for use in animals which have not been immunised.

**Sheep and goat semen**

To the knowledge of the writer, this subject has received very little attention at the research level. There is a tendency to extrapolate data from the bovine to sheep and goats; however, this is not always a good idea.

One significant study by the Ministry of Agriculture, Argentina, the Ministry of Agriculture, Canada, and the USDA involved collection of semen from males recently recovered from FMD infection and the use of such semen to inseminate females from which embryos were collected for storage and transfer to other animals (34). In this case, the semen was examined for FMDV in two tissue culture systems, including two blind passages, and none was demonstrated.

The embryos in this study were washed according to International Embryo Transfer Society (IETS) recommendations, frozen and implanted into sheep and goats negative to FMD. None of the recipients showed FMDV antibody, nor signs of infection, and all of the embryos were negative for FMDV antibody 30 days after birth. Although this is a single study, it demonstrates that in sheep and goats, as in cattle, FMDV in semen is usually associated with viraemia.

**Swine semen**

In recent years there have been improvements in the technology to collect and store swine semen, including the technology to preserve semen by freezing. This latter
development simplifies movement of semen internationally. As a result, limited quantities of boar semen have begun to move internationally and this trade will probably increase as additional progress is made in the preservation of semen. Increased international movement focuses attention on the possibilities of transmission of a large number of diseases, including FMDV, by way of swine semen.

McVicar et al. studied the possibilities of transmission of foot and mouth disease and swine vesicular disease (SVD) viruses through swine semen (23). Boars were artificially infected with FMD and SVD viruses and, in each instance, virus was detected in semen before clinical signs of the disease developed. Semen was collected for only two days because the feet of the boars became sore and the boars refused to mount. (Electro-ejaculation was not used in this test series.) It is likely that the animals would have shed virus in the semen for several more days. The sperm-poor fraction of the ejaculate was the sample of choice for virus detection. This may account for the fact that, in this study, it was not possible to infect sows by insemination. However, the study demonstrated that semen collected from boars infected with FMD or SVD is likely to contain virus, highlighting the need for precautions in selecting donor boars from which semen is to be exported to FMD or SVD-free countries. Swine react to FMDV in much the same fashion as cattle, except that a carrier state has not been demonstrated in swine. The same serological tests applied to cattle can be applied to swine to determine whether they have been infected with FMD. Swine semen is toxic to tissue cultures, thus, special precautions must be taken if samples are assayed for virus in tissue cultures (28).

Live animals

Definitive studies of the FMD carrier state of cattle and other species following infection have not been conducted in recent years, at least to the knowledge of the writer. Some of the concepts based on research and experiences are listed below and are based on investigations in Europe, Africa and North and South America (22).

- Following infection with FMDV, various types of antibody may be detected in the recovered animal. Tests currently applied include complement fixation, virus neutralisation, agar-gel and enzyme-linked immunosorbent assay (ELISA). The virus neutralising and VIA antibodies last the longest, and can usually be detected one or more years after infection.

- Following immunisation, all of the above types of tests may also be applied for the detection of the same types of antibody, except, as a rule, antibody to VIA. As this is an antibody which results from the viral enzyme polymerase, VIA is usually not present after a single immunisation with an inactivated vaccine.

- In multiply vaccinated animals, antibody to VIA may be seen soon after immunisation; however, in 30 to 60 days this antibody generally disappears.

- Cattle, sheep, goats and wild ruminants may become carriers of the virus in the soft tissues of the throat for varying periods of time after FMD infection. Data indicate that Cape buffalo remain carriers for long periods and there is circumstantial evidence that FMD has spread from this species to cattle (18, 31). Other evidence of disease spread from carriers is also largely circumstantial. Researchers have not demonstrated the spread of FMD from carriers to susceptible animals. Despite this, it is not recommended that carriers be mixed with susceptible livestock.

- The carrier state in swine recovered from FMD has not been demonstrated.
- The number of animals which become carriers and the duration of the carrier state vary according to the type and subtype of virus.

**EMBRYOS AND OVA**

**Bovine, sheep and goat embryos and ova**

The first embryo transfer was by Walter Heape, in rabbits at Cambridge (1), where significant contributions continue to be made in the field of embryo transfer and, more recently, embryo manipulation, cloning, *in vitro* fertilisation and maturation, gene transfer and mutation. The number of transfers, especially in bovines and other ruminants, continues to increase each year, primarily because of cost-effectiveness and safety in comparison to the alternatives of artificial insemination and movement of the adult animal (17).

This review will be limited to embryo transfers in relation to FMD. Movement of unfertilised ova is not practised commercially; however, as *in vitro* fertilisation and maturation techniques improve, the number of transfers of ova from one country to another will probably increase. The same precautions in relation to embryos are applicable to ova.

Singh *et al.*, McVicar *et al.*, Mebus and Singh and Villar *et al.* have studied the relationship of FMD to embryos from cattle, sheep and goats (24, 25, 30, 34). Summary statements of the findings of these authors are as follows:

- Bovine, sheep and goat embryos, with the zona pellucida intact, which were exposed to $10^6$ plaque-forming units (PFU) per ml of FMDV and then washed according to IETS standards were found to be free of virus.

- In contrast, hatched or zona pellucida-free bovine embryos remain infected when exposed to FMDV, demonstrating the necessity, from a zoosanitary point of view, of an intact zona pellucida.

- Washed zona pellucida-intact embryos collected from cattle, sheep and goats during the acute stages of FMD infection and 14 to 21 days after clinical stages of the disease were found to be free of FMDV.

- Such zona pellucida-intact embryos were washed according to IETS recommendations, and implanted into cows fully susceptible to FMDV. The recipients and offspring did not show infection or antibody to FMDV.

- Zona pellucida-intact bovine embryos resulting from insemination of cows recovered from FMD infection 90 days previously, with semen from a bull recovered 40 days previously, are free of FMDV, following application of the IETS recommendations for washing.

- As noted above, zona pellucida-intact embryos from sheep and goats resulting from insemination with semen collected from males 60 days post infection with FMDV, and used in females 60 days after recovery from FMD, were shown to be free of FMDV after washing according to the IETS recommendations. Embryos were assayed for virus by inoculation into tissue cultures, by intradermal inoculation into the tongues of susceptible cattle and by implantation into females negative to FMDV. The recipients remained negative throughout gestation and the recipients and offspring were negative for antibody to FMDV.
These experiences demonstrate that embryos from cattle, sheep and goats, when they have intact zona pellucida and when washed according to IETS recommendations, may be safely transferred from FMD-infected and recovered cattle without transmitting the infection.

Swine embryos

The technology for the handling of embryos from swine is not sufficiently developed for a commercial trade in such embryos. From the little information that is available on FMD and Aujeszky's disease, the swine embryo is very sticky, and there seems to be a tendency for FMDV and Aujeszky's disease virus to stick to the zona. Extreme caution should be exercised in moving swine embryos from a country that has such a disease to another where this disease does not exist. The subject needs intensive investigation, along with development of the technology for handling swine embryos.

MEAT AND MEAT PRODUCTS

Foot and mouth disease plays a major role in shaping the world meat trade. The world is divided into countries which have the infection and those which are free of the disease. Many meat-exporting countries are infected and fresh or frozen meat products from these countries are not welcome in other countries. Treating meat to rid it of virus is not only expensive, but frequently results in a second-rate product. In addition, treatment also increases the price of production (20).

The 1967-1968 outbreak of FMD in the United Kingdom (UK) was one of the worst outbreaks which that country has ever experienced. It was determined that the infection was probably due to an importation of frozen meat with bone-in. The circumstances of the outbreak tend to support this theory.

Following the outbreak, a high-level commission, led by the Duke of Northumberland, formulated the meat-import policy of the UK, as follows (3):

- Importation of mutton, lamb and pork from FMD-infected countries was banned.
- Imports of mutton and lamb offal and pig offal were banned, except offal which was treated to inactivate the virus.
- Imports of carcass beef and offal from FMD-infected countries were banned.
- Imports of beef from FMD-infected countries were limited to boned-out beef and processed beef offal.
- Imports of meat from well-defined areas which were FMD-free were accepted, even if such areas existed in an FMD-infected country.

Since the above policy was instituted, an outbreak of FMD attributable to an imported product has not been experienced in the UK. Actually, there have been only two outbreaks since 1968, one on the island of Jersey and the other on the Isle of Wight, both attributable to air-borne virus from Europe. It was reported by de las Carreras in 1978 (8), and again in 1989 (9), that since 1968 the UK has imported hundreds of tons of boneless beef from South America without a single incidence of the disease caused by importation of boned-out beef. In addition to beef which has gone to the UK, large quantities have also gone to several other European countries,
all of which practise vaccination against three types of FMDV. In no instance has an outbreak been thought to have been caused by imported boneless beef.

The conditions under which FMDV survives in animal tissues are of fundamental interest to all concerned with the prevention and control of the disease. During FMD infection, the virus is distributed throughout the body of the animal. After death, survival of the virus is dependent on the stage of the disease at the time of slaughter, on the characteristics of the strain of virus and on the environmental factors surrounding the carcass, especially temperature and hydrogen ion concentration.

The virus in muscle is inactivated within 24 to 72 hours after slaughter, due to the reduced pH. In contrast, the virus may survive for weeks or months in refrigerated internal organs, bone marrow, lymph and haemal nodes, glands and residual blood. Lymph nodes, bone marrow and large blood clots provide chemical barriers conducive to survival of the virus. Quick freezing suspends acid formation, hence the desirability of allowing the carcass to remain above freezing for 24 hours. Thawing of quick-frozen meat initiates the suspended acid formation at an accelerated rate and quickly produces a medium unsuitable for virus survival.

During World War II, cured beef (pieces of beef cut from the carcass and held at 4°C in brine) began entering the USA and continued to be imported after the war, the US meat industry having learned how to use such beef for the manufacture of meat products. In a study published in 1960, Cottral et al. showed that FMDV present in muscle, lymph node, clots of blood and bone marrow survived these storage conditions for 60 days (10, 11). After 60 days the muscle gave negative results in tests for the virus. As a result of these findings, this product was no longer imported into the USA. Loss of the market caused hardship to Argentina and subsequently the two governments agreed to seek a solution. In the co-operative study, a five-kilo cooked meat roll (68.5°C) was developed and large quantities of this product are now exported from Argentina and Brazil to the USA and other countries which are FMD-free (19). Other, fully cooked meat products may also be imported, including tinned meat, which is sterilised and thus stable at room temperature.

More recently, Blackwell et al. developed technology using imported ground meat, which was made into meat balls and stuffed into a five-kilo plastic bag for cooking (6). This product, because it contains or may contain lymph node pieces, must be cooked to 93°C to be eligible for entry. Each roll reaches 93°C, thus each roll has a separate indicator disc which may be examined if the inspector so desires.

These products which are cooked and then frozen enter the USA and other markets primarily for further processing. Investigations continue in several FMD-infected meat-exporting countries to develop products which FMD-free countries will accept, yet which are subjected to less rigorous treatment.

**MILK AND MILK PRODUCTS**

In recent years, several groups have studied the survival of FMDV in milk products, stimulated at least in part by the observation that milk and/or milk trucks may have been responsible for the spread of infection during the 1967-1968 outbreak in the UK (7, 14). From the research conducted before and after that outbreak, the following general conclusions can be drawn.
During FMD infection of a bovine or swine, large quantities of FMDV may be found in milk some time before the development of clinical signs of the disease. Virus in milk disappears with the development of neutralising antibody and, following infection, milk contains significant antibody (29, 36).

FMDV in milk may survive pasteurisation, probably because the virus is attached to or contained within cell debris, where the virus is protected from the heat of short or long pasteurisation. Either method reduces the titre of FMDV significantly (21).

Ultra-high temperature (UHT) processing is sufficient to inactivate FMDV in milk, including virus contained in fat (micelles) or cellular debris (13).

FMDV survives the processing of casein or caseinates. However, the processing of either product reduces viral titre and, after 30-day storage, infectivity could not be demonstrated.

FMDV survives the processing of certain cheeses. However, the infectivity remaining after processing disappears during ageing or ripening. Cheese production would, in the opinion of this writer, be a good option for disposing of milk collected during an outbreak.

In countries where cattle are immunised against FMD, milk contains large amounts of antibody, which neutralises FMDV which might enter the milk supply from an infected animal.

During outbreaks, milk from infected animals should not be fed to livestock, nor used for the processing of products which are then fed to livestock, such as dry milk powder, casein, caseinates or whey, unless such milk is UHT-processed or otherwise sterilised.

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ÉVALUATION DU RISQUE DE TRANSMISSION DU VIRUS DE LA FIÈVRE APHTEUSE PAR LES PRODUITS DU COMMERCE INTERNATIONAL - J.J. Callis.

Résumé : Les produits suivants peuvent contenir des virus de la fièvre aphteuse : semence de taureaux, de béliers, de boucs et de verrats ; ovules et embryons de ruminants et de porcins ; viande et produits carnés, lait et produits lactés. L'auteur examine les précautions à prendre pour empêcher la transmission de la fièvre aphteuse par l'intermédiaire de ces produits.


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Resumen: Entre las fuentes potenciales del virus de la fiebre aftosa se cuentan el semen de toro, carnero, chivo y verraco, así como los embriones y óvulos de rumiantes y cerdos, la carne y productos cárnicos y la leche y productos lácteos. El autor expone las precauciones que deben tomarse para prevenir la transmisión de la fiebre aftosa a través de estos productos.


REFERENCES


