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Inactivation of Foot-and-Mouth Disease Virus in Milk Products

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Introduction

Foot-and-mouth disease (FMD) is a viral infection that mainly occurs in cloven-hooved animals including commercially important species such as cattle, sheep, goats, swine and (water) buffalo. The FMD virus (FMDV) can be shed by a variety of routes, including milk.[1;2] FMDV can persist in milk products for some time, especially at refrigeration temperatures. This virus has been reported to survive in raw milk for 6 days at 18ºC and for 15 days at 4ºC.[3 cited in 1] When the milk was pasteurized before adding FMDV, the virus was detected for 30-35 days at room temperature and 50 days at 4ºC. Terbruggen (1932) reported virus survival in milk for at least 12 hours at 37ºC, 25 hours at 17-20ºC and 12 days at 5ºC.[1] In other experiments, it persisted in milk for up to 7 days at 7ºC, 5 days at 10ºC, 3 days at 15ºC or 42 hours at 20ºC.[4] While FMDV in milk products seems to present a minimal risk to humans,[5] products intended for human consumption (especially spoiled or outdated products) may be fed to animals. Other milk products may be manufactured for animal feed (e.g., whey used in calf milk replacer). Infected, nonpasteurized milk has been linked to FMD outbreaks;[6-8] however, there are still uncertainties in the level of risk to animals fed pasteurized or processed milk products. This review summarizes information in the literature regarding the inactivation of FMDV in milk and milk products. Unless otherwise noted, all references to milk products refer to milk from cattle.

FMDV levels in milk

Most research on the occurrence of FMDV in milk, and nearly all research concerning the effects of pasteurization or pH changes, has been done in cattle. FMDV can replicate in the squamous epithelial tissues of the mammary gland, resulting in high viral titers in milk.[7;9] In some experimentally infected cattle, the highest viral titers found in milk were comparable to levels detected in the pharynx.[10] FMDV may be shed in milk before the onset of clinical signs. During the 1967-1968 epizootic in England, this virus was detected in some milk bulk tanks and tankers at least 33 hours before clinical signs were reported in the affected herds.[11] In some experimentally infected cows, it was found in milk 1-4 days before vesicles developed.[12-15]

FMDV is also likely to be present at high levels in the milk of other susceptible species, although there is little research or detailed information available. It has documented in milk from goats[16 cited in 17] and sheep; [18 cited in 17] the latter study was based on the detection of nucleic acids rather than infectious virus.

Methods used to measure FMDV in milk products

Various systems have been used to measure FMDV titers. Some experiments have evaluated virus levels in cell culture, as the number of plaque forming units (pfu) in cell monolayers. Other studies describe FMDV titers in terms of the ID_{50} (infectious dose 50%), a value which is expected to be directly proportional to the number of viable virions in the sample.[19] The ID_{50} may be measured in cell cultures, as the $TCID_{50}$, i.e., the amount of virus that infects 50% of the cultures, or in laboratory animals, as the quantity that infects 50% of the inoculated animals.[19;20] The mouse lethal dose 50% (mouse LD_{50}) has also been used.[21] Test variability can be high, particularly in the animal ID_{50} determination, where practical considerations may limit the number of replicates.[20]

Measured titers can vary with the detection system. Cell types may differ in their sensitivity to FMDV.[22;23] For example, primary bovine embryo thyroid cells are reported to be more sensitive than secondary pig kidney cells.[22] Sellers (1971) estimated the overall variability between the cell types typically used for FMDV detection, other than calf thyroid cells, to be relatively low.[23] Animal inoculation is usually more sensitive in detecting FMDV than cell culture.[22;24;25] However, the sensitivity varies with the species and possibly the breed of animal.[23;26] Felkai et al. (1970) reported similar results when FMDV-contaminated milk samples were tested by inoculation into suckling mice or porcine kidney cells, but inoculation into guinea pigs was less sensitive.[26] The results reported in Kastli and Moosbrugger (1968) also suggest that guinea pigs are not a very sensitive system.[27] Inconsistent results are apparent in this study, with virus sometimes detected at higher pasteurization temperatures and/or longer incubation times, but absent at lower temperatures or shorter incubation times. A number of studies have used inoculation into cattle. Typically, these studies use relatively large volumes of milk (e.g.,30-50 ml), with a small amount of the milk (often 1-2 ml) inoculated into the tongue, and the remainder given intramuscularly. This is thought to be a particularly sensitive system, and can often detect residual FMDV when it is below the detection limit for cell culture.[22;24;25;28-33] Some authors have speculated that this system might be particularly sensitive because it reveals viruses complexed with milk components that prevent interaction with cell receptors in tissue culture.[8] Enzymes or other factors might dissociate these complexes *in vivo*.[8] Another possible reason for the sensitivity is that "interfering factors," such as interferons, may inhibit infection of cells, but be diluted *in vivo*.[8] Lastly, the residual viruses might be found simply because the volumes inoculated are high compared to the amounts used in cell culture.[8] Some authors have, however, criticized intradermolingual inoculation of cattle because it causes infection at lower viral titers than more natural methods such as aerosol or oral inoculation.[21]

Although FMDV can also be detected in milk using PCR,[34] this technique does not differentiate viable virus from fragments of inactive nucleic acids, and it is not useful for studies of FMDV inactivation.

FMDV titers shed in milk

Field studies

There is relatively little published information on the amount of FMDV typically shed in milk during outbreaks. The existence of seven FMDV serotypes (O, A, C, Asia-1, SAT-1, SAT-2 and SAT-3) and a large number of strains may complicate this assessment, as the amount of virus might vary between serotypes and strains. In a few field samples collected from infected cows, the virus titer in milk varied from trace amounts to $10^{6.6}$ $TCID_{50}$/ml.[10;11;35] One review article stated that peak virus shedding in cow's milk is $10^{6.7}$ $TCID_{50}$ /ml milk, without giving further details.[36] Alexandersen (2005) noted that, due to the limited number of field samples, it is possible that some cows shed greater amounts of FMDV than have been reported.[17]

Donaldson (1997) reasoned that milk from affected cows is likely to be diluted by milk from uninfected animals, resulting in lower virus titers in a farm's bulk milk tank, with further dilution in milk tankers and at the dairy.[6] Once most or all animals on the farm are infected, and the quantity of virus in the bulk milk tank rises, the infection is likely to be recognized and the farm quarantined.[6] Another factor likely to lower virus concentrations in the farm's bulk tank is that production is usually decreased in infected animals.[6] Dilution of the virus in milk tankers and at the dairy may not always be applicable to cheese, as some cheese is produced by cottage industries on farms.[21]

During the 1967-68 UK outbreak (serotype O_1), a field monitoring program reported trace amounts to $10^{4.0}$ mouse ID_{50} /ml and $10^{3.75}$ $TCID_{50}$ /ml in bulk tankers (3 samples), trace amounts to $10^{5.5}$ $TCID_{50}$ /ml in churns (18 samples), $10^{4.0}$ mouse ID_{50} /ml in a bottle of untreated retail milk (1 sample), $10^{4.5}$ $TCID_{50}$ /ml in a farm storage tank (1 sample), and trace amounts to $10^{2.0}$ mouse ID_{50} /ml in milk from 2 cows.[10;11] The milk storage tank with $10^{4.5}$ $TCID_{50}$ /ml was located on a farm where 3 of 55 cows were infected.[11] The bulk tanker with $10^{3.75}$ $TCID_{50}$ /ml had collected 219 gallons of milk from a farm where one of 107 cows was infected, and contained 547 gallons in total.[11] The bulk tanker with $10^{4.0}$ mouse ID_{50} /ml served a farm where 8 of 75 cows were infected, and this farm produced 136 of the 1220 gallons in the tanker.[11] From this information, it appears that some cattle may have been shedding high levels of FMDV in milk on these farms. Alternatively, some infected cows may not have been detected. No virus was found in a bulk milk tank from a farm where 1 of 65 cows was infected (the affected animal was a cow close to drying off), in some churns from a farm where 1 of 46 cows was infected , or in a storage vat from a farm where 10 of 99 cows were infected.[11] During the 1981 Isle of Wight outbreak, six asymptomatic cattle (from a herd with 32 cows) were reported to shed $10^{0.7}$-$10^{6.6}$ $TCID_{50}$/ml in milk, and $10^{2.2}$ $TCID_{50}$/ml was found in this farm's bulk milk tank.[35 cited in 6;9;17]

Experimentally infected cows: inhalation, contact and other "natural" routes

Information from experimentally infected cows also provides evidence for the level of FMDV in milk. Cows that were inoculated with a serotype O_1 virus from the 1967-68 U.K.

epizootic shed a maximum of $10^{5.2}$ "infectious particles"/ml of milk.[10] In 6 dairy cattle exposed to a serotype O_1 virus (Brugge strain) by contact with infected pigs, peak titers in individual animals ranged from $10^{2.5}$ to $10^{5.5}$ pfu/ml milk.[15] Kihm et al. (1979) reported titers up to $10^{5.5}$ $TCID_{50}$ of FMDV serotype C (Noville strain) in the milk of cattle infected by exposure to an infected ox.[14] Similarly, Orsel et al. (2007) found that titers of a serotype O_1 (NET2001) virus in cows inoculated intranasally, or exposed to these animals by contact, reached 10^5 pfu/ml in some animals.[37] Burrows et al. (1971) found that individual cows exposed by contact shed 10 to $10^{5.2}$ pfu/ml of a serotype O_1 virus (BFS 1860), and $10^{1.2}$ to $10^{4.5}$ pfu/ml of a serotype A_{22} virus (Iraq 24/64) in milk, on or before the day vesicles appeared.[13] However, one group reported peak viral titers of only $10^{2.7}$ pfu/ml of a serotype O virus (strain Weerselo) in intranasally inoculated cows.[38] The same group found that peak titers were slightly higher than $10^{3.0}$ pfu/ml in cows inoculated by the intradermolingual route.[8]

Experimentally infected cows: intramammary inoculation

A number of studies have used intramammary inoculation, often combined with intravenous inoculation, to produce FMDV-infected milk for inactivation studies. Cows infected by this route seem to shed higher levels of virus in milk, with titers often reaching 10^6 to 10^7 pfu/ml in some experiments.[13;15;38] In one study, peak FMDV titers in milk were $10^{2.5}$ to $10^{5.5}$ pfu/ml in 6 dairy cattle exposed to FMDV serotype O_1 (Brugge strain) by contact with infected pigs, but the peak titers in one cow inoculated by the intramammary route reached almost 10^7 pfu/ml.[15] Burrows et al. (1971) found that individual cows exposed by contact shed virus concentrations of 10 to $10^{5.2}$ pfu/ml milk, but cows exposed by intramammary inoculation shed $10^{0.7}$-$10^{7.2}$ pfu/ml of FMDV serotypeO_1, and $10^{0.7}$ to $10^{6.6}$ pfu/ml of serotype A_3.[13] De Leeuw et al. (1978) reported that the highest viral titer in milk was $10^{2.7}$ pfu/ml after intranasal inoculation, but a single cow inoculated by the intramammary and intravenous routes had titers up to $10^{6.1}$ pfu/ml.[38] After intramammary and intravenous inoculation, Hyde et al. (1975) found titers of $10^{6.7}$ to $10^{7.5}$ pfu/ml on day 1 PI, and $10^{3.1}$ to $10^{5.8}$ pfu/ml on the following 5 days.[29] De Leeuw et al. (1979) reported peak FMDV titers of $10^{4.4}$ pfu/ml to $10^{6.8}$ pfu/ml, with a mean of $10^{5.9}$ pfu/ml,[8] while Cunliffe et al. (1979) found that the titers in milk varied from $10^{3.7}$ pfu/ml to $10^{6.4}$ pfu/ml.[33] Two groups reported that FMDV shedding in milk was biphasic in some animals, with the highest viral titers occurring on the first day after inoculation, and a second, lower peak a few days later.[8;29]

Other species

Other FMDV-susceptible species probably shed significant amounts of virus in milk, but there is little detailed information on the levels.[17] High titers of FMDV have been demonstrated in goat milk,[16 cited in 17] and RT-PCR demonstrated the occurrence of nucleic acids in milk from experimentally infected sheep.[18 cited in 17]

Vaccinated animals

Vaccinated animals are likely to shed less FMDV in milk, but some virus may still be present.[6;17] In one study, this virus could not be isolated from the milk of vaccinated cows after challenge by intranasal inoculation.[38] Similarly, Orsel et al. (2007) did not detect FMDV in the milk of vaccinated dairy cattle, although peak virus titers shed by some nonvaccinated cows reached at least 10^5 pfu/ml.[37] Sadir et al. (1980) found that type A, C or O FMDV added to whole or skim milk from vaccinated cows was inactivated rapidly during the first 120 minutes of storage (4ºC), then reached a plateau, with further inactivation proceeding at a much slower rate.[39] The authors speculated that the virus was inactivated by anti-FMDV antibodies in the milk, although other milk components may have played a role.[39]

Summary

FMDV titers ranging from trace amounts to $10^{6.6}$ $TCID_{50}$ have been reported in the field from infected cows, and peak titers as high as $10^{5.5}$ pfu/ml or $10^{5.5}$ $TCID_{50}$/ml have been found in experimentally infected cows exposed by contact or inhalation. Cows infected by intramammary and intravenous inoculation can shed higher FMDV titers in milk, with levels often reaching approximately 10^6 to 10^7 pfu/ml. FMDV titers as high as $10^{4.5}$ $TCID_{50}$ /ml have been reported in farm storage tanks, and titers up to $10^{4.0}$ mouse ID_{50} /ml and $10^{3.75}$ $TCID_{50}$ /ml were found in milk from bulk tankers. Churns were reported to contain up to $10^{5.5}$ $TCID_{50}$ /ml, and one bottle of untreated retail milk had $10^{4.0}$ mouse ID_{50} /ml. Information on FMDV shedding in the field is limited, and higher titers might occur. Vaccinated animals are likely to shed much less virus in milk than unvaccinated animals. There is little published information on FMDV titers in the milk of species other than cattle.

Routes of inoculation used in experiments

Many studies on FMDV inactivation use milk from cows that have been infected by intramammary and intravenous inoculation.[40] This route has been criticized by some, because it is not expected to be common in the field,[40] and because it seems to result in particularly high virus titers in milk.[13;15;38] However, there is a consensus that such high titers constitute a worst case scenario, and inactivation demonstrated under these conditions may provide a safety margin.[40] A few studies collected milk from cattle inoculated intranasally.[15;41]

Some studies use milk from noninfected animals, and add FMDV from cell culture or other sources. De Leeuw et al. (1980) reported that heat treatment inactivated FMDV more rapidly in this system, compared to milk taken from the same cows after they were infected with the virus.[22] This was especially true during the first few minutes (at 56ºC); after this time, the rate of inactivation was similar. Infected animals might shed a proportion of the viruses within cells [7;42] as well as within fat globules and casein micelles,[28] which is expected to partially protect the virus from inactivation procedures.

Commercial milk processing and the effects of various processes on FMDV survival

Commercial processing of dairy products includes clarification to remove debris, heating, and various other procedures such as homogenization, evaporation, pH changes, drying or filtration.[43] Although the vast majority of research has been on the effects of heat, and to a lesser extent pH, other processes could also affect the amount of residual FMDV in contaminated milk products.

Clarification

Clarification of milk removes somatic cells(leukocytes and epithelial cells), bacteria, sediment and other small debris.[43] Clarification may remove some FMDV that is intracellular or associated with cellular debris,[7;42;44] but there is no published research on how much this might reduce FMDV titers in milk.

Homogenization and standardization of milk fat

During processing, milk is preheated to 50°C and separated into the cream and skim milk fractions, the latter containing casein, lactose and whey proteins dispersed in water.[28;43] The milk fat content is standardized by adding varying amounts of cream back to the skim milk fraction before pasteurization.[28] Many products are also homogenized, which reduces the size of the fat globules in milk.[28;44] Homogenization is performed on the reconstituted milk product, and takes place between the regeneration and heating stages in the HTST pasteurizers used in modern processing plants.[28;44] Because FMDV contained within fat globules is thought to be more heat-resistant,[24;28;29] homogenization is expected to improve virus inactivation.[28;44] However, no published studies have examined the effect of homogenization on FMDV survival.

Protective effects of milk fat and protein on FMDV survival

Raw milk from cattle is 87-88% water by weight.[43] It also contains approximately 4% fat, in globules of 1-4 μm surrounded by a membrane, casein micelles (2.5% by weight), lactose (4.6% by weight), and whey proteins (0.8% by weight).[43] Fat droplets and casein micelles are thought to help protect FMDV from inactivation.[28] During pasteurization, heat must be transferred from the bulk of the milk to the interior of the fat globule, liquefying the fat in the process.[28] Higher temperatures increase the rate of heat transfer. Alternatively, longer holding times at a fixed temperature allow more time for heat transfer. Milk fat and protein are also thought to protect FMDV from inactivation by changes in pH.[7] Elevated fat and protein content is a particular concern for virus inactivation in products such as cream, butter, casein and cheese. Conversely, the low fat and protein content in products such as whey may facilitate inactivation.

A few studies compared inactivation in products with a higher or lower fat content. Terbruggen (1932) found that FMDV survived longer in stored cream than in skim milk, when samples were held for prolonged periods at 5°C, 17-20°C or 37°C.[1] Blackwell and Hyde (1976) reported that FMDV survived more readily in whole milk compared to skim milk, in pasteurization trials performed at 85°C for 0.25 min.[24] In another study, FMDV was still detectable by animal inoculation after heating whole milk at 72ºC for 3.0 min, followed by evaporation, but not in skim milk heated at 72ºC for 0.5 minutes then evaporated.[29] Tomasula et al. (2007) stated that pasteurization inactivated FMDV more readily in 2% milk compared to whole milk containing 6% fat.[28] However, there may be no significant difference in virus inactivation when milk is pasteurized at higher temperatures.[40] De Leeuw (1979) reported similar results in whole and skim milk at temperatures of 100°C or greater.[8] High fat milk products, such as butter or cream, may afford more protection to the virus even at high temperatures.

Filtration

Filtration is usually used for purposes other than food safety, and includes microfiltration, which is used to clarify or concentrate milk and remove bacteria; ultrafiltration, which separates soluble milk or whey proteins (e.g., in producing whey concentrates); and nanofiltration, which is used to separate soluble whey molecules.[9;44] There is no information about the effects of these filtration techniques on FMDV titers.[44]

Effect of pH on FMDV inactivation

FMDV inactivation by pH in aqueous solutions

FMDV is reported to be most resistant to inactivation between pH 7 and 7.5.[45] In a series of experiments, a serotype A virus (strain 119) was stable in cell culture fluids at pH 7 to 7.5, but increasingly rapid inactivation was seen as the solution was made more acidic or basic.[45] A 90% reduction in viral infectivity occurred in 3 weeks at pH 8, in 1 week at pH 9, every 14 hours at pH 6.5 or pH 10, and every minute at pH 5 or 6. Inactivation was too rapid to be measured at pH 4 or below. Using a more sensitive method, a small amount of residual, pH-resistant virus (approximately one-millionth of the original amount) was detected for at least 30 to 60 minutes at pH 5 to 6; however, this residual population disappeared in 45 seconds at pH 3 or pH 4, and no virus was detectable after this time.[45] It should be noted that the detection method used was cell culture, which is less sensitive than injection into steers.

Components of milk appear to protect FMDV from inactivation by changes in pH. In one experiment, the FMDV titer decreased by $10^{4.5}$-10^{5} in an aqueous solution acidified to pH 6 for 30 minutes (4ºC), but the titer decreased by only $10^{2.2}$ if the test material contained 40% milk.[46 cited in 17]

Milk pH in FMDV-infected cows

Because pH affects the stability of FMDV, it is important to know whether infected cows secrete milk with an altered pH. The normal pH of milk from cattle is usually 6.6 to 6.8, although a range of 6.4 to 6.9 has been reported from individual animals at various stages of lactation.[47] Sellers (1969) stated that the pH of milk from FMDV-infected cows varies from 6.7 to 7.7, but the source of this statement was not given.[42] Some experiments documented a rise in milk pH, when cows were infected with FMDV by combined intramammary and intravenous inoculation.[8;22;28;29;29] Hyde et al. (1975) reported a mean pH of 7.15,[29] and de Leeuw et al. (1980) documented individual pH values of 7.0-7.4, one day after inoculation.[22] Tomasula et al. (2007) reported a pH change from 6.7 to 7.1 after infection.[28] However, some studies reported that milk pH did not rise when cows were inoculated by more natural routes.[8;13] Burrows et al. (1971) found that the pH of infectious milk remained between 6.5 and 6.9 in animals infected by contact.[13] De Leeuw et al. (1979) noted that the milk pH was 6.5-6.8 in cows inoculated by contact, intranasal inoculation, or intradermolingual injection, but the pH rose transiently when cows were inoculated by combined intramammary and intravenous inoculation.[8] The pH of milk from the latter animals had a range of 6.6 to 7.4, with a mean of 7.0, between 16 and 32 hours post-inoculation.[8] It remained between 6.5 and 6.8, before and after this time.[8]

Blending infectious milk with noninfected milk, during milk collection and at dairies, is expected to lower the pH of milk if it is elevated.[28] However, it should be noted that the pH of field samples was reported to be 7.0-7.5 during the 1967-68 U.K. outbreak.[11] Sources reported in this paper included two cows, a bottle of retail milk, more than a dozen churns, a bulk milk tank, and milk tankers. It is unclear from the paper whether the milk samples tested for pH came from all of these sources or only from individual cows.

FMDV inactivation in milk products by changes in pH (Table 1)

Altering the pH of an FMDV-containing solution of pH 7.0-7.5 to become more acidic or basic is expected to increase the inactivation rate of FMDV.[45] Conversely, elevating the pH of normal milk (pH 6.6-6.8) to 7.0-7.5 would be expected to have a protective effect on the virus, during inactivation procedures such as heating. The specific effects of pH changes may be difficult to quantify in milk.[17] Some alterations in pH that would be expected to increase virus inactivation can precipitate milk components, which protects the virus.[48] Milk also resists changes in pH compared to aqueous solutions.[46;48 cited in 17]

Sellers (1969) estimated the time needed to inactivate 99.999% of an O_1 FMDV in whole milk, by adding the virus to uninfected milk and adjusting the milk pH to 2.0, 4.0, 5.5, 5.8, 11.0, 12.0 or 13.0, for 0.5 hours to 18 hours.[42] At 4°C, this level of inactivation was estimated to occur within 1 minute at pH 2.0, 2 minutes at pH 4.0, 30 minutes at pH 5.5, 18 hours at pH 5.8, 2 hours at pH 11.0, and 2.5 minutes at pH 12.0 or 13.0. These and other data were then used to extrapolate the estimated time for 99.999% inactivation at various combinations of pH and temperature. With increased temperature and increased distance from pH 7.0-7.5, the rate of inactivation is expected to increase. At 56°C, 99.999% inactivation was estimated to require 30 minutes at pH 7.0, 7.3 or 7.6, but at pH 6.7 it would occur in 6 minutes. At 63°C, this level of inactivation would occur in 1

minute at pH 6.7, 1.2 minutes at pH 7.0, 1.4 minutes at pH 7.3 and 2 minutes at pH 7.6. At 72°C, it would require 17 seconds at pH 6.7, 40 seconds at pH 7.0, 50 seconds at pH 7.3 and 55 seconds at pH 7.6. At 80°C or 85°C, 99.999% inactivation was estimated to occur in less than 5 seconds at any pH between 6.7 and 7.6. The presence of residual virus, which was not inactivated by changes in pH, was also noted.

Sonder at al. (1990) reported that FMDV in skim milk could survive the addition of 0.1%-0.3% of consumable acids (citric acid or propionic acid) or 0.1%-0.3% hydrogen peroxide for 6 hours at 5°C.[48]This was true both for artificially contaminated milk, and for milk from infected cows. In these experiments, adding 0.1% citric or propionic acid to skim milk decreased the pH to 6.14, on average, and 0.3% citric or propionic acid lowered the pH to 4.62. When milk from different sources was tested with a constant amount of acid, the resulting pH varied between sources. In this study, 0.1% propionic or citric acid had only a marginal effect on the virus titer, while 0.2% or 0.3% citric or propionic acid inactivated FMDV in some trials but not others. In trials using skim milk from infected cows, adding 0.3% propionic acid resulted in immediate precipitation of the milk proteins.[48] Inactivation of FMDV by pH change was non-linear, and residual infectivity was detected by cell culture even after 2 days of treatment.

The same authors treated FMDV-contaminated skim milk with 0.1%-0.3% hydrochloric acid, acetic acid, fumaric acid, sulphuric acid or sodium hydroxide, to determine the effect of more extreme changes in pH for 6 hours.[48] In this study, complete inactivation of FMDV (measured in cell culture) was seen only between pH 5.2 and 5.5, and did not occur consistently. A large amount of residual virus was found when the pH was higher than 5.5, while milk proteins began to precipitate below pH 5.5 (precipitation always occurred below pH 5.2). The precipitated milk components apparently protected the virus, resulting in less effective inactivation. Residual FMDV could even be found in some samples where the pH had been lowered to 1.97 for 6 hours with 0.3% sulphuric acid. Incomplete inactivation of FMDV was observed at pH of 7.39 to 10.51 (1% to 4% 1M sodium hydroxide,); however, virus could no longer be detected by cell culture if the pH was raised to 12.88 or higher. This level of alkaline treatment rendered the milk unfit for consumption.

Using noninfected milk with FMDV added, Gorskii (1972) reported that culture at 38ºC with a 5% acidophilic starter accelerates inactivation of the virus.[4] Based on detection in mice and guinea pigs, residual virus could not be found in milk fat after acidophyllic fermentation (pH 4.1-4.9) for 4-5 hours at 38°C. Similarly, Terbruggen (1932) found that FMDV disappeared from acidified cream before the cream was manufactured into butter, but the virus persisted for up to 45 days in butter made from non-acidified cream.[1]

Virus inactivation in stored milk has been explained by the acidification of the milk as it spoils.[1;3 cited in 1;4] Terbruggen (1932) reported that FMDV in stored milk did not fully disappear until well into the acidification process, based on virus detection in guinea pigs.[1] In one batch of raw whole milk held at 37°C, the virus was still present after 12 hours, when the pH had reached 4.7. It was no longer detected at 24 hours, at a pH of 2.3. In another milk sample held at 37°C, FMDV also disappeared by 24 hours; however, the

pH of this sample was 5.6. At 17-20°C, the virus was detected at 24-25 hours, at a pH of 4.0-4.8, but it was not found after 27-34 hours, when the milk pH was 4.5. At 5°C, the virus could still be detected after 12 days (pH 5.9), but not 13 days (pH 5.6).

Commercial batch and continuous pasteurization

Most milk products are pasteurized, but some (e.g., milk used to make aged cheeses) are heated to sub-pasteurization temperatures.[43] Currently, the most common method of pasteurization for milk is the high-temperature, short-time (HTST) method.[44] The specific time and temperature requirements for HTST pasteurization vary with the milk product, and are established by regulatory agencies for a country, but are typically a minimum of 72°C for at least 15 seconds.[9;43;49] (see below for mandated temperature/time combinations in the U.S., Canada and the E.U.) The pasteurization requirements for products with a higher fat content (e.g., cream), added sugar (e.g., flavored milks), or added stabilizers (e.g., ice cream mix) are greater than for milk.[43;49]

The two basic pasteurization methods are batch and continuous pasteurization. In the batch method, the product is heated, with agitation, in a vat for a specific holding time.[43] The heating method may include steam, water, or heating coils with steam or water. This process is not usually used for milk, but it can be used for products made in smaller amounts such as cream and flavored milks.[43] It is also used for ice cream.

Continuous pasteurization is usually used to treat milk, typically in the form of a HTST pasteurizer with plate heat exchangers.[43] Raw milk is pre-warmed, then pumped through the pasteurizer under positive pressure. After being heated to at least 72ºC in the heater section, it flows through the holding tube, where it is maintained at the desired temperature for the pre-determined time. Milk that has not maintained the cutoff temperature is not allowed to continue to the cooldown stage, but is automatically diverted back to the holding tank to be re-pasteurized.

Ultra-high temperature (UHT) processing also uses a continuous flow process, but with higher temperatures (e.g., 135°C) to completely eliminate spoilage organisms.[43] The product may undergo either direct heating (e.g., injection of steam, followed by the removal of excess water) or indirect heating by mechanisms such as plate heat exchangers or tubular heat exchangers.[43] Cream is often processed by UHT pasteurization to extend its shelf life.[28]

Pasteurization standards

The temperature-time conditions used for pasteurization in the dairy industry were chosen to ensure milk safety but retain the quality and flavor of the milk.[50 cited in 28]

U.S. standards

The U.S. Public Health Service (USPHS)/Food and Drug Administration (FDA) publishes recommended pasteurization standards for Grade "A" milk and milk products

produced in the U.S.[49] In addition to milk, buttermilk and evaporated milk from cows, sheep, goats and other hooved mammals, these standards apply to cottage cheese, whey and whey products, and modified versions of these products, but not to sweetened condensed milk, infant formula, butter, cheese, puddings and ice cream or other frozen desserts.

The current U.S. pasteurization standards for Grade A milk and milk products, to be applied to all particles and held continuously at or above the given temperature for at least the corresponding specified time are given in the chart below.[49] In batch processors, all particles of milk or milk products must be held continuously for at least 30 minutes at the minimum pasteurization temperature or greater.

U.S. Milk and Milk Product Pasteurization Conditions

- 63ºC (145ºF)* for 30 minutes
- 72ºC (161ºF)* for 15 seconds (HTST)
- 89ºC (191ºF) for 1.0 second
- 90ºC (194ºF) for 0.5 seconds
- 94ºC (201ºF) for 0.1 seconds
- 96ºC (204ºF) for 0.05 seconds
- 100ºC (212ºF) for 0.01 seconds

*The temperature is to be increased by 3ºC (5ºF) if the fat content is 10% or greater, or total solids$ 18% or if the product contains added sweeteners.[49]

Eggnog Pasteurization Conditions

- 69ºC (155ºF) for 30 minutes
- 80ºC (175ºF) for 25 seconds
- 83ºC (180ºF) for 15 seconds

Buttermilk should be made from butter produced with pasteurized Grade "A" cream.[49] Whey can be made from cheese produced from pasteurized grade A milk, or from cheese produced after heating milk to at least 64ºC (147ºF) for at least 21s or at least 68ºC (153ºF) for at least 15s.[49]

In the U.S., UHT is defined as thermal processing at or above 138°C (280°F) for at least two (2) seconds.[49]

Canadian standards

The temperature/ time combinations for HTST pasteurization of milk in Canada are the same as in the U.S. Milk-based products that have less than 10% milk fat (e.g., milk and whey) are processed at 72°C for 15 seconds.[51] Milk-based products that have a milk fat content of at least 10% (e.g., cream) or have added sugar (e.g., chocolate milk), are heated at 75°C for 15 seconds. Eggnog and frozen dairy product mixes pasteurized by HTST are heated at 80°C for 25 seconds, or 83°C for 15 seconds.

In Canada, the generally accepted pasteurization conditions for batch pasteurization are 63°C for 30 minutes, if the milk-based product has less than 10% milk fat; 66°C for 30 minutes if the product has added sugar or at least 10% milk fat; and 69°C for 30 minutes for eggnog and frozen dairy product.[51]

European Union standards

Several regimens are allowed for milk and milk product pasteurization in E.U. legislation; however, HTST treatment is usually 72ºC to 75ºC for 15 to 30 s, and UHT treatment is 135ºC to 150ºC for a holding time that achieves commercial sterility (usually 2 to 3 seconds).[9]

OIE standards for international trade, for milk products from FMD-infected countries

The World Organization for Animal Health (OIE) publishes standards for the inactivation of FMDV in milk and milk products.[52] The recommendations are more stringent if these products will be fed to animals than if they are intended for human consumption. If milk products are imported from an FMD-infected country or zone with an official control program, the OIE recommends that the importing country require an international veterinary certificate that states:

- that the herd or flock of origin was not infected with FMDV, or suspected of being infected, when the milk was collected,
- that the products were processed in accordance with the procedures below, and
- after processing, the proper procedures were followed to prevent contact between the processed product and possible sources of FMDV.

If milk and cream are intended for human consumption, the OIE recommends that they be processed with one of the following time/ temperature combinations:[52]

- A minimum temperature of 132°C for at least one second (UHT)
- A minimum temperature of 72°C for at least 15 seconds (HTST), provided that the pH of the milk is less than 7.0
- A minimum temperature of 72°C for at least 15 seconds (HTST), applied twice, if the pH of the milk is 7.0 or higher

If milk is intended for animal consumption, it should be processed with one of the following time/ temperature combinations:[52]

- A minimum temperature of 72°C for at least 15 seconds (HTST), applied twice
- This HTST process, combined with another physical treatment. Examples given by the OIE for the second treatment are maintaining the milk at pH 6 for at least one hour, or additional heating to at least 72°C combined with desiccation.
- A minimum temperature of 132°C for at least one second (UHT), combined with another physical treatment, as above.

Pasteurization methods used in studies of FMDV inactivation

Most studies of FMDV inactivation have used the batch method, usually performed with laboratory equipment rather than a commercial pasteurizer. Typically, the milk is placed in tubes or bottles and immersed in a heated water bath for the specified time, followed by immediate chilling. This technique has been used to simulate HTST pasteurization as well as pasteurization at lower temperatures. The come-up and cool-down times are not precise, and they are difficult to distinguish from the holding time.[28] Because some inactivation may also occur during the come-up and cool-down times, these methods tend to overestimate the inactivation time needed in commercial (continuous) HTST pasteurization.[28] There may also be issues with evaporative cooling or splashing of milk, which might underestimate the effectiveness of the pasteurization conditions.[28]

A few studies have used laboratory apparatus to simulate continuous flow pasteurization, and one study used commercial pasteurizing equipment. The come-up and cool-down times are relatively short in continuous flow pasteurization, and can be distinguished from the holding time.[28] Flow may affect the inactivation of FMDV by time and temperature.[44] In commercial HTST processing, the flow conditions ensure that all particles of milk are held for the same period in the holding tube.[44]

Only a few studies have included temperatures (132ºC or higher) in the range used for UHT pasteurization. Both batch and continuous methods have been employed.

Some storage conditions used in experiments might affect FMDV inactivation. In one study, skim milk stored at -70°C before pasteurization was inactivated more rapidly, especially during the initial stage of heating.[22]

Experimental inactivation of FMDV by heat

FMDV inactivation in aqueous solution by heat

In clarified cell culture fluid, 90% inactivation of FMDV occurred in 0.5 minute at 61ºC, 2 minutes at 55ºC, 1 hour at 49ºC, 7 hours at 43ºC, 21 hours at 37ºC, 11 days at 21ºC, and 18 weeks at 14ºC.[45] Bachrach et al (1960) demonstrated the existence of a heat-resistant fraction of FMDV virus, and reported that it is possible to enrich the virus population for this fraction by continuous heat selection in cell culture. [53]

FMDV inactivation by heat in whole and skim milk

A number of studies examined the effect of heat on FMDV inactivation in skim and whole milk. Whether residual FMDV could be detected after heating varied with the time and temperature conditions, the pH of the milk, and the system used to detect the virus.

Batch pasteurization studies, whole milk, FMDV added to milk (Table 2)

Using inoculation into suckling mice or cell cultures, Felkai et al. (1970) reported that virus could be detected after heating FMDV-contaminated whole milk at temperatures between 50ºC and 75ºC for up to 70 seconds, or at 80ºC or 85ºC for 35 seconds.[26] No virus was found in whole milk heated at 80ºC for 70 seconds, or 90ºC for 35 seconds. There was good agreement between the two detection systems. The pH of the milk averaged 6.7 in this experiment, with a range of 6.6-7.2. The same authors reported that FMDV was not found in a sample pasteurized for a few seconds at 85ºC , using the less sensitive method of guinea pig inoculation to detect the virus.[26] Akopyan (1967) also used guinea pigs, and reported that all virus in FMDV-contaminated whole milk was inactivated after heating at 85-90ºC for 2 minutes, but some virus remained after 30 seconds or 1 minute.[54] In contrast, Kastli and Moosbrugger (1968) found that heating milk at 55°C for 10 seconds was unable to inactivate the virus, but 65°C for 30 seconds was effective, using a guinea pig inoculation system.[27] ScherningThiesen (1979) used mouse inoculation to detect FMDV, and reported virus inactivation at relatively low time and temperature combinations.[55] In FMDV-contaminated milk (pH 6.7) heated at 50-73ºC for 15 seconds, only one sample contained trace amounts of virus at 60ºC, and no virus could be detected at temperatures of 65ºC or 73ºC. Reid et al. (2006) reported that FMDV could not be found in whole milk heated at 72ºC for 25 seconds or 95ºC for 5 seconds, using cell culture to detect the virus.[34]

Sellers (1969) used cell culture for detection, and found that FMDV was inactivated at lower time/temperature combinations when the pH was 6.7 than when it was raised to 7.6.[42] In this experiment, virus was no longer detected in whole milk (pH 6.7) containing $10^{4.6}$ to $10^{5.7}$ pfu./ml, after heating at 56ºC for 10, 15 or 30 minutes; however, FMDV could still be found in pH 7.6 milk. At 63ºC, pH 7.6 milk required treatment for at least 2 minutes to decrease FMDV to undetectable levels, but 1 minute was sufficient if the milk was at pH 6.7. At 72ºC, some virus was still viable after 15 seconds, but not 30 seconds or longer, at pH 6.7. In contrast, pH 7.6 milk contained detectable levels of virus until the treatment time was 60 seconds. At 80ºC, treatment for 10 seconds but not 5 seconds inactivated the virus in milk with a pH of either 6.7 or 7.6, and 5 seconds was sufficient at 85ºC.

It should be noted that, in all of the experiments described above, virus was added to milk from noninfected cows.[26;34;42] FMDV appears to be more readily inactivated in this system than when the milk is taken from FMDV-infected cows.[22]

Batch pasteurization studies, whole milk, milk from FMDV-infected cows (Table 2)

Other studies used milk from experimentally infected cows. FMDV was occasionally found in such milk after pasteurization at 72ºC for 16 seconds, when cell culture was used to detect the virus.[15;24;29] In one set of experiments, treating milk at 72ºC for times varying from 16 seconds to 5 minutes usually resulted in undetectable levels of FMDV in cell culture, but occasional samples (including one heated for 4 minutes) still contained

viable virus.[24] Residual FMDV could be found by inoculating the treated milk into steers, even when virus was undetectable using cell culture.[15;24]
Hyde et al. (1975) reported that FMDV could still be detected after pasteurization for 16 seconds at 80ºC, using cell culture to detect the virus.[29] The virus was also reported to survive in infected milk treated at 85ºC for 16 seconds.[24] The detection system was not stated in the last instance, and may have been either cell culture or (more likely) inoculation into steers.

De Leeuw et al. (1979) tested milk held at temperatures of 100°C or greater, using inoculation into steers to detect the virus.[8] In these studies, residual virus was found in milk from FMDV-infected cows after pasteurization at 100ºC for 3 or 9 minutes, but not 100ºC for 27 minutes, 110ºC for 3 minutes, 120ºC for 30 seconds, or 135ºC for 17 seconds.[8]

One study used field samples from infected cows in Egypt, and reported that FMDV could be isolated from 40% of the milk samples after they had been pasteurized at 63ºC for 30 minutes.[56] In this study, virus could no longer be found after the milk was boiled for an unspecified time.

Continuous pasteurization studies, whole milk, FMDV added to milk (Table 2)

Commercial continuous pasteurization, usually used for HTST treatment of milk, is more accurately simulated with a similar system.[28;44] Bohm et al. (1979) pasteurized FMDV-contaminated milk at temperatures of 66ºC to 78ºC at holding times of 40 seconds.[57] Although the phosphatase test indicated that pasteurization was incomplete in the 66ºC sample, FMDV was not detected by cell culture or inoculation into steers. Samples pasteurized at 68ºC to 78ºC, which were tested only by cell culture, were also negative for virus.

Continuous pasteurization studies, whole milk, milk from FMDV-infected cows (Table 2)

Bohm et al. (1982) could not detect FMDV in milk heated at 62ºC for 40 seconds or 73ºC for 40 seconds, using either cell culture or animal inoculation.[41] However, the virus titer in this experiment was probably quite low: the milk came from intranasally infected cows, and this infectious milk was mixed at a ratio of 40:105 or 13:37 with whole and skim milk from noninfected cows before pasteurization.

Using continuous pasteurization, Tomasula et al. (2007) found that heating infected whole milk (pH 7.1) at 72ºC or 80°C and holding times of either 18.6 or 36 seconds reduced FMDV titers by 99.9% to 99.99%, but did not eliminate all infectivity.[28] In these experiments, FMDV was not detected in treated milk if cell culture was used to detect the virus; however, there was sufficient residual virus to infect some steers, even in samples that originally contained the least amount of virus (1.92×10^2 pfu/ml). Based on the number of steers that became infected, continuous HTST pasteurization appears to be

more effective than the batch pasteurization methods used in some previous experiments (e.g., Hyde et al., 1975 and Blackwell and Hyde, 1976).[28] Despite the increased efficiency, this experiment corroborated these reports in demonstrating the existence of residual, low level FMDV in whole milk after HTST pasteurization.

Tomasula et al. (2007) also tested temperatures of 80°C to 95°C for 36 seconds, applied to whole milk, and 72°C to 95°C for 36 seconds, applied to milk containing 2% fat, and found that FMDV titers were reduced by at least 99.99%, but some residual infectivity remained by animal inoculation.[28] As with lower temperatures, continuous flow pasteurization appeared to be more effective than the laboratory batch pasteurization methods used in a previous experiment (Blackwell and Hyde, 1976). Nevertheless, this experiment also confirmed that residual virus can survive temperatures of 80°C to 95°C for 36 seconds in whole milk.

Using a cruder continuous pasteurization system, Cunliffe et al. (1979) tested the effect of pasteurizing whole milk from experimentally infected cows for 2-3 seconds at 103°C, 123°C, 130°C, 138°C or 148°C.[33] All temperatures except 123°C reduced virus titers to undetectable levels in cell culture; however, residual virus could be detected by inoculation into steers at temperatures lower than 148°C. In general, the number of steers that became infected decreased as the temperature increased.

Batch pasteurization studies, skim milk, FMDV added to milk (Table 3)

Using guinea pigs for virus detection, Kastli and Moosbrugger (1968) reported that heating skim milk at 65°C for 5-30 seconds was unable to inactivate FMDV, but 73°C for 10 seconds or longer was effective.[27]

Batch pasteurization studies, skim milk, milk from FMDV-infected cows (Table 3)

Theoretically, FMDV may be inactivated more rapidly in skim milk, because it is not protected by fat. De Leeuw (1980) found that FMDV could no longer be detected in cell culture if infected skim milk (pH 7.4) was heated at 60ºC for 4 minutes, but some virus remained at holding times up to 2 minutes.[22] At 63ºC, the virus was still present in milk held for up to 1 minute but not 2 minutes.[22] Whole milk was not tested in this experiment. Using inoculation into mice to detect FMDV, Dhennin and Labie (1976) found that there was very little decrease in the virus titer when skim milk from infected cows was heated at 50ºC for times between 5 seconds and 10.7 minutes.[58] At 60ºC, however, the titer decreased rapidly at holding times greater than 80 seconds, and the virus was nearly undetectable after 5.3 minutes. When the milk was held for 20 seconds at temperatures of 58ºC to 68ºC, the amount of residual virus decreased rapidly as the temperature increased between 60ºC and 68ºC.

Several studies reported that FMDV could not be detected by cell culture after infected skim milk had been heated at 72ºC for 15 seconds or longer,[15;24;32] and one study reported that it was undetectable after heating the milk at 72ºC for 12 seconds.[22] Residual virus could still be

found by inoculation into steers, although fewer animals became infected when milk was treated for 2 minutes compared to 1 minute.[24] Dhennin and Labie (1976) were able to detect very low levels of virus by inoculation into mice, after pasteurization of skim milk at temperatures of 70ºC, 73ºC, 75ºC, 78ºC, 83ºC, 85ºC or 90ºC, with a holding time of 20 seconds.[58] De Leeuw et al. (1979) also detected residual FMDV, using inoculation into steers, after heating skim milk at 80ºC for up to 5 minutes, 90ºC or 100ºC for up to 3 minutes, or 110ºC for 30 seconds.[8] Virus could no longer be found after heating the milk at 110ºC for 3 minutes, or 120ºC or 135ºC for 30 seconds. The results were similar for whole and skim milk heated at high temperatures of 100ºC (3 min), 110ºC (3 min), 120ºC (30 s) and 135ºC.

Continuous pasteurization studies, skim milk, milk from infected cows (Table 3)

Bohm et al. (1982) could not detect FMDV in infectious skim milk pasteurized at 73ºC for 40 seconds, using either cell culture or animal inoculation.[41]

The thermal death curve for FMDV in whole or skim milk

Walker et al. (1984) generated a curve for FMDV inactivation at temperatures of 80ºC to 150ºC, using a large number of samples tested at the Plum Island Animal Disease Center (PIADC) in the U.S. and the Central Veterinary Institute (CVI) in the Netherlands.[40] The FMDV viruses A_3 (strain Mecklenburg) and O_1 (strain Weerselo) were used, and the FMDV concentration in the milk was $10^{5.9}$ pfu/ml (mean), with a range of $10^{3.7}$ $10^{6.8}$ pfu/ml. Residual virus was detected by inoculating the treated milk samples into cattle (2 ml intradermally into the tongue and 48 ml by the intramuscular route). Because skim and whole milk had similar thermal sensitivity at high temperatures,[8] data from both types of milk were plotted together.[40] The authors noted that, because only a limited number of data points were used to generate this curve, it has limited statistical significance.[40]

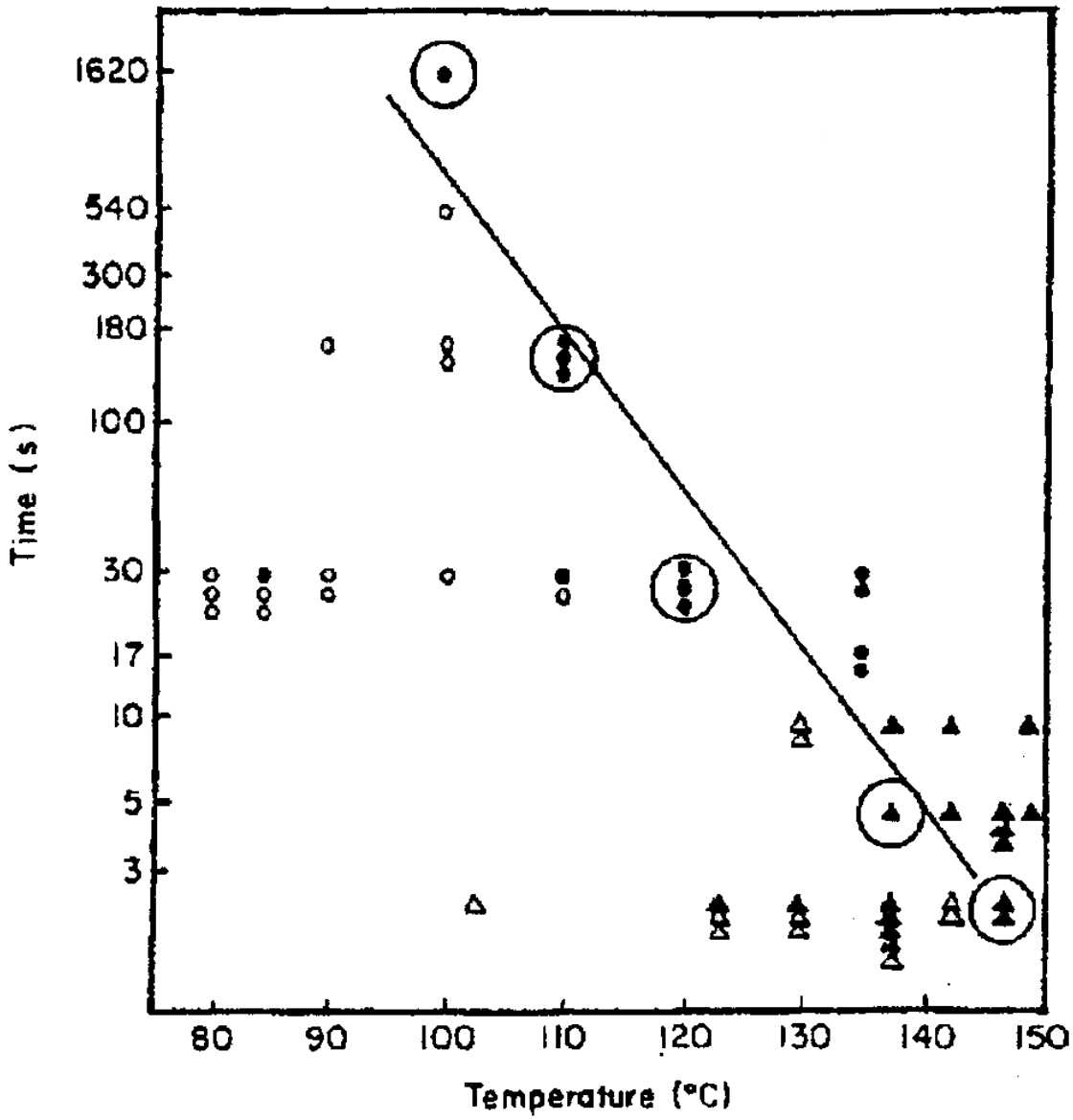

Figure 1: Thermal death time curve of FMD virus in milk, semi log plot by time. Data points obtained at the CVI: ●FMD-virus inactivated; ○FMD virus survived. Data points obtained at the PIADC: ▲FMD-virus inactivated; Δ FMD virus survived. Point ⊙,⊙, used to hand fit line for thermal death time curve. *Reprinted from Journal of Biological Standardization, Vol. 12, J.S. Walker, P.W. de Leeuw, J.J. Callis, J.G. van Bekkum, The thermal death time curve for foot-and-mouth disease virus contained in primarily infected milk, Pages 185-189, Copyright (1984), with permission from Elsevier.*

In this thermal death curve, points to the right of the curve would be expected to result in FMDV inactivation, while points to the left would not.[40] Some individual data points on this graph demonstrate virus inactivation in 3-5 seconds at approximately 138ºC. Based on the fitted curve, however, complete inactivation would require more than 3 minutes at 110ºC, more than 30 seconds at 125ºC, more than 17 seconds at 130ºC, more than 5

seconds at 140ºC, and more than 3 seconds at 145ºC. The data from this curve also suggest that there may be no clear endpoint for virus inactivation.[40]

FMDV inactivation by heat in cream (Table 4)

The high fat content of cream appears to protect FMDV from inactivation. Blackwell and Hyde (1976) reported that, when three cream samples containing $10^{1.8}$ to $10^{6.3}$ pfu/ml were heated at 72ºC for 0.25 minutes, virus was still detectable by cell culture except in the sample with $10^{1.8}$ pfu/ml.[24] All three samples were able to infect steers by inoculation. In this study, and in another experiment with virus concentrations of $10^{5.1}$ to $10^{5.8}$ pfu/ml, FMDV was undetectable by cell culture in all samples pasteurized for 0.25 minutes at 93ºC, but residual virus was still found by animal inoculation.[15;24] In another experiment, cream samples contained $10^{6.9}$ to $10^{7.6}$ pfu/ml, and the virus was still detected in cell culture after pasteurization for 0.25 minutes at 93ºC.[30]

Double pasteurization

Double pasteurization has not been evaluated in controlled experiments.[44] However, field experience suggests that it is effective. During the 1982 FMD outbreak in Denmark, milk was heated at 72°C for 15 seconds, then 80°C for 3 seconds, sometimes followed by lowering the pH to less than 4.5.[6;17] No outbreaks were reported when approximately18 million kg of this treated milk was fed to domesticated animals.[6;17]

Heat stability of different FMDV serotypes

Most experiments have used only a single serotype of FMDV, often type A or type O. Felkai et al. (1970) compared the heat resistance of the serotypes O_1 (Bacsalmas) , A (Riems), and C (Loupoigne).[26] Based on detection in guinea pigs, all three serotypes showed similar heat resistance in milk or cream, although it appeared that the O_1 virus was least thermoresistant, the serotype C virus was the most thermoresistant, and the serotype A virus was intermediate between these two types. There were no significant differences in the thermoresistance of these three serotypes when milk was tested for residual virus in suckling mice or porcine kidney cells.

Scherning-Thiesen (1979) heated milk contaminated with three European serotype O strains, two European serotype A strains, and two European serotype C strains at 50ºC, 55ºC, 60ºC, 65ºC or 73ºC for 15 seconds.[55] Milk contaminated with one serotype A strain retained a trace amount of infectivity after heating at 60ºC, no residual virus was detected in any other samples after heating at this temperature, and no FMDV could be detected in any milk samples heated at 65ºC or 73ºC for 15 seconds. However, the serotype C strains appeared to be more stable than the serotype O or serotype A viruses when heated at 50ºC for 15 seconds.

There do not appear to be any published reports on the inactivation of Asia-1, SAT-1, SAT-2 or SAT-3 viruses by heat.

Evaporation

Evaporation heats milk to the boiling point to remove water and concentrate the product.[43] Evaporation may be used in the production of liquid products such as evaporated milk, or as a method of concentrating a milk product before spray-drying.[43]

Whole milk pasteurized at 72ºC or 80ºC for 16 seconds , then evaporated at 65ºC for 1 hour, did not contain detectable virus when tested in cell culture; however, the samples still contained residual virus by inoculation into steers.[29] Whole milk pasteurized at 72ºC for 3 minutes, then evaporated, also remained infectious by animal inoculation.[24] Skim milk pasteurized at 72ºC for 15 seconds, then evaporated at 65ºC for 1 hour, contained residual virus; however, skim milk pasteurized at 72ºC for 30 seconds before evaporation did not infect steers inoculated with the samples.[24] It should be noted that commercial evaporators are much more sophisticated than the simple system used in these experiments.

Dehydration/ drying

The effect of drying milk products is difficult to assess.[17] Although this process is expected to decrease FMDV infectivity by 10-fold or more,[17] this has not been formally tested, and the effects are expected to vary with the drying method.[17] The material is often heated to facilitate drying, and this probably also reduces infectivity.[17]

If residual virus survives drying, it might be stable for an extended period. Milk proteins appear to help stabilize FMDV. In a study of freeze-drying, the addition of 10% skim milk powder helped maintain virus viability.[59] Some of these FMDV preparations lost little infectivity when stored for up to 168 days at 4ºC, and maintained residual activity for at least 84 days at 37ºC. FMDV in dried milk powder was reported to survive for up to two years.[60 cited in 29;61]

Felkai et al. (1970) examined the situation where dried FMDV might contaminate milk before pasteurization.[26] They found that the heat resistance of FMDV that had been dried for 2-3 days, before being added to whole milk, was similar to FMDV grown in cell cultures and not dried, if milk was pasteurized for 35 or 70 seconds, and infectivity was evaluated in cell cultures and suckling mice. Neither dried nor fresh virus could be detected in milk heated at 90ºC for 35 seconds, or 80ºC for 70 seconds. However, differences in stability were found when the milk was pasteurized for only a few seconds, and guinea pigs were used for virus detection.[26] The fresh virus could not be found when the milk was heated momentarily at 85-90ºC, but significant amounts of the dried virus could still be detected even at 100ºC.

FMDV inactivation during the production of butter and buttermilk (Table 5)

Cream used to produce butter is usually pasteurized at 95ºC or greater, to destroy the enzymes and microorganisms that would reduce its keeping quality.[43] Blackwell (1978) reported that FMDV could still be detected, in cell culture, after cream from infected

cows was pasteurized at 93ºC for 16 seconds.[30] The virus titer dropped below the limits of detection after the cream was churned into butter in the laboratory. However, samples of butter and butter oil (prepared from the butter), still contained infectious virus when inoculated into cattle, even after the butter had been stored at 4ºC for 45 days. At this time, the pH of the product, which was 5.9 after butter production, had decreased to 5.4.

Using inoculation into guinea pigs, Terbruggen (1932) detected virus for up to 8 days in unsalted butter made from FMDV-contaminated raw cream, when the butter was frozen for 11 hours, then stored at 17ºC.[1] Under the same conditions, the virus was found for up to 9 days in unsalted butter. When the butter was not frozen before storage, FMDV persisted for 26 hours but not 4 days in unsalted butter, and for up to 4 days in salted butter. Survival was much longer when the butter was stored at refrigeration temperatures. In some replicates, FMDV was recovered for up to 26 days in unsalted butter and 45 days in salted butter stored at 5ºC. In contrast, no virus could be found in butter made from acidified cream.[1] The cream was stored for 18-24 hours at 16-18ºC before butter manufacture, and virus was undetectable by the end of this storage time.

In the experiments above, both Blackwell (1978) and Terbruggen (1932) detected FMDV in buttermilk, which was a by-product of butter manufacture.[1;30]

FMDV inactivation in the production of cheese (Table 6)

During cheese manufacture, milk is clarified and either pasteurized or heated to sub-pasteurization temperatures (the latter is used for aged cheeses), before being processed into cheese.[43] In some parts of the world, cheeses such as Brie, Camembert (France), and Queijo da Ilha (Azores) are sometimes made from raw cow's milk.[9] Common steps in cheese production include coagulation (by bacterial fermentation and the addition of rennet) in temperature controlled vats, and the removal of whey.[43] The specific manufacturing process, including the temperature, pH and salt content, varies between cheeses. In the E.U., cheeses made from raw milk must be aged for at least 60 days if they are allowed in trade.[62]

Blackwell (1976) examined virus survival in cheddar cheese made from both raw and heat-treated milk from FMDV-infected cows.[25] In cheddar made from raw milk, the amount of virus decreased from $10^{4.2}$-$10^{5.4}$ pfu/ml in the milk to $10^{2.5}$ pfu/ml in the curd, and 10 to 100 pfu/ml in the whey after salting, and to levels undetectable by cell culture in both curds and whey after pressing. FMDV could still be detected at this time by animal inoculation, and continued to be detected during curing of the cheddar at 2ºC for up to 60 days. It was no longer present at 120 days. In parallel experiments using heat-treated milk, FMDV was undetectable by cell culture after heating the milk at 67ºC for 10 seconds, 15 seconds or 1 minute, although some virus could still be found in milk heated at 63ºC for 6 seconds.[25] Some samples of 1-day-old cheddar made from this milk, including 2 of 3 batches made from milk heated at 67 ºC for 1 minute, still contained FMDV by inoculation into steers. However, the virus could no longer be detected in any cheddar samples after curing for 30 days. Scherning-Thiesen (1979) reported that FMDV was detected in cheddar cheese 8 hours after beginning the manufacturing process, which included heating the milk at 63ºC for 15 minutes before processing.[55] The virus could no

longer be detected after 14 days of curing. FMDV was added to milk from noninfected cows in this study, and the detection system was inoculation into mice.

Blackwell (1976) reported that FMDV was undetectable by either cell culture or animal inoculation in mozzarella cheese (pH 5.1) made from milk pasteurized at 72ºC for 16 seconds.[25] The authors speculated that the combination of the acidic environment and processing conditions (e.g., heating the curd in hot water before kneading and stretching) was responsible for the rapid inactivation of the virus.

FMDV survived the manufacturing process for camembert cheese (pH 5.2) made from milk pasteurized at 72ºC for 16 seconds.[25] In camembert, FMDV was still detectable by animal inoculation after 21 days of ripening at 4ºC, but not after 35 days. The survival of FMDV in this acidic product may have been enhanced by its protein and milk fat content. In contrast, Scherning-Thiesen (1979) reported that FMDV could not be detected in camembert cheese (pH 5.2) after 2-3 days of ripening, although it was still present one day after cheese manufacture.[55] In this study, the virus was added to noninfected milk, and the milk was heated at 63ºC for 15 minutes before processing. Inoculation into mice was used for virus detection. Bohm et al. (1982) found that FMDV could not be detected in camembert cheese at any stage of processing, when it was made from milk pasteurized at 73ºC for 40 seconds.[41] This milk came from cows inoculated by the intranasal route, and it was mixed with whole and skim milk from noninfected cows at a ratio of 13:37 before pasteurization; thus, the virus content was likely to be low.

Kihm et al. (1979) reported that FMDV persisted in emmenthal cheese made from the milk of infected cows through the piling stage, but it was no longer found 1, 2 or 5 days after the cheese was manufactured.[14] Virus detection was by cell culture and inoculation into steers. Factors that may have contributed to virus inactivation included heating to 53ºC during manufacture, together with the very low pH during the first 24 hours after manufacture (the pH is then maintained at 5.2 to 5.6 during the 4 months this cheese is usually stored).[14] In earlier experiments, FMDV was not detected in emmenthal cheese during manufacturing;[63 cited in 14] however, the detection techniques were not as sensitive, and the milk was contaminated with FMDV rather than being collected from infected cows.[14]

Bohm et al. (1982) could not detect FMDV in a hard cheese made from milk heated at 62ºC for 40 seconds, or in edam made from milk pasteurized at 73ºC for 40 seconds.[41] In both cases, virus was not detected by cell culture or animal inoculation in the pasteurized or heated milk, or at any stage of cheese manufacture. However, the milk for both cheeses was obtained from intranasally infected cows, and this milk was mixed with whole and skim milk from noninfected cows before pasteurization and cheese manufacture. The ratio of infectious milk to noninfected milk was 40:105 for the hard cheese, and 13:37 for edam.

During an FMD outbreak in Egypt, FMDV was found in 6.7% of samples of kareish cheese, a soft cheese made from skim milk.[64]

FMDV inactivation in the production of casein (Table 6)

Cunliffe et al. (1977) reported that FMDV survived in casein and sodium caseinate produced from a variety of sources, including raw skim milk from infected cows, HTST pasteurized (72ºC x 15 s) skim milk from infected cows, and HTST pasteurized milk that had $10^{5.2}$-$10^{6.6}$ pfu/ml FMDV added before pasteurization.[32] The amount of FMDV in the casein was low, whether it was made from raw or pasteurized milk. The virus titer was generally below the limits of detection in cell culture, and residual virus infected some but not all inoculated steers. In a follow-up study, FMDV was reported to persist in some batches of dried casein (made from HTST pasteurized, infectious skim milk) stored at ambient temperature for at least 42 days, but not in casein stored for 84 days.[65] Based on animal inoculation, the amount of virus in dried casein appeared to be very low.[65] Blackwell (1978) also reported that FMDV could be detected in casein made from milk collected from infected cows.[31] In contrast, Bohm et al. (1982) did not detect FMDV in acid casein or rennet casein made from infectious skim milk that had been pasteurized at 73ºC for 40 seconds.[41] Although acid conditions (pH 4.5-4.7) are used to make casein, FMDV might be protected within the casein micelles formed during aggregation and precipitation of the product.[32]

FMDV inactivation in the production of whey (Table 6)

Acid whey (pH 4.5-4.6) and sweet whey (pH 6.1 to 6.7) are by-products made during the production of various cheeses.[9] Acid whey is also produced during casein manufacture.[9;31] Whey has an average dry matter content of 6.6%, with lactose (4.9%) as the main component.[9] It contains negligible amounts of fat or casein, which protect FMDV from inactivation.[31] Products that may be made from whey include lactose and whey powder.[9] Whey powder is a main ingredient in calf milk replacer.[9]

Blackwell (1978) evaluated the survival of FMDV in sweet whey produced during the manufacture of cheddar and camembert cheeses.[31] In this experiment, the cheddar was made from infectious milk that had been heated at 67ºC for 1 minute, and the camembert was produced from milk pasteurized at 72ºC for 16 seconds. Using cell culture, FMDV was undetectable in the sweet whey from camembert manufacture. However, residual virus was found in the whey from both cheeses, by inoculation into steers. Kihm et al. (1979) detected FMDV in whey from the manufacture of emmenthal cheese.[14] In contrast, Bohm et al. (1982) did not find FMDV in whey from camembert, edam or a hard cheese, by either cell culture or animal inoculation.[41] The camembert and edam were made from milk pasteurized at 73ºC for 40 seconds, and the hard cheese was made from milk heated at 62ºC for 40 seconds.[41] As discussed previously (see cheese section), the milk used to manufacture these cheeses had been mixed with milk from noninfected cattle.

Kastli and Moosbrugger (1968) reported that FMDV added to pH 6.8 whey (made from noninfected milk) was no longer detected after heating the whey at 55ºC to 73ºC for at least 10 seconds.[27] This study used inoculation into guinea pigs to detect the virus.

FMDV is less likely to survive in acid whey than sweet whey.[9] This virus was not found in acid whey made from casein, using both cell culture and inoculation into steers to detect the virus.[31] Similarly, no virus was detected in acid whey made from casein, when the casein was manufactured from skim milk pasteurized at 73ºC for 40 seconds.[41]

FMDV inactivation in the production of purified whey constituents (Table 7)

Lactose, α-lactalbumin and β-lactoglobulin produced from infectious sweet whey did not contain viable virus, even by inoculation into steers.[31] These products were generated by continuous heating, precipitation and solubilization at pH extremes, which probably inactivated the residual virus.[31]

FMDV inactivation in the production of yogurt (Table 8)

The low pH of fermented products such as yogurt, combined with the temperatures used during their production, may be sufficient to inactivate FMDV.[44] Using field samples of milk from naturally infected cows in Egypt, El-Alfy (1998) found that FMDV did not survive after the milk was made into yogurt.[56] The yogurt was manufactured by traditional techniques that included culture with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Further details were not given.

FMDV inactivation in milk and milk products from species other than cattle (Table 9)

Milk from animals other than cattle can be found in commercial production; however, there is very little information on FMDV inactivation in such milk products. In one experiment, an Egyptian serotype O virus was added to buffalo milk collected from uninfected animals.[64] The virus survived heating at 63ºC for 30 minutes, but not boiling for 5 minutes, when cell culture was used to detect the virus. In a similar study using cow's milk, no FMDV was detected in milk treated at 63ºC for 2 minutes or longer, using the same cell type for virus detection.[42] Compared to cow's milk, buffalo milk has higher acidity, contains greater quantities of milk solids, and has a higher fat content (with larger fat globules), and a higher casein content. All of these factors could affect FMDV inactivation. In Italy, buffalo milk (sometimes unpasteurized) is used to produce some mozzarella cheeses.[9]

There is no published information on FMDV inactivation in milk from sheep or goats. FMDV infections are often subclinical in small ruminants, which increases the risk that virus may be present in milk products before an outbreak becomes apparent.[62] While there is very little international trade in sheep and goat milk, some countries export significant quantities of sheep or goat milk cheeses.[62] Unpasteurized milk is used in many traditional recipes for these cheeses, including Roquefort (France) and Serra da Estrela (Portugal).[9] In the E.U., such cheeses must be aged for a minimum of 60 days if they are allowed in trade.[62]

Risk analyses for FMDV survival in milk

Several published risk analyses have evaluated the risks of feeding FMDV-contaminated milk products to animals. These analyses are based on published reports for FMDV inactivation in various milk products, together with the estimated infectious doses for animals by various routes, and the volume of milk that can be consumed by various species.

Evidence from the field

Infected, nonpasteurized milk has been linked to virus transmission in FMDV outbreaks.[6-8] However, there is no field evidence to indicate whether products pasteurized by ordinary methods are likely to cause outbreaks. De Leeuw (1979) noted that, during the period when FMD was endemic in Europe (and vaccination was conducted), outbreaks were never linked to exported milk products that had been heat treated in any way.[8] During the 1982 FMD outbreak in Denmark, milk was heated to 72°C for 15 seconds, then 80°C for 3 seconds, sometimes followed by lowering the pH to less than 4.5.[6;17] No outbreaks were associated with this milk, although approximately18 million kg was fed to domesticated animals.[6;17]

The minimum infectious dose for FMDV

The minimum infectious dose has been estimated for some species of animals, although there is still some uncertainty in these values.[17;19;23] Such experiments are often based on small numbers of animals.[23] In some studies, only a single animal was exposed to each dose.[23] Species may differ in their susceptibility to different strains,[23] and variability is also expected between individual animals.[21] Factors such as mouth abrasions can affect the dose needed to infect an animal by ingestion.[17]

Cattle are relatively difficult to infect by ingestion,[23;36] and have an estimated minimum infectious dose of 10^5-10^6 $TCID_{50}$.[17] In unpublished results cited by Sellers (1971), less than half of the cattle fed $10^{5.8}$ to $10^{6.8}$ ID_{50} became infected within 72 hours. [23] In another study, cattle did not become infected when the tongue was exposed to $10^{7.8}$ ID_{50} for 10 minutes unless the tongue epithelium was damaged.[66 cited in 23] However, abrasions around the mouth may reduce the amount of virus needed for infection.[67 cited in 17] Calves might also become infected by the insufflation of virus when drinking milk.[23;36] Cattle are very susceptible to infection in aerosols, and may be infected by intranasal doses as low as 10 ID_{50}[68 cited in 23] or 12.5 $TCID_{50}$.[67] Pigs are more sensitive to the ingestion of FMDV than cattle, and the minimum infectious dose is estimated to be 10^4-10^5 $TCID_{50}$.[17] While some studies reported that pigs did not become infected unless they were fed at least $10^{5.0}$ ID_{50},[23] one group reported infection with $10^{3.9}$ ID_{50} of a pig-adapted type C strain.[69 cited in 23]

The minimum infectious dose should be used as an indicator of susceptibility, rather than an absolute value.[17] This value is often defined as the minimum dose that is necessary to

result in infection (or disease), implying that exposure lower doses will not result in infection.[19] However, this is not accurate, as the probability that at least one animal will become infected depends on the number of animals as well as the amount of virus.[17;19;20] As the number of exposed animals increases, there is a greater chance that at least one will become infected.[19] Because FMDV is highly infectious, infection of a single animal could result in an outbreak. Thus, the defined minimum infectious doses from experiments are probably the doses that have a low probability of resulting in infection and/or disease, and large populations could probably become infected with even lower doses.[17;19;20]

Risk analyses

A risk assessment published by the Australian Quarantine and Inspection Service (AQIS) for the importation of dairy products from countries not free of FMD concluded that conventional pasteurization was inadequate to inactivate FMDV in cattle, and only commercially sterilized milk products would be acceptable.[70] This analysis was based on an assessment of published studies documenting residual virus in milk products (including virus detected by injection into steers). It did not consider the minimum infectious dose for animals, the routes of inoculation, or the FMDV levels likely to be present in milk.

In one widely-cited analysis, Donaldson (1997) considered a possible scenario in which 10% of a herd of dairy cattle becomes infected with FMDV, and the milk from these animals contains $10^{6.6}$ ID_{50} FMDV/ ml.[6] Given hypothetical but credible dilution factors of a 10-fold dilution when the infectious milk is mixed with milk from uninfected cows, an additional 5-fold dilution when the farm's milk is mixed with uninfected farms, and a further 10-fold reduction at the dairy, he estimated that the milk would contain $10^{3.9}$ ID_{50}/ml before pasteurization. Based on a 4-5 $\log_{10}$ unit reduction in infectivity by HTST pasteurization (72°C for 15 seconds) reported in some experiments, [42;55 cited in 6] the final concentration of virus would be $10^{1.9}$ to $10^{2.9}$ $1D_{50}$ /liter.[6] Assuming an oral minimum infectious dose of $10^{5.0}$ ID_{50} for pigs and $10^{6.0}$ ID_{50} for calves, Donaldson concluded that a single pig that drank this milk would have to ingest at least 125 liters and a single calf would have to ingest at least 1,250 liters for a high probability of infection. The daily intake of milk is 0.5 to 9 liters for calves, and 0.5 to 4.5 liters for pigs,[23] and these volumes would be physically impossible for the animal to ingest.[6] Although it would be theoretically possible for an animal to become infected if large numbers were exposed, Donaldson concluded that the risk of infection from pasteurized milk products is low. Based on similar reasoning, he estimated that the possibility of infection from milk treated at higher temperatures (e.g., UHT) or HTST followed by an additional heat treatment or acid treatment, is remote. In this analysis, Donaldson assessed the residual viruses detected by cattle inoculation but not cell culture as having little relevance to risk assessments for feeding milk products to animals, although he felt they could be relevant for milk components used by the pharmaceutical industry. This assessment was based on the high minimum infectious dose for animals by ingestion.

Rather than estimating the amount of milk needed to infect one pig, Sutmoller and Vose (1997) used the values from Donaldson's example to estimate the probability that larger

or smaller groups of animals would become infected by drinking infectious milk.[20] They concluded that, if 20 pigs were each fed one liter of milk containing $10^{1.9}$ to $10^{2.9}$ $1D_{50}$ /liter (approximately 500 pfu/ l), and the minimum infectious dose by the oral route in pigs is 10^5, the probability of one pig becoming infected is approximately 6.7%. If 100 pigs were fed 1 liter of the milk, the probability that at least one pig would become infected is approximately 29%.

A 2006 analysis by the Animal Health and Welfare panel of the European Food Safety Authority (EFSA) assessed the risk of transmitting FMDV in unpasteurized and pasteurized milk products, using published research and models.[9] The risk was evaluated as a worst case scenario, assuming that FMDV-contaminated milk is not diluted by milk from uninfected herds. The model used animal susceptibility data, together with estimates of the amount of product that would be fed to animals, and the risk of a single animal becoming infected and transmitting virus to others. In addition to raw milk, the risk was considered to be moderate to high for butter from pasteurized cream, unpasteurized cheese with a pH that was greater than 6 during its manufacture, and sweet whey generated when making cheese from raw milk. Products considered to have a very low to low risk included HTST (72ºC for 15s) pasteurized milk; unpasteurized cheese if the pH was < 6 during its manufacture; pasteurized cheese if the pH> 6 was during this process; and sweet whey (pH > 6) from pasteurized cheese processing. Products considered to have a negligible to very low risk included milk pasteurized by UHT methods using a temperature higher than 132ºC and time greater than 1 second; pasteurized cheese if the pH was less than 6 during cheese-making; unpasteurized cheese with a pH< 5; acid whey (pH < 6) from either pasteurized or unpasteurized cheese processing; whey powder; and yogurt. In this analysis, HTST treatment was expected to decrease FMDV concentrations in whole milk by 4-5 $\log_{10}$ units of infectivity, on average, assuming that the pH of milk was within the normal range and not elevated. The risk for feeding whey (not dried) was evaluated as equivalent to the cheese it was made from. For cheese, additional virus may be inactivated during the ripening process, but this was not assessed.

Vaccinated cattle

One risk analysis on the importation of milk from countries that vaccinate dairy cattle suggested that this poses a negligible risk.[2] No outbreaks have been traced to this source.[2]

Summary

There are a number of challenges in assessing the risks of feeding milk products from FMDV-infected animals. One is the great variety of milk products, with varying pH, milk fat and protein composition, which can be obtained from various species of animals. Most studies have tested milk and skim milk from cattle, with a smaller number examining butter, certain cheeses, casein, whey and purified components from milk products. A single Egyptian study reported that FMDV was inactivated during yogurt production; however, detailed information on the production method was not provided.[56] Milk products with added sugar, eggnog, many types of cheese, sour cream, ice cream and other products have not been tested. One study examined FMDV inactivation in milk

from water buffalo,[64] but there are currently no reports concerning other non-bovine products such as sheep or goat cheeses, or mozzarella from buffalo milk.

A second challenge is the relevance of experiments to commercial processes conducted on milk products from naturally infected animals. Considerations in experimental design include the use of FMDV-contaminated milk vs. milk from infected animals, the viral titer before treatment, and possibly the serotype and strain of virus. In many early studies, milk samples were collected from small numbers of infected animals with varying amounts of FMDV in their milk. Most studies have used intramammary and intravenous inoculation, which appears to result in higher virus titers in milk than intranasal inoculation or contact exposure.[13;15;38] While some authors argue that this provides a margin of safety, others feel this may overestimate the treatment conditions needed.[40] In addition, there is relatively little published information on actual FMDV titers in milk from the field,[17] which adds an element of uncertainty to the discussion.

Many early experiments used laboratory equipment to simulate commercial pasteurization conditions. There is some concern that this might not accurately measure the effects of modern commercial pasteurizers on FMDV titers.[28] In addition, the effects of some processes such as clarification, homogenization and dehydration have not been measured in experiments.[17;44] The sensitivity of the detection method also has a significant influence on the results. In particular, the importance of the residual heat-resistant or pH-resistant virus detected by injection into cattle is controversial. Lastly, when conducting risk analyses, there is some uncertainty in the minimum infectious dose.[17;19;23] In particular, there is little information for species such as sheep, goats, water buffalo and exotic ruminants. Even data for cattle and pigs are based on relatively few replicates, and individual susceptibility may differ from the norm.[17;19;23] An additional consideration in risk analysis is the number of animals that are likely to be exposed.[17;19;20] Given the wide variety of experimental and natural variables, it is likely that the FMDV risk of a product can be evaluated only in terms of its probability of safety, rather than in absolute terms.

Risk analyses vary in their assessments, and there may be disagreements on the importance of the residual virus detected only by animal inoculation. Some analyses, such as that by the Animal Health and Welfare panel of the EFSA,[9] have identified some milk products as "very low to low risk" or "negligible to very low risk," while other products are considered to be of higher risk. In general, lower risk products include those processed at low pH and/or higher temperatures. The OIE standards for the inactivation of FMDV in milk and milk products, if the product is intended to be fed to animals, currently include the following time/ temperature combinations:[52]

- A minimum temperature of 72°C for at least 15 seconds (HTST), applied twice
- This HTST process, combined with another physical treatment. Examples given by the OIE for the second treatment are maintaining the milk at pH 6 for at least one hour, or additional heating to at least 72°C combined with desiccation.
- A minimum temperature of 132°C for at least one second (UHT), combined with another physical treatment, as above.

While there appears to be no formal proof of these procedures from published controlled experiments, results from the field during the 1982 outbreak in Denmark suggest that they are effective. In this outbreak, milk was heated to 72°C for 15 seconds, then 80°C for 3 seconds, sometimes followed by lowering the pH to less than 4.5.[6;7] No outbreaks were associated with this milk, even though approximately18 million kg was fed to domesticated animals.[6;7] In addition, we are not aware of any reports where milk and milk products treated to OIE standards for animal consumption have resulted in outbreaks of FMD.

The OIE recommendations also include the important provision that the importing country require an international veterinary certificate stating that the herd or flock of origin was not infected with FMDV, or suspected of being infected, at the time the milk was collected.[52] This additional safeguard helps ensure that the amount of virus present in milk, if any, is already low before processing. The recommendations also stipulate that proper procedures should be followed to prevent contact between the processed product and possible sources of FMDV. It should be noted that the OIE recommendations for milk fed to animals are more stringent than those for milk and cream intended for human consumption.[52] This is consistent with the EFSA analysis, which considers milk treated by single HTST treatment (acceptable for human but not animal consumption in OIE guidelines, if the milk pH is less than 7) to be "very low to low" risk if fed to animals, but does not place it in the "negligible to very low" category.[9] It also implies that stringent precautions should be in place to prevent milk products imported for human consumption from being fed to animals. All member countries of the OIE have agreed that the OIE standards for treatment of milk and milk products render the products acceptable for human or animal consumption in FMD free countries.

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References

(1) Terbruggen F. Ueber die Haltbarkeit des Maul- und Klauenseuchevirus in Milch und Molkereiprodukten. I. Mitteilung. *Dtsch Tieraerztl Wochenschr* 1932;40:129-134.

(2) Sutmoller P, Casas OR. The risks posed by the importation of animals vaccinated against foot and mouth disease and products derived from vaccinated animals: a review. *Rev Sci Tech* 2003;22:823-835.

(3) Galloway LA. The survival of virus in milk and dried milk and the effect of heat on the virus suspended in milk or contained in dried milk. 248. 1931. 4th Progress report of the Foot-and-Mouth-Disease Research Committee.

(4) Gorskii BV. [Disinfection of skim milk, whey and buttermilk in foot and mouth disease]. Uch Zap Kazan Vet Inst 1972; 112:6-11.

(5) Bauer K. Foot- and-mouth disease as zoonosis. *Arch Virol Suppl* 1997;13:95-97.

(6) Donaldson AI. Risks of spreading foot and mouth disease through milk and dairy products. *Rev Sci Tech* 1997;16:117-124.

(7) Hyslop NS. The epizootiology and epidemiology of foot and mouth disease. *Adv Vet Sci Comp Med* 1970;14:261-307.

(8) de Leeuw PW, van Bekkum JG. Some aspects of foot and mouth disease virus in milk. Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Lindholm, Denmark; 12-14 Jun 1979. Rome: Food and Agriculture Organization (FAO), 1979. p. 79-87.

(9) Animal Health and Welfare panel of EFSA. Animal health risks of feeding animals with ready-to-use dairy products without further treatment. *EFSA Journal* 2006;347:1-21.

(10) Report of the Committee of Inquiry on Foot-and-Mouth Disease 1968. Part 1. *Vet Rec* 1969;84:471-473.

(11) Hedger RS, Dawson PS. Foot-and-mouth disease virus in milk: an epidemiological study. *Vet Rec* 1970;87:186-188.

(12) Burrows R. The persistence of foot-and-mouth disease virus in sheep. *J Hyg (Lond)* 1968;66:633-640.

(13) Burrows R, Mann JA, Greig A, Chapman WG, Goodridge D. The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. *J Hyg (Lond)* 1971;69:307-321.

(14) Kihm U, Bommeli W, Kurmann N. Persistence of FMD virus in Emmentaler cheese. Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Lindholm, Denmark; 12-14 Jun 1979. Rome: Food and Agriculture Organization (FAO), 1979. p. 75-78.

(15) Blackwell JH, McKercher PD, Kosikowski FV, Carmichael LE, Gorewit RC. Concentration of foot-and-mouth disease virus in milk of cows infected under simulated field conditions. *J Dairy Sci* 1982;65:1624-1631.

(16) McVicar JW, Sutmoller P. Foot-and-mouth disease in sheep and goats: early virus growth in the pharynx and udder. *Proc Annu Meet US Anim Health Assoc*.1971; 75:194-199.

(17) Alexandersen S.Virus inactivation kinetics. Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Greifswald Insel-Riems, Germany; 20-23 Sept 2005. p. 192-200. Available at: http://www.fao.org/ag/againfo/commissions/en/eufmd/resgr_riems.html. Accessed 12 Mar 2012.

(18) Callens M, de Clercq K, Gruia M, Danes M. Detection of foot-and-mouth disease by reverse transcription polymerase chain reaction and virus isolation in contact sheep without clinical signs of foot-and-mouth disease. *Vet Q* 1998;20 Suppl 2:S37-S40.

(19) French NP, Kelly L, Jones R, Clancy D. Dose response relationships for foot and mouth disease in cattle and sheep. *Epidemiol Infect* 2002;128:325-332.

(20) Sutmoller P, Vose DJ. Contamination of animal products: the minimum pathogen dose required to initiate infection. *Rev Sci Tech* 1997;16:30-32.

(21) Ryan E, MacKay D, Donaldson A. Foot-and-mouth disease virus concentrations in products of animal origin. *Transbound Emerg Dis* 2008;55:89-98.

(22) de Leeuw PW, Tiessink JW, van Bekkum JG. Aspects of heat inactivation of foot-and-mouth disease virus in milk from intramammarily infected susceptible cows. *J Hyg (Lond)* 1980;84:159-172.

(23) Sellers RF. Quantitative aspects of the spread of foot and mouth disease. *Vet Bull* 1971;41:431-440.

(24) Blackwell JH, Hyde JL. Effect of heat on foot-and-mouth disease virus (FMDV) in the components of milk from FMDV-infected cows. *J Hyg (Lond)* 1976;77:77-83.

(25) Blackwell JH. Survival of foot-and-mouth disease virus in cheese. *J Dairy Sci* 1976;59:1574-1579.

(26) Felkai V, Sólyom F, Szent-Iványi M, Wagner A. A száj és körömfájás vírus hőtűrése tejben. *Magyar Allatorvosok* 1970;25:378-380.

(27) Kästli P, Moosbrugger GA. La destruction du virus aphteux par la chaleur dans les produits laitiers. *Schweiz Arch Tierheilkd* 1968;110:89-94.

(28) Tomasula PM, Kozempel MF, Konstance RP et al. Thermal inactivation of foot-and-mouth disease virus in milk using high-temperature, short-time pasteurization. *J Dairy Sci* 2007;90:3202-3211.

(29) Hyde JL, Blackwell JH, Callis JJ. Effect of pasteurization and evaporation on foot-and-mouth disease virus in whole milk from infected cows. *Can J Comp Med* 1975;39:305-309.

(30) Blackwell JH. Persistence of foot-and-mouth disease virus in butter and butter oil. *J Dairy Res* 1978;45:283-285.

(31) Blackwell JH. Potential transmission of foot-and-mouth disease in whey constituents. *J Food Prot* 1978;41:631-633.

(32) Cunliffe HR, Blackwell JH. Survival of foot-and-mouth disease virus in casein and sodium caseinate produced from the milk of infected cows. *J Food Prot* 1977;40:389-392.

(33) Cunliffe HR, Blackwell JH, Walker JS. Inactivation of milkborne foot and mouth virus at ultra-high temperature. *J Food Prot* 1979;42:135-137.

(34) Reid SM, Parida S, King DP et al. Utility of automated real-time RT-PCR for the detection of foot-and-mouth disease virus excreted in milk. *Vet Res* 2006;37:121-132.

(35) Donaldson AI, Gloster J, Harvey LD, Deans DH. Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreaks in Brittany, Jersey and the Isle of Wight in 1981. *Vet Rec* 2012;110:53-57.

(36) Kitching RP. Clinical variation in foot and mouth disease: cattle. *Rev Sci Tech* 2002;21:499-504.

(37) Orsel K, Dekker A, Bouma A, Stegeman JA, de Jong MC. Quantification of foot and mouth disease virus excretion and transmission within groups of lambs with and without vaccination. *Vaccine* 2007;25:2673-2679.

(38) de Leeuw PW, van Bekkum JG, Tiessink JW. Excretion of foot-and-mouth disease virus in oesophageal-pharyngeal fluid and milk of cattle after intranasal infection. *J Hyg (Lond)* 1978;81:415-425.

(39) Sadir AM, Cacchione R, Pérez H, Rivenson S. Curvas de inactivacion del virus de la fiebre aftosa en leche total y descremada de vacas vacunadas. *Gaceta Veterinaria* 1980;42:32-37.

(40) Walker JS, de Leeuw PW, Callis JJ, van Bekkum JG. The thermal death time curve for foot-and-mouth disease virus contained in primarily infected milk. *J Biol Stand* 1984;12:185-189.

(41) Bohm HO. Inaktivierung von MKS-Viren in kasein, milch und milchprodukten. *Deutsche Molkerei-Zeitung* 1982;103:68, 70-72.

(42) Sellers RF. Inactivation of foot-and-mouth disease virus in milk. *Br Vet J* 1969;125:163-168.

(43) Goff HD. Dairy product processing equipment. In: Kutz M, ed. *Handbook of farm, dairy, and food machinery*. Norwich, NY: William Andrew Publishing; 2007;193-214.

(44) Tomasula PM, Konstance RP. The survival of foot-and-mouth disease virus in raw and pasteurized milk and milk products. *J Dairy Sci* 2004;87:1115-1121.

(45) Bachrach HL, Breese SS, Callis JJ, Hess WR, Patty RE. Inactivation of foot-and-mouth disease virus by pH and temperature changes and by formaldehyde. *Proc Soc Exp Biol Med* 1957;95:147-152.

(46) Sellers RF. The inactivation of foot-and-mouth disease virus by chemicals and disinfectants. *Vet Rec* 1968;83:504-506.

(47) Tsioulpas A, Lewis MJ, Grandison AS. A study of the pH of individual milk samples. *International Journal of Dairy Technology* 2007;60:96-97.

(48) Sonder E, Ackermann M, McCullough KC, Kihm U. Inactivation of foot and mouth disease virus in skimmed milk with propionic acid, citric acid and hydrogen peroxide. *Rev Sci Tech* 1990;9:1139-1155.

(49) Food and Drug Administration, US Department of Health and Human Services. Pasteurized milk ordinance. 2009. Washington, D.C. . Available at:http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/MilkSafety/NationalConferenceonInterstateMilkShipments NCIMSModelDocuments/UCM209789.pdf. Accessed 20 Feb 2012.

(50) Holsinger VH, Rajkowski KT, Stabel JR. Milk pasteurization and safety: A brief update. *Rev Sci Tech Off Int Epiz* 1997;16:451.

(51) Canadian Food Inspection Agency. Dairy establishment inspection manual. Available at: http://www.inspection.gc.ca/english/fssa/dailai/man/estman/contentse.shtml. Accessed Apr. 11, 2012.

(52) World Organization for Animal Health (OIE). Foot and mouth disease. *Terrestrial Animal Health Code*. Paris, France: OIE; 2010.

(53) Bachrach HL, Patty RE, Pledger RA. Thermal resistant populations of foot-and-mouth disease virus. *Proc Soc Exp Biol Med* 1960;103:540.

(54) Akopyan ESh. Resistance to heat of foot and mouth disease virus in milk. *Tr Vses Inst Vet Sanit* 1967;28:65-67.

(55) Scherning-Thiesen K. Survival of foot-and-mouth disease virus in milk and cheese. Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Lindholm, Denmark; 12-14 Jun 1979. Rome: Food and Agriculture Organization (FAO), 1979. p. 68-71.

(56) El-Alfy MB. Chemical and bacteriological characteristics of milk from foot- and mouth-disease (FMD) infected cows. *Ann Agr Sci, Moshtohor* 1998;36:949-956.

(57) Bohm HO, Krebs H, Kehrer E. Pasteurisierungsversuche mit milch nach kontamination mit maul-klauenseuchevirus. *Milchwissenschaft* 1979;34:253-256.

(58) Dhennin L, Labie J. Thermoresistance du virus de la fievre aphteuse dans le lait de vaches infectees. *Bull Acad Vet de France* 1976;19:243-249.

(59) Ferris NP, Philpot RM, Oxtoby JM, Armstrong RM. Freeze-drying foot-and-mouth disease virus antigens. I. Infectivity studies. *J Virol Methods* 1990;29:43-52.

(60) Nikitin EE, Vladimirov AG. ^{60} *Veterinariya* 2012;42:99-101.

(61) Cottral GE. Persistence of foot-and-mouth disease virus in animals, their products and the environment. *Bull Off Int Epizoot* 1969;71:549-568.

(62) Sherman DM. The spread of pathogens through trade in small ruminants and their products. *Rev Sci Tech* 2011;30:207-217.

(63) Terbruggen F. Ueber die Haltbarkeit des Maul- und Klauenseuchevirus in Milch und Molkereiprodukten. II. Mitteilung-Kase und Molken. *Dtsch Tieraerztl Wochenschr* 1932;40:529-538.

(64) Deeb AAM, Ahmmed AF, Salem SAH, El-Hassanine MIM. Detection of foot and mouth disease virus (FMDV) in milk and kareish cheese with trials to control in milk. *Assiut Vet Med J* 2010;56:144-155.

(65) Cunliffe HR, Blackwell JH, Walker JS. Persistence of foot and-mouth disease virus in dried casein. *J Food Prot* 1978;41:706-707.

(66) Cottral GE, Patty RE, Gailiunas P, Scott FW. Sensitivity of cell cultures, cattle, mice, and guinea-pigs for detection of nineteen foot-and-mouth disease viruses. *Bull Off Int Epizoot* 1965;63:1607-1625.

(67) Donaldson AI, Gibson CF, Oliver R. Infection of cattle by airborne foot-and-mouth disease virus: Minimal doses with O_1 and SAT2 strains. *Res Vet Sci* 1987;43:339-346.

(68) Eskildsen MK. Esperimental pulmonary infection of cattle with foot-and-mouth-disease virus. *Nord Vet Med* 1969;21:86-91.

(69) Nathans I. Vaccinatie van varkens tegen monden klauwzeer met geinactiveerd virus bevattende entestoffen, Thesis, Rijksuniver. Utrecht; 1965.

(70) Heng NH, Wilson DW. Risk assessment on the importation of milk and milk products (excluding cheese) from countries not free from foot and mouth disease. *Rev Sci Tech* 1993;12:1135-1146.

Appendix: Tables

Table 1: FMDV inactivation in cow's milk: Effect of changing pH

Virus Serotype, Subtype, Strain	Product and Source of FMDV	Treatment Conditions	Effect of Treatment	Detection Method	Reference
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 2.0 held for 1 min (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 4.0 held for 2 min (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 5.5 held for 0.5 h, 2 h, 4 h, or 6 h (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 5.8 held for 2 h (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to $10^{3.3}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 5.8 held for 4 h (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to $10^{3.0}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV	pH 5.8 held for 6 h (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to $10^{2.9}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969

	added				
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 5.8 held for 18 h (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 11.0 held for 0.5 h (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to $10^{3.5}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 11.0 held for 2 h, 4 h, 6 h or 18 h (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 12.0 held for 2.5 min (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows; FMDV added	pH 13.0 held for 2.5 min (4ºC)	Decrease from $10^{7.1}$ pfu./ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, Lausanne	Milk, skim; from noninfected cows; FMDV added	Treated with 0.1% (v/v) citric acid; 6 h at 5ºC	Decrease from approx. 10^5 - 10^8 $TCID_{50}$/10 ml before treatment to approx. 10^3 - 10^7 $TCID_{50}$/ 10 ml after treatment	Cell culture (BHK-21/C13 cells)	Sonder et al., 1990
O_1, Lausanne	Milk, skim	Treated with 0.1% (v/v) propionic acid; 6 h at 5ºC	Decrease from approx. 10^5 - 10^8 $TCID_{50}$/10 ml before treatment to approx. 10^3 - 10^5 $TCID_{50}$/ 10 ml after treatment	Cell culture (BHK-21/C13 cells)	Sonder et al., 1990
O_1,	Milk, skim;	Treated	Decrease from	Cell	Sonder et al.,

Lausanne	from noninfected cows; FMDV added	with 0.2% citric or propionic acid (v/v); 6 h at 5ºC	approx. 10^5 - 10^8 $TCID_{50}$/10 ml to undetectable levels in 56-59% of trials; In other trials, up to 10^3 $TCID_{50}$/10 ml (citric acid) or up to 10^4 $TCID_{50}$/10 ml (propionic acid) remained	culture (BHK-21/C13 cells)	1990
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Treated with 0.2% propionic acid (v/v); 6 h at 5 ºC	Decrease from approx. 10^3 - 10^5 $TCID_{50}$/ 10 ml before treatment to ≤ 10 $TCID_{50}$/ 10 ml after treatment	Cell culture (BHK-21/C13 cells)	Sonder et al., 1990
O_1, Lausanne	Milk, skim; from noninfected cows; FMDV added	Treated with 0.3% citric or propionic acid (v/v); 6 h at 5ºC	Decrease from approx. 10^5 - 10^8 $TCID_{50}$/10 ml to undetectable levels in 65-80% of trials; In other trials, up to 10^3 $TCID_{50}$/10 ml (citric acid) or up to 10^2 $TCID_{50}$/ 10 ml (propionic acid) remained	Cell culture (BHK-21/C13 cells)	Sonder et al., 1990
O_1, Lausanne	Milk, skim; from noninfected cows; FMDV added	Treated with 0.1% to 0.3% (v/v) H_2O_2; 6 h at 5ºC	No effect on virus titer	Cell culture (BHK-21/C13 cells)	Sonder et al., 1990
O_1, Lausanne	Milk, skim; from noninfected cows; FMDV added	Treated with 0.2% (v/v) H_2O_2; 72 h at 5ºC	Some inactivation of FMDV	Cell culture (BHK-21/C13 cells)	Sonder et al., 1990
O_1, Lausanne	Milk, skim; from noninfected cows;	Treated with 1% (v/v) H_2O_2; 4-5 h at 5ºC	99% decrease in FMDV levels	Cell culture (BHK-21/C13	Sonder et al., 1990

	FMDV added			cells)	
O_1, Lausanne	Milk, skim; from noninfected cows; FMDV added	Treated with 3% (v/v) H_2O_2; 4-5 h at 5ºC	99.999% decrease in FMDV levels	Cell culture (BHK-21/C13 cells)	Sonder et al., 1990

** Detection limit of cell culture system in this experiment

Table 2: FMDV inactivation in whole milk from cattle: Heat treatment

Virus Serotype, Subtype, Strain	Product and Source of FMDV	Treatment Conditions	Effect of Treatment	Detection Method	Reference
Temperatures less than 72ºC, Batch pasteurization					
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Momentary pasteurization (laboratory, batch process) at 50ºC	Virus still detected	Inoculation into guinea pigs	Felkai et al., 1970
O, three European strains (86, 105, 110)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 50ºC x 15 s	Decrease from 10^4 - $10^{4.5}$ mouse ID_{50} before treatment to $10^{2.4}$- $10^{3.5}$ mouse ID_{50} after treatment	Inoculation into mice	Scherning-Thiesen, 1979
A, two European strains (101, 107-115)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 50ºC x 15 s	Decrease from $10^{2.5}$ - 10^3 mouse ID_{50} before treatment to $10^{1.5}$- $10^{2.8}$ mouse ID_{50} after treatment	Inoculation into mice	Scherning-Thiesen, 1979

C, two European strains (90,116)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 50ºC x 15 s	Decrease from $10^{3.3}$ - $10^{4.1}$ mouse ID_{50} before treatment to $10^{3.3}$ – $10^{3.6}$ mouse ID_{50} after treatment	Inoculation into mice	Scherning-Thiesen, 1979
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 50ºC x 70 s	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Momentary pasteurization (laboratory, batch process) at 55ºC	Virus still detected	Inoculation into guinea pigs	Felkai et al., 1970
O	Milk, whole, from noninfected cows, FMDV added; pH 6.3	Pasteurization (laboratory, batch process) at 55ºC x 5-10 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Milk, whole, from noninfected cows, FMDV added; pH 6.3	Pasteurization (laboratory, batch process) at 55ºC x 15-30 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O, three European strains (86,	Milk, whole; from	Pasteurization (laboratory, batch	Decrease from 10^{4} - $10^{4.5}$ mouse	Inoculation into mice	Scherning-Thiesen, 1979

105, 110)	noninfected cows, FMDV added; pH 6.7	process) at 55ºC x 15 s	ID_{50} before treatment to 10-32 mouse ID_{50} after treatment		
A, two European strains (101, 107-115)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 55ºC x 15 s	Decrease from $10^{2.5}$ - 10^{3} mouse ID_{50} before treatment to 10-20 mouse ID_{50} after treatment	Inoculation into mice	Scherning-Thiesen, 1979
C, two European strains (90,116)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 55ºC x 15 s	Decrease from $10^{3.3}$ - $10^{4.1}$ mouse ID_{50} before treatment to $10^{2.3}$ - $10^{2.8}$ mouse ID_{50} after treatment	Inoculation into mice	Scherning-Thiesen, 1979
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 55ºC x ≤ 70 s	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 56ºC x 5 min	Decrease from $10^{4.6}$ - $10^{5.7}$ pfu/ml before treatment to < 10** to $10^{1.5}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected	Pasteurization (laboratory, batch process) at	Decrease from $10^{4.5}$- $10^{6.0}$ pfu/ml before	Cell culture (BHK21 cells)	Sellers, 1969 c

	cows, FMDV added; pH 7.6	56ºC x 5 min	treatment to $10^{3.4}$ - $10^{5.0}$ pfu/ml after treatment		
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 56ºC x 10-30 min	Decrease from $10^{4.6}$ - $10^{5.7}$ pfu/ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 56ºC x 10 min	Decrease from $10^{4.5}$ - $10^{6.0}$ pfu/ml before treatment to $10^{2.7}$ - $10^{4.2}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969 c
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 56ºC x 15 min	Decrease from $10^{4.5}$ - $10^{6.0}$ pfu/ml before treatment to $10^{2.5}$ - $10^{3.6}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969 c
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 56ºC x 30 min	Decrease from $10^{4.5}$ - $10^{6.0}$ pfu/ml before treatment to $10^{1.7}$ - $10^{3.2}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969 c
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Momentary pasteurization (laboratory, batch process) at 60ºC	Virus still detected	Inoculation into guinea pigs	Felkai et al., 1970
O	Milk,	Pasteurization	Virus not	Inoculation	Kastli and

	whole, from noninfected cows, FMDV added; pH 6.3	(laboratory, batch process) at 60ºC x 5-30 s	detected	into guinea pigs	Moosbrugger, 1968
O, three European strains (86, 105, 110)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 60ºC x 15 s	Decrease from 10^4 - $10^{4.5}$ mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
A, two European strains (101, 107-115)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 60ºC x 15 s	Decrease from $10^{2.5}$ - 10^3 mouse ID_{50} before treatment, to trace amounts or undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
C, two European strains (90,116)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 60ºC x 15 s	Decrease from $10^{3.3}$ - $10^{4.1}$ mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 60ºC x ≤ 70 s	Virus still detected	Inoculation into suckling mice or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, British Field Strain 1860	Milk, whole; from noninfected cows,	Pasteurization (laboratory, batch process) at 63ºC x	Decrease from $10^{4.9}$ - $10^{6.1}$ pfu/ml before treatment to	Cell culture (BHK21 cells)	Sellers, 1969

	FMDV added; pH 6.7	1-15 min	< 10** pfu/ml after treatment		
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 63ºC x 1 min	Decrease from $10^{4.0}$ - $10^{6.0}$ pfu/ml before treatment to < 10** - $10^{3.7}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 63ºC x 2-15 min	Decrease from $10^{4.0}$ - $10^{6.0}$ pfu/ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
Serotype not identified	Milk; field samples from FMDV-infected cows in Egypt	Pasteurization at 63ºC x 30 min	Virus survived in 40% of samples	Cell culture (bovine kidney cells)	El-Alfy, 1998
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Momentary pasteurization (laboratory, batch process) at 65ºC	Virus still detected	Inoculation into guinea pigs	Felkai et al., 1970
O	Milk, whole, from noninfected cows, FMDV added; pH 6.3	Pasteurization (laboratory, batch process) at 65ºC x 5 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Milk, whole,	Pasteurization (laboratory,	Virus not detected	Inoculation into guinea	Kastli and Moosbrugger,

	from noninfected cows, FMDV added; pH 6.3	batch process) at 65ºC x 10-30 s		pigs	1968
O, three European strains (86, 105, 110)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 65ºC x 15 s	Decrease from 10^4 - $10^{4.5}$ mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
A, two European strains (101, 107-115)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 65ºC x 15 s	Decrease from $10^{2.5}$ - 10^3 mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
C, two European strains (90,116)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 65ºC x 15 s	Decrease from $10^{3.3}$ - $10^{4.1}$ mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 65ºC x ≤ 70 s	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added;	Momentary pasteurization (laboratory, batch process) at 70ºC	Virus not detected	Inoculation into guinea pigs	Felkai et al., 1970

	pH 6.6-7.2 (mean 6.7)				
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 70ºC x ≤ 70 s	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
Temperatures less than 72ºC; Continuous pasteurization					
O_1, Kaufbeuren	Milk, whole (fat content 3.7-3.9%) from noninfected cows; FMDV added; pH 6.5-6.7	Pasteurization at 66ºC x 40 s	Decrease from $10^{6.2}$ to $10^{6.5}$ $TCID_{50}$ before treatment, to undetectable levels by cell culture or animal inoculation after treatment	1. Cell culture (BHK21 cells) 2. Inoculation into steers (1-2 ml intradermal into tongue + 40 ml intramuscular	Bohm et al., 1979
O_1, Kaufbeuren	Milk, whole (fat content 3.7-3.9%) from noninfected cows; FMDV added; pH 6.5-6.7	Pasteurization at 68ºC x 40 s	Decrease from $10^{6.2}$ to $10^{6.5}$ $TCID_{50}$ before treatment, to undetectable levels by cell culture after treatment	Cell culture (BHK21 cells)	Bohm et al., 1979
O_1, Kaufbeuren	Milk, whole (fat content 3.7-3.9%) from noninfected cows; FMDV added; pH 6.5-6.7	Pasteurization at 70ºC x 40 s	Decrease from $10^{6.2}$ to $10^{6.5}$ $TCID_{50}$ before treatment, to undetectable levels by cell culture after	Cell culture (BHK21 cells)	Bohm et al., 1979

			treatment		
Temperature 72-73ºC; Batch pasteurization					
O_1, Brugge	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 15 s	Decrease from $10^{4.2}$ - $10^{5.4}$ pfu/ml before treatment to < $10^{0.4}$** - $10^{2.0}$ pfu/ml after treatment	Cell culture (primary bovine kidney cells)	Blackwell et al., 1982
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows; mean pH 7.15	Pasteurization (laboratory, batch process) at 72ºC x 16 s	Decrease from $10^{6.7}$ - $10^{7.5}$ pfu/ml before treatment to $10^{1.6}$ - $10^{3.0}$ pfu/ml after treatment	Cell culture (primary bovine kidney cells)	Hyde et al., 1975
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 72ºC x 15 s	Decrease from $10^{4.4}$- $10^{6.3}$ pfu/ml before treatment to < 10** - $10^{1.6}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 72ºC x 15 s	Decrease from $10^{4.4}$ - $10^{6.2}$ pfu/ml before treatment to < 10** - $10^{4.5}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 72ºC x 30-60 s	Decrease from $10^{4.4}$ - $10^{6.3}$ pfu/ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British	Milk,	Pasteurization	Decrease	Cell culture	Sellers, 1969

Field Strain 1860	whole; from noninfected cows, FMDV added; pH 7.6	(laboratory, batch process) at 72ºC x 30 s	from $10^{4.4}$ - $10^{6.2}$ pfu/ml before treatment to < 10** - $10^{3.4}$ pfu/ml after treatment	(BHK21 cells)	
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 72ºC x 45 s	Decrease from $10^{4.4}$ - $10^{6.2}$ pfu/ml before treatment to < 10** - $10^{1.7}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 72ºC x 60 s	Decrease from $10^{4.4}$ - $10^{6.2}$ pfu/ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 0.25 to 5.0 min	Decrease from $10^{5.0}$ - $10^{6.4}$ pfu/ml before treatment to < $10^{0.4}$** to $10^{1.2}$ pfu/ml after treatment, by cell culture; Virus still detected in 3/3 samples by inoculation into steers	1. Cell culture (primary bovine kidney cells); 2.. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell and Hyde, 1976
O, UKG 34/2001	Milk, whole; from noninfected	Pasteurization (laboratory, batch process) at	Decrease from $10^{6.2}$ $TCID_{50}$/ml before	Cell culture (primary bovine thyroid cells)	Reid et al., 2006

	cows, FMDV added	72ºC x 25 s to 5 min	treatment to undetectable levels		
O	Milk, whole, from noninfected cows, FMDV added; pH 6.3	Pasteurization (laboratory, batch process) at 73ºC x 5 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Milk, whole, from noninfected cows, FMDV added; pH 6.3	Pasteurization (laboratory, batch process) at 73ºC x 10-30 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O, three European strains (86, 105, 110)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 73ºC x 15 s	Decrease from 10^4 - $10^{4.5}$ mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
A, two European strains (101, 107-115)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 73ºC x 15 s	Decrease from $10^{2.5}$ - 10^3 mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
C, two European strains (90,116)	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 73ºC x 15 s	Decrease from $10^{3.3}$ - $10^{4.1}$ mouse ID_{50} before treatment to undetectable levels	Inoculation into mice	Scherning-Thiesen, 1979
Temperatures 72-73ºC; Continuous pasteurization					
O_1, Kaufbeuren	Milk, whole (fat	Pasteurization at 72ºC x 40 s	Decrease from $10^{6.2}$	Cell culture (BHK21	Bohm et al., 1979

	content 3.7-3.9%) from noninfected cows; FMDV added; pH 6.4-6.7		to $10^{6.5}$ $TCID_{50}$ before treatment, to undetectable levels by cell culture after treatment	cells)	
O, 01/ UK	Milk, whole (fat content 6%); from FMDV-infected cows; pH 7.1	Pasteurization at 72ºC x 18.6 s at flow rate of 10L/h (continuous, commercial-type, HTST, plate and frame pasteurizer)	Decrease from $10^{2.3}$ – $10^{4.0}$ pfu/ml before treatment, to undetectable levels, by cell culture; Virus still detected in some steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if mice negative (2 ml intradermal into tongue + 35 ml intramuscular)	Tomasula et al., 2007
O, 01/ UK	Milk, whole (fat content 6%);from FMDV-infected cows; pH 7.1	Pasteurization at 72ºC x 36 s at flow rate of 20L/h (continuous, commercial-type, HTST, plate and frame pasteurizer)	Virus not detected by cell culture or animal inoculation (however, residual infectivity was detected in samples pasteurized at 80ºC x 36 s)	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if mice negative (2 ml intradermal into tongue + 35 ml intramuscular)	Tomasula et al., 2007
O_1	Milk, whole; from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 13 (infectious milk): 37 (noninfected milk); Mixed milk	Virus not detected	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml	Bohm et al., 1982

		pasteurized (tubular pasteurizer) at 73ºC for 40 s		intramuscular)	
Temperatures 75ºC to 99°C; Batch pasteurization					
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 75ºC x ≤ 70 s	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 80ºC x 5 s	Decrease from $10^{6.3}$ pfu/ml before treatment to $10^{1.1}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 7.6	Pasteurization (laboratory, batch process) at 80ºC x 5 s	Decrease from $10^{6.3}$ pfu/ml before treatment to $10^{1.1}$ pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 80ºC x 10-15 s	Decrease from $10^{6.3}$ pfu/ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added;	Pasteurization (laboratory, batch process) at 80ºC x 10-15 s	Decrease from $10^{6.3}$ pfu/ml before treatment to < 10** pfu/ml after	Cell culture (BHK21 cells)	Sellers, 1969

	pH 7.6		treatment		
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows; mean pH 7.15	Pasteurization (laboratory, batch process) at 80ºC x 16 s	Decrease from $10^{6.7}$ - $10^{7.5}$ pfu/ml before treatment to $10^{0.91}$ - $10^{2.1}$ pfu/ml after treatment	Cell culture (primary bovine kidney cells)	Hyde et al., 1975
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 80ºC ≤ 35 s	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 80ºC x 70 s	Virus not detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Momentary pasteurization (laboratory, batch process) at 85ºC	Virus not detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, British Field Strain 1860	Milk, whole; from noninfected cows, FMDV added; pH 6.7	Pasteurization (laboratory, batch process) at 85ºC x 5-15 s	Decrease from $10^{6.0}$ pfu/ml before treatment to < 10** pfu/ml after treatment	Cell culture (BHK21 cells)	Sellers, 1969
O_1, British Field Strain	Milk, whole;	Pasteurization (laboratory,	Decrease from $10^{6.0}$	Cell culture (BHK21	Sellers, 1969

1860	from noninfected cows, FMDV added; pH 7.6	batch process) at 85ºC x 5-15 s	pfu/ml before treatment to < 10** pfu/ml after treatment	cells)	
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 85ºC x 0.25 min	Virus still detected	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell and Hyde, 1976
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 85ºC x 35 s	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O	Milk, whole; from noninfected cows; FMDV added	Pasteurization (laboratory, batch process) at 85-90ºC x 0.5 to 1 min	Virus still detected	Inoculation into guinea pigs	Akopyan, 1967
O	Milk, whole; from noninfected cows; FMDV added	Pasteurization (laboratory, batch process) at 85-90ºC x 2 min	Virus not detected	Inoculation into guinea pigs	Akopyan, 1967
O_1, Bacsalmas; A, Riems; C,	Milk, whole; from	Pasteurization (laboratory, batch	Virus not detected	Inoculation into suckling mice;	Felkai et al., 1970

Loupoigne	noninfected cows, FMDV added; pH 6.6-7.2 (mean 6.7)	process) at 90ºC x 35 s		or Cell culture (porcine kidney cells)	
O, UKG 34/2001	Milk, whole; from noninfected cows, FMDV added	Pasteurization (laboratory, batch process) at 95ºC x 5 s	Decrease from $10^{6.2}$ $TCID_{50}$/ml before treatment to undetectable levels	Cell culture (primary bovine thyroid cells)	Reid et al., 2006
Temperatures 75ºC to 99°C; Continuous pasteurization					
O_1	Milk, whole; from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 40 (infectious milk): 105 (noninfected milk); Mixed milk pasteurized (tubular pasteurizer) at 62ºC for 40 s	Virus not detected	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
O_1, Kaufbeuren	Milk, whole (fat content 3.7-3.9%) from noninfected cows; FMDV added; pH 6.4-6.6	Pasteurization at 74ºC x 40 s	Decrease from $10^{6.2}$ to $10^{6.5}$ $TCID_{50}$ before treatment, to undetectable levels by cell culture after treatment	Cell culture (BHK21 cells)	Bohm et al., 1979
O_1, Kaufbeuren	Milk, whole (fat content 3.7-3.9%) from noninfected	Pasteurization at 76ºC x 40 s	Decrease from $10^{6.2}$ to $10^{6.5}$ $TCID_{50}$ before treatment, to	Cell culture (BHK21 cells)	Bohm et al., 1979

	cows; FMDV added; pH 6.4-6.6		undetectable levels by cell culture after treatment		
O_1, Kaufbeuren	Milk, whole (fat content 3.7-3.9%) from noninfected cows; FMDV added; pH 6.4-6.6	Pasteurization at 78ºC x 40 s	Decrease from $10^{6.2}$ to $10^{6.5}$ $TCID_{50}$ before treatment, to undetectable levels by cell culture after treatment	Cell culture (BHK21 cells)	Bohm et al., 1979
O, 01/ UK	Milk, whole(fat content 6%); from FMDV-infected cows; pH 7.1	Pasteurization at 80ºC x 18.6 s at flow rate of 10L/h (continuous, commercial-type, HTST, plate and frame pasteurizer)	Decrease from $10^{2.3}$ – $10^{4.0}$ pfu/ml before treatment, to undetectable levels, by cell culture; Virus still detected in some steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if mice negative (2 ml intradermal into tongue + 35 ml intramuscular)	Tomasula et al., 2007
O, 01/ UK	Milk, whole(fat content 6%);; from FMDV-infected cows; pH 7.1	Pasteurization at 80ºC x 36 s at flow rate of 20L/h (continuous, commercial-type, HTST, plate and frame pasteurizer)	Decrease from $10^{2.3}$ – $10^{4.0}$ pfu/ml before treatment, to undetectable levels, by cell culture; Virus still detected in some steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into suckling mice if cell culture negative; 3. Inoculation into steers if mice negative (2 ml intradermal into tongue + 35 ml intramuscular)	Tomasula et al., 2007
O, 01/ UK	Milk,	Pasteurization	Decrease	1. Cell culture	Tomasula et al.,

	whole(fat content 6%); from FMDV-infected cows; pH 7.1	at 95ºC x 36 s at flow rate of 20L/h (continuous, commercial-type, HTST, plate and frame pasteurizer)	from $10^{4.5}$ pfu/ml before treatment, to undetectable levels, by cell culture; Virus still detected in 1 of 2 steers	(primary bovine kidney cells); 2. Inoculation into suckling mice if cell culture negative 3. Inoculation into steers if mice negative (2 ml intradermal into tongue + 35 ml intramuscular)	2007
Temperatures 100ºC or higher					
O_1, Weerselo	Milk, whole; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 100ºC x 3-9 min	Virus titer $10^{5.8}$-$^{6.4}$ pfu/ ml before treatment; Virus still detected after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
O_1, Weerselo	Milk, whole; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 100ºC x 27 min	Decrease from $10^{5.7}$ pfu/ ml before treatment to undetectable levels after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, continuous process) at 102ºC x 2-3 s	Decrease from $10^{6.1}$ pfu/ml before treatment, to undetectable levels by cell culture; Virus still detected in steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers (40 ml intradermal into tongue + 48 ml intramuscular)	Cunliffe et al., 1979
O_1, Weerselo	Milk, whole;	Pasteurization (laboratory,	Virus titer $10^{5.7}$-$10^{5.8}$	Inoculation into steers (5	De Leeuw et al., 1979

	from FMDV-infected cows; pH 6.7-7.4	batch process) at 110ºC x 3 min	pfu/ ml before treatment; Virus not detected after treatment	ml intradermal into tongue + 20-25 ml intramuscular)	
O_1, Weerselo	Milk, whole; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 120ºC x 0.5 min	Virus titer $10^{6.4}$ pfu/ ml before treatment; Virus not detected after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, continuous process) at 123ºC x 2-3 s	Virus titer $10^{5.1}$ - $10^{6.3}$ pfu/ml before treatment; undetectable levels to $10^{0.9}$ pfu/ml after treatment, by cell culture; Virus (some batches of milk) still detected in steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers (40 ml intradermal into tongue + 48 ml intramuscular)	Cunliffe et al., 1979
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, continuous process) 130ºC x 2-3 s	Decrease from $10^{4.9}$ - $10^{6.3}$ pfu/ml before treatment, to undetectable levels after treatment, by cell culture; Virus still detected in 2 of 3 batches of	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers (40 ml intradermal into tongue + 48 ml intramuscular)	Cunliffe et al., 1979

			milk, by inoculation into steers		
O_1, Weerselo	Milk, whole; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 135ºC x 17 s	Virus titer $10^{6.0}$-$^{6.5}$ pfu/ml before treatment; Virus not detected after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, continuous process) at 138ºC x 2-3 s	Virus titer $10^{3.7}$ -$10^{6.4}$ pfu/ml before treatment; not detected by cell culture after treatment; Virus still found in 1 of 5 batches of milk by inoculation into steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers (40 ml intradermal into tongue + 48 ml intramuscular)	Cunliffe et al., 1979
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, continuous process) at 148ºC x 2-3 s	Decrease from 4.4-5.2 $\log_{10}$ pfu/ml before treatment, to undetectable levels in cell culture or steers after treatment	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers (40 ml intradermal into tongue + 48 ml intramuscular)	Cunliffe et al., 1979
Experiments with dried FMDV added to milk					
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, Dried FMDV added;	Momentary pasteurization (laboratory, batch process) at 80ºC	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970

	pH 6.6-7.2 (mean 6.7)				
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, Dried FMDV added; pH 6.6-7.2 (mean 6.7)	Momentary pasteurization (laboratory, batch process) at 90ºC	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, Dried FMDV added; pH 6.6-7.2 (mean 6.7)	Momentary pasteurization (laboratory, batch process) at 100ºC	Virus still detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
O_1, Bacsalmas; A, Riems; C, Loupoigne	Milk, whole; from noninfected cows, Dried FMDV added; pH 6.6-7.2 (mean 6.7)	Pasteurization (laboratory, batch process) at 90ºC x 35 s	Virus not detected	Inoculation into suckling mice; or Cell culture (porcine kidney cells)	Felkai et al., 1970
Pasteurized evaporated whole milk					
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 16 s; followed by evaporation at 65ºC approx 1 h	Virus not detected in cell culture, but detected by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each	Hyde et al., 1975

				flank)	
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, batch process) 72ºC x 3.0 min, followed by evaporation	Virus still detected	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell and Hyde, 1976
A_3, Mecklenburg	Milk, whole; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 80ºC x 16 s; followed by evaporation at 65ºC approx 1 h	Virus not detected in cell culture, but detected by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Hyde et al., 1975

** Detection limit of cell culture system in this experiment

Table 3: FMDV inactivation in reduced fat milk from cattle: Heat treatment

Virus Serotype, Subtype, Strain	**Product and Source of FMDV**	**Treatment Conditions**	**Effect of Treatment**	**Detection Method**	**Reference**
Milk, 2% fat					
O, 01/ UK	Milk, 2% fat; from FMDV-infected cows; pH 7.1	Pasteurization at 95ºC x 36 s at flow rate of 20L/h (continuous, commercial-type, HTST,	Decrease from $10^{3.7}$ pfu/ml before treatment, to undetectable levels after	1. Cell culture (primary bovine kidney cells); 2. Inoculation into suckling mice if cell	Tomasula et al., 2007

		plate and frame pasteurizer)	treatment, by cell culture; Virus still detected in 1 of 2 steers	culture negative; 3. Inoculation into steers if mice negative (2 ml intradermal into tongue + 35 ml intramuscular)	
Skim milk, temperatures less than 72°C					
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 50ºC x 5-640 s	Virus titer 10^5 mouse ID_{50} before treatment; Little or no decrease in titers after treatment ($10^{4.4}$ $10^{5.4}$-mouse ID_{50})	Inoculation into suckling mice	Dhennin and Labie, 1976
O	Milk, skim, from noninfected cows, FMDV added	Pasteurization (laboratory, batch process) at 55ºC x 5-30 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Milk, whole, from noninfected cows, FMDV added	Pasteurization (laboratory, batch process) at 55ºC x 60 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Milk, skim, from noninfected cows, FMDV added	Pasteurization (laboratory, batch process) at 60ºC x 5-10 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Milk, whole, from noninfected cows, FMDV	Pasteurization (laboratory, batch process) at 60ºC x 15-30 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968

	added				
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 7.4	Pasteurization (laboratory, batch process) at 60ºC x 0.2 – 2 min	Virus titer $10^{6.2}$ pfu/ ml before treatment; Virus still detected after treatment	Cell culture (primary bovine embryo thyroid cells)	De Leeuw et al., 1980
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 7.4	Pasteurization (laboratory, batch process) at 60ºC x 4 min	Decrease from $10^{6.2}$ pfu/ ml before treatment to undetectable levels after treatment	Cell culture (primary bovine embryo thyroid cells)	De Leeuw et al., 1980
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 60ºC x 5 s	Decrease from 10^5 mouse ID_{50} before treatment to $10^{4.6}$ mouse ID_{50} after treatment	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 60ºC x 10 s	Decrease from 10^5 mouse ID_{50} before treatment to $10^{4.4}$ mouse ID_{50} after treatment	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 60ºC x 20 s	Decrease from 10^5 mouse ID_{50} before treatment to $10^{4.5}$ mouse ID_{50} after treatment	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 60ºC x 40 s	Decrease from 10^5 mouse ID_{50} before treatment to	Inoculation into suckling mice	Dhennin and Labie, 1976

			$10^{3.6}$ mouse ID_{50} after treatment		
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 60ºC x 80 s	Decrease from 10^5 mouse ID_{50} before treatment to $10^{3.4}$ mouse ID_{50} after treatment	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 60ºC x 160 s	Decrease from 10^5 mouse ID_{50} before treatment to $10^{2.3}$ mouse ID_{50} after treatment	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 60ºC x 320-640 s	Decrease from 10^5 mouse ID_{50} before treatment to nearly undetectable levels after treatment	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 7.4	Pasteurization (laboratory, batch process) at 63ºC x 0.2 - 1 min	Virus titer $10^{6.2}$ pfu/ ml before treatment; Virus still detected after treatment	Cell culture (primary bovine embryo thyroid cells)	De Leeuw et al., 1980
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 7.4	Pasteurization (laboratory, batch process) at 63ºC x 2 min	Decrease from $10^{6.2}$ pfu/ ml before treatment to undetectable levels after treatment	Cell culture (primary bovine embryo thyroid cells)	De Leeuw et al., 1980
O	Milk, skim, from	Pasteurization (laboratory,	Virus still detected	Inoculation into guinea	Kastli and Moosbrugger,

	noninfected cows, FMDV added	batch process) at 65ºC x 0-30 s		pigs	1968
Skim milk, temperatures of 72-73ºC					
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 70ºC x 20 s	Virus still detected after treatment, but at very low levels	Inoculation into suckling mice	Dhennin and Labie, 1976
A_3	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) 72ºC x 15 s)	Decrease from $10^{5.2}$ - $10^{6.4}$ pfu/ml before treatment to $< 10^{0.7}$** $TCID_{50}$/ml after treatment	Cell culture (primary bovine kidney cells)	Cunliffe and Blackwell, 1977
O_1, Brugge	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 15 s	Decrease from $10^{4.6}$ - $10^{5.5}$ pfu/ml before treatment to $< 10^{0.4}$** pfu/ml after treatment	Cell culture (primary bovine kidney cells)	Blackwell et al., 1982
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 7.4	Pasteurization (laboratory, batch process) at 72ºC x 0.2 – 0.5 min	Decrease from $10^{6.2}$ pfu/ ml before treatment to undetectable levels after treatment	Cell culture (primary bovine embryo thyroid cells)	De Leeuw et al., 1980
A_3, Mecklenburg	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 0.25 to 1.0 min	Decrease from $10^{4.6}$ - $10^{6.3}$ pfu/ml before treatment, to $< 10^{0.4}$** to $10^{0.8}$ pfu/ml after treatment, by cell	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue +	Blackwell and Hyde, 1976

			culture; Virus still detected in 3/3 samples by inoculation into steers	35 ml intramuscular into each flank)	
A_3, Mecklenburg	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 2.0 min	Decrease from $10^{4.6}$ - $10^{6.3}$ pfu/ml before treatment to < $10^{0.4}$** pfu/ml after treatment, by cell culture; Virus still detected in 1/3 samples by inoculation into steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell and Hyde, 1976
O	Milk, skim, from noninfected cows, FMDV added	Pasteurization (laboratory, batch process) at 73ºC x 5 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 73ºC x 20 s	Virus still detected after treatment, but at very low levels	Inoculation into suckling mice	Dhennin and Labie, 1976
O	Milk, skim, from noninfected cows, FMDV added	Pasteurization (laboratory, batch process) at 73ºC x 10-30 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O_1	Milk, skim; from FMDV-infected cows	Milk pasteurized (tubular pasteurizer) at 73ºC for	Virus not detected	Cell culture (BHK21 cells); Inoculation into mice	Bohm et al., 1982

		40 s		Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	
Skim milk, temperatures 75-99ºC					
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 75ºC x 20 s	Virus still detected after treatment, but at very low levels	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 78ºC x 20 s	Virus still detected after treatment, but at very low levels	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 7.4	Pasteurization (laboratory, batch process) at 80ºC x 0-5 min	Virus titer $10^{6.2}$ pfu/ ml before treatment; Virus still detected after treatment	Inoculation into steers(2 ml intradermal into tongue + 25 ml intramuscular)	De Leeuw et al., 1980
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 83ºC x 20 s	Virus still detected after treatment, but at very low levelst	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 85ºC x 20 s	Virus still detected after treatment, but at very low levels	Inoculation into suckling mice	Dhennin and Labie, 1976
O_1, Lausanne	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 90ºC x 20 s	Virus still detected after treatment, but at very low levels	Inoculation into suckling mice	Dhennin and Labie, 1976

O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 90ºC x 0.5 min to 3 min	Virus titer $10^{6.1}$-$10^{6.8}$ pfu/ ml before treatment; Virus still detected after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
Skim milk, temperatures greater than 100ºC					
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 100ºC x 0.5 min to 3 min	Virus titer $10^{5.8}$-$10^{6.7}$ pfu/ ml before treatment; Virus still detected after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 110ºC x 0.5 min	Virus titer $10^{5.9}$-$10^{6.1}$ pfu/ ml before treatment; Virus still detected after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 110ºC x 3 min	Decrease from $10^{5.8}$ pfu/ ml before treatment, to undetectable levels after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
O_1, Weerselo	Milk, skim; from FMDV-infected cows; pH 6.7-7.4	Pasteurization (laboratory, batch process) at 120ºC x 0.5 min	Decrease from $10^{4.7}$ - $10^{6.2}$ pfu/ ml before treatment, to undetectable levels after treatment	Inoculation into steers (5 ml intradermal into tongue + 20-25 ml intramuscular)	De Leeuw et al., 1979
O_1, Weerselo	Milk, skim; from	Pasteurization (laboratory,	Decrease from $10^{4.7}$ -	Inoculation into steers (5	De Leeuw et al., 1979

	FMDV-infected cows; pH 6.7-7.4	batch process) at 135ºC x 0.5 min	$10^{6.1}$ pfu/ ml before treatment, to undetectable levels after treatment	ml intradermal into tongue + 20-25 ml intramuscular)	
Pasteurized evaporated skim milk					
A_3, Mecklenburg	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 0.25 min; followed by evaporation	Virus detected after treatment in 1/3 samples	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell and Hyde, 1976
A_3, Mecklenburg	Milk, skim; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 72ºC x 0.5 min; followed by evaporation	Virus not detected after treatment (3/3 samples)	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell and Hyde, 1976

** Detection limit of cell culture system in this experiment

Table 4: FMDV inactivation in cream from cow's milk: Heat treatment

Virus Serotype, Subtype, Strain	**Product and Source of FMDV**	**Treatment Conditions**	**Effect of Treatment**	**Detection Method**	**Reference**
A_3,	Cream;	Pasteurization	Decrease from	1. Cell culture	Blackwell

Mecklenburg	from FMDV-infected cows	(laboratory, batch process) at 72ºC x 0.25 min	$10^{1.8}$ - $10^{6.3}$ pfu/ml before treatment, to < $10^{0.4}$** to 10 pfu/ml after treatment, by cell culture; Virus still detected in 3/3 samples by inoculation into steers	(primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	and Hyde, 1976
O_1, Brugge	Cream; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 93ºC x 0.25 min	Decrease from $10^{5.1}$ - $10^{5.8}$ pfu/ml before treatment to < $10^{0.4}$** pfu/ml after treatment	Cell culture (primary bovine kidney cells)	Blackwell et al., 1982
A_3, Mecklenburg	Cream; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 93ºC x 0.25 min	Decrease from $10^{1.8}$ - $10^{6.3}$ pfu/ml before treatment, to < $10^{0.4}$** pfu/ml after treatment, by cell culture; Virus still detected in 3/3 samples by inoculation into steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell and Hyde, 1976
A_3, Mecklenburg	Cream; from FMDV-infected cows	Pasteurization (laboratory, batch process) at 93ºC x 16 s	Decrease from $10^{6.9}$ - $10^{7.6}$ pfu/ml before treatment to $10^{0.85}$ - $10^{1.5}$ pfu/ml after treatment	Cell culture (primary bovine kidney cells)	Blackwell, 1978a
A_3, Mecklenburg	Cream; from FMDV-infected cows	Milk stored at 4ºC for 18 h; Followed by pasteurization of cream (laboratory,	Decrease from $10^{5.4}$ pfu/ml before treatment to 10 pfu/ml after treatment	Cell culture (primary bovine kidney cells)	Blackwell, 1978a

		batch process) at 93ºC x 16 s			

** Detection limit of cell culture system in this experiment

Table 5: FMDV inactivation in butter and buttermilk from cow's milk

Virus Serotype, Subtype, Strain	Product and Source of FMDV	Treatment Conditions	Effect of Treatment	Detection Method	Reference
Butter					
	Butter, unsalted; made from FMDV-contaminated cream	Unpasteurized cream manufactured into butter; butter stored at 5ºC	Replicate 1: Virus detected at 26 d of storage, but not 29 d; Replicate 2: Virus detected at 20 d of storage, but not 21 d	Inoculation into guinea pigs	Terbrüggen, 1932
	Butter, salted; made from FMDV-contaminated cream	Unpasteurized cream manufactured into butter; butter stored at 5ºC	Replicate 1: Virus detected at 26 d of storage, but not 29 d; Replicate 2: Virus detected at 45 d of storage, but not 46 d	Inoculation into guinea pigs	Terbrüggen, 1932
	Butter, unsalted; made from FMDV-contaminated cream	Unpasteurized cream manufactured into butter; butter stored at 17ºC	Virus detected at 26 h of storage, but not 4 d	Inoculation into guinea pigs	Terbrüggen, 1932
	Butter, salted; made from FMDV-contaminated cream	Unpasteurized cream manufactured into butter; butter stored at 17ºC	Virus detected at 4 d of storage, but not 5 d	Inoculation into guinea pigs	Terbrüggen, 1932
	Butter, unsalted;	Unpasteurized cream	Virus detected at 8 d of	Inoculation into guinea	Terbrüggen, 1932

	made from FMDV-contaminated cream	manufactured into butter; butter frozen for 11 h then stored at 17ºC	storage, but not 9 d	pigs	
	Butter, salted; made from FMDV-contaminated cream	Unpasteurized cream manufactured into butter; butter frozen for 11 h then stored at 17ºC	Virus detected at 9 d of storage, but not 10 d	Inoculation into guinea pigs	Terbrüggen, 1932
	Butter, unsalted; made from FMDV-contaminated cream	Unpasteurized cream acidified with a lactic acid bacterial starter culture (in milk) x 18-24 h at 16-18ºC; acidified cream manufactured into butter; butter stored at 17ºC	Virus not detected in acidified cream after 18-24 h or in butter	Inoculation into guinea pigs	Terbrüggen, 1932
A_3, Mecklenburg	Butter, salted; made from cream from FMDV-infected cows	Cream pasteurized (laboratory, batch process) at 93ºC x 16 s; followed by butter manufacture; butter stored at 4ºC x 1 to 30 d	Virus titer in cream $10^{6.9}$ - $10^{7.6}$ pfu/ml before pasteurization; Decrease to $10^{0.85}$ - $10^{1.5}$ pfu/ml after pasteurization of cream; Further decrease to < $10^{0.4}$** pfu/ml after churning into butter (pH 5.9); Virus still detected after 30 d (pH 5.4-	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 6 ml intramuscular)	Blackwell, 1978a

			5.5) by inoculation into steers		
A_3, Mecklenburg	Butter, salted; made from cream from FMDV-infected cows	Cream frozen at 70ºC; thawed cream pasteurized (laboratory, batch process) at 93ºC x 16 s; followed by butter manufacture; butter stored at 4ºC x 1 to 30 d	Virus titer in cream $10^{4.8}$ - $10^{4.9}$ pfu/ml before pasteurization; Decrease to $10^{2.6}$ - $10^{4.5}$ pfu/ml in cream after pasteurization; Further decrease to < $10^{0.4}$** - $10^{4.3}$ pfu/ml after churning into butter (pH 5.9); Virus still detected at 1 d by inoculation into steers; Virus not detected at 30 d (pH 5.4) by inoculation into steers	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 6 ml intramuscular)	Blackwell, 1978a
A3, Mecklenburg	Butter oil from butter, salted; made from cream from FMDV-infected cows	Cream pasteurized (laboratory, batch process) at 93ºC x 16 s; followed by butter manufacture; butter stored at 4ºC x 1 to 45 d	Virus titer in cream $10^{7.6}$ pfu/ml before pasteurization; Decrease to $10^{1.1}$ to $10^{1.5}$ pfu/ml in cream after pasteurization; Further decrease to < $10^{0.4}$** pfu/ml in butter oil prepared from butter; Virus detected (by inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 6 ml intramuscular)	Blackwell, 1978a

			into steers) in butter oil made from butter stored for 45 d		
A_3, Mecklenburg	Butter oil from butter, salted; made from cream from FMDV-infected cows	Milk stored at 4ºC for 18 h; Cream pasteurized (laboratory, batch process) at 93ºC x 16 s; followed by butter manufacture; Butter stored at 4ºC x 1 to 45 d	Virus titer in cream $10^{5.4}$ pfu/ml before pasteurization; Decrease to 10 pfu/ml in cream after pasteurization; Further decrease to < $10^{0.4}$** pfu/ml after preparation of butter oil from butter; Virus detected (by inoculation into steers) in butter oil made from butter stored for 45 d	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 6 ml intramuscular)	Blackwell, 1978a
Buttermilk					
	Buttermilk, after manufacture of butter from FMDV-contaminated cream	Unpasteurized cream manufactured into butter; buttermilk is by-product of this process	Virus detected	Inoculation into guinea pigs	Terbrüggen, 1932
A_3, Mecklenburg	Buttermilk, after butter manufacture; made from cream from FMDV-infected cows	Cream pasteurized (laboratory, batch process) at 93ºC x 16 s; followed by butter manufacture; buttermilk is by-product of	Virus detected	Cell culture (primary bovine kidney cells)	Blackwell, 1978a

		this process			

** Detection limit of cell culture system in this experiment

Table 6: FMDV inactivation in cheese, casein and whey from cow's milk

Virus Serotype, Subtype, Strain	Product and Source of FMDV	Treatment Conditions	Effect of Treatment	Detection Method	Reference
Cheese, cheddar					
A_3, Mecklenburg	Cheese, cheddar; pH 5.1; made from milk from FMDV-infected cows	Raw milk; cheese sampled during and after processing	Virus titer $10^{4.2}$ - $10^{5.4}$ pfu/ml in raw milk; Decrease to < $10^{0.4}$** pfu/ml in cheese curds and whey after pressing stage of cheese manufacture; Virus still detected by animal inoculation	Inoculation into steers (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
A_3, Mecklenburg	Cheese, cheddar; pH 5.1; made from milk from FMDV-infected cows	Raw milk; cheese cured 2ºC x 30-60 d after manufacture	Virus detected by animal inoculation in cheese cured for 60 d	Inoculation into steers (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
A_3, Mecklenburg	Cheese, cheddar; pH 5.1; made from milk from FMDV-infected cows	Raw milk; cheese cured 2ºC x 120 d after manufacture	Virus not detected by any method in cheese cured for 120 d	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal	Blackwell, 1976

				into tongue + 35 ml intramuscular into each flank)	
A_3, Mecklenburg	Cheese, cheddar; pH 5.1; made from milk from FMDV-infected cows	Milk heated at 63ºC x 6s; cheese sampled 1 d after manufacture	Virus titer $10^{6.2}$ pfu/ml in raw milk; Decrease to $10^{1.6}$ pfu/ml in milk after heat treatment; Virus detected in cheese by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
A_3, Mecklenburg	Cheese, cheddar; pH 5.1; made from milk from FMDV-infected cows	Milk heated at 63ºC x 6s; cheese cured 2ºC x 30 d after manufacture	Virus not detected by any method in cheese cured for 30 d	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
O, Switzerland 1966	Cheese, cheddar; pH 5.9; FMDV added to milk from noninfected cows	Milk heated at 63ºC x 15 min; cheese sampled during processing	Decrease in virus titer over first 8 h of processing, but virus still detected at 8 h;	Inoculation into mice	Scherning-Thiesen, 1979
O, Switzerland 1966	Cheese, cheddar; pH 5.9; FMDV added to milk from noninfected	Milk heated at 63ºC x 15 min; cheese cured 14 d after manufacture	Virus not detected after curing for 14 d	Inoculation into mice	Scherning-Thiesen, 1979

	cows				
A_3, Mecklenburg	Cheese, cheddar; pH 5.1; made from milk from FMDV-infected cows	Milk heated at 67ºC x 1 min; cheese sampled 1 d after manufacture	Virus titer $10^{4.4}$ - $10^{6.2}$ pfu/ml in raw milk; Decrease to < $10^{0.4}$** pfu/ml in milk after heat treatment; Virus detected in cheese by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
A_3, Mecklenburg	Cheese, cheddar;; pH 5.1; made from milk from FMDV-infected cows	Milk heated at 67ºC x 1min; cheese cured 2ºC x 30 d after manufacture	Virus not detected by any method in cheese cured for 30 d	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
Cheese, camembert					
O, Switzerland 1966	Cheese, camembert; pH 5.2; FMDV added to milk from noninfected cows	Milk heated at 63ºC x 15 min; cheese cured 21 d after manufacture	Traces of virus detected 1 d after cheese manufacture; Virus not detected after curing for 2-3 d	Inoculation into mice	Scherning-Thiesen, 1979
A_3, Mecklenburg	Cheese, camembert; pH 5.2; made from milk from FMDV-infected	Milk pasteurized (laboratory, batch process) at 72ºC x 16s; cheese	Virus titer $10^{4.2}$ - $10^{4.7}$ pfu/ml in raw milk; Decrease to < $10^{0.4}$** pfu/ml in milk after	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture	Blackwell, 1976

	cows	sampled 1 d after manufacture	pasteurization; Virus detected in cheese by animal inoculation	negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	
A_3, Mecklenburg	Cheese, camembert; pH 5.2; made from milk from FMDV-infected cows	Milk pasteurized (laboratory, batch process) at 72ºC x 16s; cheese ripened 4ºC x 21 d after manufacture	Virus detected by animal inoculation in cheese ripened for 21 d	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
A_3, Mecklenburg	Cheese, camembert; pH 5.2; made from milk from FMDV-infected cows	Milk pasteurized (laboratory, batch process) at 72ºC x 16s; cheese ripened 4ºC x 35 d after manufacture	Virus not detected by any method in cheese ripened for 35 d	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
O_1	Cheese, camembert; made from milk from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 13 (infectious milk): 37 (noninfected milk); Mixed milk	Virus not detected in pasteurized milk or at any stage of cheese manufacture	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml	Bohm et al., 1982

		pasteurized (tubular pasteurizer) at 73ºC for 40 s		intramuscular)	
Cheese, mozzarella					
A_3, Mecklenburg	Cheese, mozzarella; pH 5.1; made from milk from FMDV-infected cows	Milk pasteurized (laboratory, batch process) at 72ºC x 16s; cheese sampled after manufacture	Virus not detected by any method in cheese	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 35 ml intramuscular into each flank)	Blackwell, 1976
Cheese, emmenthal					
C, Noville	Cheese, emmenthal; made from milk from FMDV-infected cows	Milk heated at 53ºC for > 45 min during processing; cheese sampled during processing	Virus detected through piling stage of cheese manufacture	Cell culture and inoculation into steers; details not given	Kihm et al., 1979
C, Noville	Cheese, emmenthal; pH 5.2-5.6; made from milk from FMDV-infected cows	Milk heated at 53ºC for > 45 min during processing; cheese (pH 5.2-5.6) stored 1-5 days	Virus not detected in cheese 1 d, 2 d or 5 d after manufacture	Cell culture and Inoculation into steers; details not given	Kihm et al., 1979
Cheese, Edam					
O_1	Cheese, edam; made from milk from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 13 (infectious milk): 37 (noninfected	Virus not detected in pasteurized milk or at any stage of cheese manufacture	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal	Bohm et al., 1982

		milk);Milk pasteurized (tubular pasteurizer) at 73ºC for 40 s		into tongue and 20-40 ml intramuscular)	
Cheese, hard cheese					
O_1	Hard cheese; made from milk from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 40 (infectious milk): 105 (noninfected milk); Mixed milk heated (tubular pasteurizer) at 62ºC for 40 s	Virus not detected in pasteurized milk or at any stage of cheese manufacture	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
Cheese, kareish					
	Cheese, kareish	Commercially made from raw milk in outbreak area	Virus detected	Cell culture (BHK-21/C13 cells)	Deeb et al., 2010
Casein and sodium caseinate					
A_3, Mecklenburg	Casein; made from milk from FMDV-infected cows	Casein produced by isoelectric precipitation, (pH 4.7) hydrochloric acid	Virus detected	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 8 ml intramuscular)	Blackwell, 1978b
	Acid casein, made from skim milk from FMDV-infected cows	Skim milk pasteurized (tubular pasteurizer) at 73ºC for 40 s; Casein produced by acid	Virus not detected in pasteurized milk or at any stage of casein manufacture	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml	Bohm et al., 1982

		precipitation.		intradermal into tongue and 20-40 ml intramuscular)	
	Dried acid casein, made from skim milk from FMDV-infected cows	Skim milk pasteurized (tubular pasteurizer) at 73ºC for 40 s; Casein produced by acid precipitation.	Virus not detected in pasteurized milk or at any stage of casein manufacture	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
	Rennet casein, made from skim milk from FMDV-infected cows	Skim milk pasteurized (tubular pasteurizer) at 73ºC for 40 s	Virus not detected in pasteurized milk or at any stage of casein manufacture	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
	Dried rennet casein, made from skim milk from FMDV-infected cows	Skim milk pasteurized at 73ºC for 40 s	Virus not detected in pasteurized milk or at any stage of casein manufacture	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
A_3	Casein; made from skim milk from FMDV-infected	Casein produced by precipitation, (pH 4.6) hydrochloric acid	Virus titer $10^{3.0}$ - $10^{4.0}$ pfu/ml in raw milk; Titer < $10^{0.7}$** $TCID_{50}$/ml in	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if	Cunliffe and Blackwell, 1977

	cows		casein, by cell culture; Virus detected by animal inoculation in 1 of 2 batches of casein	cell culture negative (2 ml intradermal into tongue + 20-40 ml intramuscular)	
A_3	Casein; made from skim milk from noninfected cows, FMDV added	Skim milk pasteurized at 72ºC x 15s; Casein produced from skim milk by precipitation, (pH 4.5) hydrochloric acid	Virus titer $10^{5.5}$ pfu/ml in raw milk; Decrease to < $10^{0.5}$ $TCID_{50}$/ml in milk after pasteurization; Virus titer $10^{0.7}$ $TCID_{50}$/ml in casein; Virus also detected in casein by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 20-40 ml intramuscular)	Cunliffe and Blackwell, 1977
A_3	Casein, made from skim milk from FMDV-infected cows	Skim milk pasteurized at 72ºC x 15s; Casein produced from skim milk by precipitation, (pH 4.5) hydrochloric acid	Virus titer $10^{5.2}$ - $10^{6.4}$ pfu/ml in raw milk; Decrease to < $10^{0.7}$** $TCID_{50}$/ml in milk after pasteurization; Virus detected in some batches of casein by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 20-40 ml intramuscular)	Cunliffe and Blackwell, 1977
A_3, Mecklenburg	Casein; made from skim milk from FMDV-infected cows	Skim milk pasteurized at 72ºC x 15s; Casein produced from skim milk by precipitation,	Virus titer $10^{5.5}$ - $10^{6.4}$ pfu/ml in raw milk; Decrease to < 10** pfu/ml in milk after pasteurization;	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml	Cunliffe et al., 1978

		(pH 4.5) hydrochloric acid; casein stored at ambient temperatures 1 to 42 d	Virus detected in some batches of casein by animal inoculation	intradermal into tongue + 20-40 ml intramuscular)	
A_3, Mecklenburg	Casein, made from skim milk from FMDV-infected cows	Skim milk pasteurized at 72ºC x 15s; Casein produced from skim milk by precipitation, pH 4.5) hydrochloric acid; casein stored at ambient temperatures 84 d	Virus titer $10^{5.5}$ - $10^{6.4}$ pfu/ml in raw milk; Decrease to < 10** pfu/ml in milk after pasteurization; Virus not detected in casein by any method	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 20-40 ml intramuscular)	Cunliffe et al., 1978
A_3	Sodium caseinate; from casein, which was made from skim milk from FMDV-infected cows	Skim milk pasteurized at 72ºC x 15s; Casein produced from skim milk by precipitation, (pH 4.5) hydrochloric acid	Virus titer $10^{5.3}$ - $10^{6.4}$ pfu/ml in raw milk; Decrease to < $10^{0.7}$** $TCID_{50}$/ml in milk after pasteurization; Virus detected in some batches of sodium caseinate by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 20-40 ml intramuscular)	Cunliffe and Blackwell, 1977
A_3	Sodium caseinate; from dried casein, which was made from skim milk from	Skim milk pasteurized at 72ºC x 15s; Casein produced from skim milk by precipitation,	Virus titer $10^{5.2}$ pfu/ml in raw milk; Decrease to < $10^{0.7}$** $TCID_{50}$/ml in milk after pasteurization;	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml	Cunliffe and Blackwell, 1977

	FMDV-infected cows	(pH 4.5) hydrochloric acid	Virus detected in some batches of sodium caseinate by animal inoculation	intradermal into tongue + 20-40 ml intramuscular)	
A_3	Sodium caseinate; from casein, which was made skim milk from noninfected cows, FMDV added	Skim milk pasteurized at 72ºC x 15s; Casein produced from skim milk by precipitation, (pH 4.5) hydrochloric acid	Virus titer $10^{5.2}$ - $10^{6.6}$ pfu/ml in raw milk; Decrease to < $10^{0.5}$** - $10^{0.7}$ $TCID_{50}$/ml in milk after pasteurization; Virus titer < $10^{0.7}$ $TCID_{50}$/ml in sodium caseinate; Virus detected in some batches of sodium caseinate by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 20-40 ml intramuscular)	Cunliffe and Blackwell, 1977
Sweet whey					
A_3, Mecklenburg	Whey, sweet; from cheddar cheese manufacture; milk for cheese was from FMDV-infected cows	Milk heated at 67ºC x 1 min; whey is by-product of cheese manufacture	Virus titer $10^{5.8}$ pfu/ml in raw milk; Titer was $10^{0.05}$ pfu/ml in whey [NB: This value should probably be $10^{0.5}$ pfu/ml, as detection limit for FMDV in cell culture was $10^{4.0}$ pfu/ml]; Virus detected in whey by	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 8 ml intramuscular)	Blackwell, 1978b

			animal inoculation		
A_3, Mecklenburg	Whey, sweet; from camembert cheese manufacture; milk for cheese was from FMDV-infected cows	Milk pasteurized at 72ºC x 16s; whey is by-product of cheese manufacture	Virus titer $10^{4.4}$ pfu/ml in raw milk; Titer < $10^{0.4}$** pfu/ml in whey; Virus detected in whey by animal inoculation	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 8 ml intramuscular)	Blackwell, 1978b
C, Noville	Whey, from emmenthal cheese manufacture; milk for cheese from FMDV-infected cows	Milk heated at 53ºC for > 45 min during processing; whey is by-product of cheese manufacture	Virus detected in whey through piling stage of cheese manufacture	Cell culture and inoculation into steers; details not given	Kihm et al., 1979
O_1	Whey, from hard cheese manufacture; milk for cheese from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 40 (infectious milk): 105 (noninfected milk); Mixed milk heated (tubular pasteurizer) at 62ºC for 40 s	Virus not detected	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
O_1	Whey, from camembert cheese manufacture; milk for cheese from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 13 (infectious milk): 37 (noninfected	Virus not detected	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal	Bohm et al., 1982

		milk); Mixed milk pasteurized (tubular pasteurizer) at 73ºC for 40 s		into tongue and 20-40 ml intramuscular)	
O_1	Whey, from edam cheese manufacture; milk for cheese from FMDV-infected cows	Milk diluted with skim and whole milk from noninfected cows (ratio of 13 (infectious milk): 37 (noninfected milk); Mixed milk pasteurized (tubular pasteurizer) at 73ºC for 40 s	Virus not detected	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
O	Whey, FMDV added to whey; pH 6.8	Pasteurization (laboratory, batch process) at 55ºC x 5 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Whey, FMDV added to whey; pH 6.8	Pasteurization (laboratory, batch process) at 55ºC x 10-30 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Whey, FMDV added to whey; pH 6.8	Pasteurization (laboratory, batch process) at 60ºC x 5-30 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Whey; FMDV added to whey; pH 6.8	Pasteurization (laboratory, batch process) at 65ºC x 5 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Whey, FMDV added to whey;	Pasteurization (laboratory, batch process) at	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968

	pH 6.8	65ºC x 10-30 s			
O	Whey, FMDV added to whey; pH 6.8	Pasteurization (laboratory, batch process) at 73ºC x 5 s	Virus still detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
O	Whey, FMDV added to whey; pH 6.8	Pasteurization (laboratory, batch process) at 73ºC x 10-30 s	Virus not detected	Inoculation into guinea pigs	Kastli and Moosbrugger, 1968
Acid whey					
O_1	Whey, from casein manufacture; milk from FMDV-infected cows	Milk pasteurized (tubular pasteurizer) at 73ºC for 40 s	Virus not detected	Cell culture (BHK21 cells); Inoculation into mice Inoculation into steers (2 ml intradermal into tongue and 20-40 ml intramuscular)	Bohm et al., 1982
A_3, Mecklenburg	Whey, acid, from casein manufacture; pH 4.7; from FMDV-infected cows	Casein produced by isoelectric precipitation, (pH 4.7) hydrochloric acid; whey is by-product of casein manufacture	Virus not detected	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 8 ml intramuscular)	Blackwell, 1978b

** Detection limit of cell culture system in this experiment

Table 7: FMDV inactivation in purified components from infected cow's milk

Virus Serotype,	Product and Source of	Treatment Conditions	Effect of Treatment	Detection Method	Reference

Subtype, Strain	FMDV				
A3, Mecklenburg	α-lactalbumin; purified from sweet whey; milk from FMDV-infected cows	Precipitation and crystallization	Virus not detected	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 8 ml intramuscular)	Blackwell, 1978b
A3, Mecklenburg	β-lactoglobulin; purified from sweet whey; milk from FMDV-infected cows	Precipitation and crystallization	Virus not detected	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 8 ml intramuscular)	Blackwell, 1978b
A3, Mecklenburg	Lactose; purified from sweet whey; milk from FMDV-infected cows	Precipitation and crystallization	Virus not detected	1. Cell culture (primary bovine kidney cells); 2. Inoculation into steers if cell culture negative (2 ml intradermal into tongue + 8 ml intramuscular)	Blackwell, 1978b

Table 8: FMDV inactivation in yogurt from cow's milk

Virus Serotype, Subtype, Strain	Product and Source of FMDV	Treatment Conditions	Effect of Treatment	Detection Method	Reference

Serotype not identified	Yogurt; made from field samples of milk from FMDV-infected cows in Egypt	Traditional yogurt culture with *Streptococcus thermophilus* & *Lactobacillus bulgaricus*	Virus not detected	Cell culture (bovine kidney cells)	El-Alfy, 1998

** Detection limit of cell culture system in this experiment

Table 9: FMDV inactivation in water buffalo milk

Virus Serotype, Subtype, Strain	**Product and Source of FMDV**	**Treatment Conditions**	**Effect of Treatment**	**Detection Method**	**Reference**
O	Milk; from noninfected buffalo, FMDV added	Pasteurization at 63ºC x 30 min	Virus still detected	Cell culture (BHK-21/C13 cells)	Deeb et al., 2010
O	Milk; from noninfected buffalo, FMDV added	Boiling x 5 min	Virus not detected	Cell culture (BHK-21/C13 cells)	Deeb et al., 2010
O	Milk; from noninfected buffalo, FMDV added	Storage at 4ºC x 7 d	Virus still detected	Cell culture (BHK-21/C13 cells)	Deeb et al., 2010