Inactivation of foot and mouth disease virus in milk and milk products

By Salwa A. ALY[1] and Amal S. GABER[2]

[1]Food Hygiene Department Faculty Veterinary Medicine, Cairo University, Cairo, Egypt. E-mail
[2]Virology Department, Animal Health Research Institute, Dokki, Cairo

Buffalo, cow, sheep and goat raw milk samples (30 each) were examined for Foot and mouth disease virus (FMDV). The mean titer value was 10^3 ±0.02, 10^3 ± 0.01, 10^4 ± 0.03 and 10^3 ± 0.10 PFU/ml, respectively. Thermal processing of milk at 50°C for 10 min, long-time pasteurization at 63°C for 30 min, short-time pasteurization 72°C for 15 s, double set pasteurization at 72°C for 30 s, ultra-heat treatment (UHT) at 135°C for 1 s and sterilization at 121°C for 15 min were applied. Only UHT and double set pasteurization proved to be a reliable method for inactivating the virus in milk. Cheese manufacture could not guarantee the complete inactivation of FMD virus: however, the virus survived curing for 90 d (pH 4.9) but not for 120 d (pH 4.5) during refrigerated storage. Additionally, FMD virus was completely inhibited in yoghurt and could not be detected either when fresh or during cold storage (pH 4.4).

Inaktivierung des Virus der Maul- und Klauenseuche in Milch und Milchprodukten

Rohmilchproben von Büffeln, Kühen, Schafen und Ziegen (jeweils 30) wurden auf das Virus der Maul- und Klauenseuche (FMDV) untersucht. Die durchschnittlichen Titer betrugen 10^3±0,0 $2{,}10^3$±0,0 $1{,}10^4$±0,03 bzw. 10^3±0,10 PFU/ml. Die thermische Behandlung der Milch erfolgt bei 50°C über 10 Minuten, durch Dauererhitzung bei 63°C über 30 Minuten, Kurzzeiterhitzung bei 72°C über 15 Sekunden, verlängerte Kurzzeiterhitzung bei 72°C über 30 Sekunden, Ultrahocherhitzung bei 135°C für die Dauer 1 Sekunde und Sterilisation bei 121°C über 15 Minuten Dabei zeigte sich, dass lediglich die Ultrahocherhitzung und die Kurzzeiterhitzung über 30 Sekunden zuverlässige Verfahren zur Inaktivierung des Virus in Milch waren. Die Käseherstellung garantiert nicht die vollständige Inaktivierung des Erregers. Das Virus überlebte die Reifung über 90 Tage bei einem pH-Wert von 4,9, nicht jedoch über 120 Tage bei einem pH-Wert von 4,5 (Kühllagerung). Das Virus das Maul und Klauenseuche wurde vollständig in Joghurt inaktiviert und konnte weder in frischen Produkten noch während einer Kühllagerung nachgewiesen werden (pH-Wert 4,4).

06 Foot and mouth disease (virus inactivation in milk) **06 Maul- und Klauenseuche** (Virusinaktivierung in Milch)

1. Introduction

Milk and milk products are very important commodities in the international trade. Their quality and safety are a major field of concern for producers, consumers and public health official worldwide. At the dairy plant, the quality of the final product depends on the level of contamination from raw milk, types of microorganisms introduced and efficiency of processing (1). They have been implicated as a vehicle for the transmission of disease agents including foot and mouth disease virus (2). Foot and mouth disease (FMD) is an economically significant, highly contagious viral disease of cloven footed animals such as cattle, sheep and goat. It is endemic in many countries, including much of Africa, Asia, and South America. The causative agent, FMD virus, is a member of the family Picornaviridae, Genus Aphtho virus. There are 7 different types and many subtypes of FMD virus. The animal can become infected by one or more than one virus type. Recovered animals can suffer repeated attacks of the disease because immunity to one type does not protect against other types. FMD is spread by animals, people, and materials that bring the virus into physical contact with susceptible animals. The viral agent can persist for considerable periods in tissues of infected animals and survive in number of products produced from them. The risk of the introduction of foot and mouth disease virus into non-infected countries through contaminated animal products is the basis for regulations controlling the importation of dairy products from countries where FMD exists (3, 4). The possibility of the transmission of foot and mouth disease virus (FMD) by milk has been known from the beginning of this century. The virus appeared in milk with an average of 14 d before clinical sign appeared. This milk can serve as a vector for spreading the disease both at farm level, during milk transport to the processing plant, distribution and processing (5, 6).

The economical impact of foot-and-mouth disease can be very drastic. Not all animals die from the disease, but the blisters that form on the mouth make it painful for the animal to eat or drink. As a result, the animal could lose up to 20% of its body weight in about 2 weeks, and the blisters formed on the teat lead to chronic mastitis. In dairy industry, high losses occur from decreased milk production of about 60% and a reduced milk price (7, 8).

Milk and milk products play an important role in the distribution of the infection among humans. Hazard to public health by FMD virus may occur either by consumption or handling of contaminated raw milk or milk products. The symptoms in humans are fever, sore throat, and blisters on the feet, mouth and tongue. Some complications can be associated with meningitis - the person has headache, stiff neck or back pain as well as endocardity mainly fatal in infants. This risk assessment clearly demonstrates the potential significance of FMD virus transmission via milk and milk products (9).

The aim of the present work is to study the prevalence of FMD virus in raw milk of different animals and

to observe the persistence of foot and mouth disease virus in raw milk during heating and processing into soft cheese and yoghurt.

2. Materials and methods

2.1 Collection of milk samples and milk treatment

A total of 120 (30 each) milk samples of apparently healthy buffalo, cow, sheep and goat were collected from different farms in Giza governorate, Egypt. Milk was treated according to (10).

2.2 Stability of foot and mouth disease virus in milk

One liter of raw milk free from virus (previously tested) was divided into 10 portions, 100 ml each. Each portion was artificially contaminated with foot and mouth disease virus in a concentration of 10^5 PFU/ml. The infected milk was heat-treated at 50°C for 10 min, long pasteurized at 63°C for 30 min, short pasteurized at 72°C for 15 s, double set pasteurized at 72°C for 30 s, ultra-heat treated at 135°C for 1 s and sterilized at 121°C for 15 min using polyscience microprocessor controlled water bath Dual chamber 5/10 L Model 28 L, catalogue No. 040661 according to (11)

2.3 Cheese manufacture

Soft cheese was manufactured with some modifications according to (12). The manufactured soft cheese was stored at 4°C in soldered tins, filled with boiled salted whey (3%) and analyzed when fresh and after 15, 30, 45, 60, 90 and 120 d of cold storage.

2.4 Yoghurt manufacture: according to (13)

2.5 Cell culture

Baby hamster kid hey cell (BHK) was received from the Egyptian organization for biological and vaccine production, Cairo. They were subcultured using Eagles minimum essential medium (MEM), Sigma Chemical Co., according to the method applied by (14).

2.6 Virus detection

Milk, cheese and yoghurt samples were mixed mechanically in Hanks balanced solution (pH 7.8) containing 0.5% lactalbumin hydrolysate (HLH). Serial 10- fold dilutions were prepared in chilled HLH and 0.1 ml of suspension was inoculated onto each of the primary bovine kidney cell cultures. The plaque forming unit (PFU)/ml was enumerated according to (15).

2.7 Determination of pH

The pH was determined by using pH meter (Metrom pH meter 604 electrode No. 6.0212.000.

3. Results

Results recorded in Table 1 revealed that foot and mouth disease virus was detected in 66.6, 53.3, 50 and 43.3% of buffalo, cow, sheep and goat raw milk samples, respectively, with a mean value of 10^3, 10^3, 10^4 and 10^3 PFU/ml, respectively. The high incidence of infection may be due to some animals away from vaccination and thus affected by the disease (16, 17). The titer as high as 10^4 and 10^5 minimum infectious dose (ID50/ml) is sufficient to infect humans and animals, respectively. Nearly similar findings were recorded by (18). Higher results were obtained by (19).

Table 1: Incidence of Foot and Mouth disease virus in raw milk of different animal species

Milk from species	Total no.	Positive no.	%	Mean titer (PFU/ml
Buffalo	30	20	66.6	10^3±0.02
Cow	30	16	53.3	10^3±0.01
Sheep	30	15	50.0	10^4±0.03
Goat	30	13	43.3	10^3±0.10

Table 2: Stability of foot and mouth disease virus during heat treatment of milk experimentally infected by initial dose 10^5 PFU/ml treatment

Heat treatment	°C	Time	Virus titer (PFU/ml
Thermization	50	10 min	10^5±0.01
Long pasteurization	63	30 min	10^3±0.30
Short pasteurization	72	15 s	10^3±0.02
Double set pasteurization	72	30 s	ND
Ultra-heat treatment	135	1 s	ND
Sterilization	121	15 min	ND

ND: not detected

Heat treatment of raw milk by high temperature short time (HTST) pasteurization is now a standard practice in developed countries and has eliminated the threat of several infectious diseases transmitted by milk including salmonellosis and tuberculosis (20). Therefore, we evaluated the survival of foot and mouth disease virus after different heat treatments of milk. Table 2 shows the effect of thermization of raw milk artificially infected with 10^5 PFU/ml at 50°C for 15 s: long pasteurization at 63°C for 30 min, short pasteurization at 72°C for 15 s, double set pasteurization at 72°C for 30 s, ultra-heat treatment at 135°C for 1 s and sterilization at 121 °C for 15 min. Ultra heat treatment (UHT) produces complete inactivation of the virus. The milk samples heated at 72° C for 15 s still harbor health hazard, and inactivation was limited at 15 s, but complete at 30 s as well as at UHT and sterilization. Nearly similar findings were reported by (21,22). Virus inactivation was incomplete after short time pasteurization (72°C/15 s). Therefore, a protective effect of milk on the infectivity of FMDV is proven. Protective substances as casein, fat, sugar and ions (8, 22) may be present. Short time pasteurization of milk of less than 30 s cannot be regarded as reliable method for inactivation of FMDV in milk. The further increase in time and temperature of milk may lead to the denaturation of milk protein and to complete inactivation of the virus. Nearly similar findings were recorded by (23). Current minimum pasteurization standards may be adequate to eliminate bacterial pathogens, but not for foot and mouth disease virus in milk (24). Double set pasteurization of milk is difficult to implement in commercial high temperature short time method plants. However, (25) reported that at FMDV outbreak in Denmark, milk was subjected to two heat treatments, one at 72°C for 15 s and the second at 80°C for 3 s. Previous results on the survival of FMD virus in heated milk warranted the examination of milk products prepared from heat-treated milk. We anticipated that FMD virus would survive in milk heated at sub-pasteurization temperatures.

Table 3: Stability of FMDV during processing and storage of soft cheese

Storage period (d)	Virus titer PFU/ml	pH
0 (fresh)	10^5	5.7
15	10^5	5.5
30	10^5	5.4
45	10^5	5.2
60	10^4	5.0
90	10^2	4.9
120	ND	4.5

ND: not detected

The survival of FMD virus in soft cheese was investigated (Table 3). FMD virus was detected in the curd immediately after processing (pH 5.7) and after 90 d (pH 5.2) of storage at 4°C. Storage of soft cheese over a period of 90 d at low temperature (4°C) did not inactivate foot and mouth disease virus. There is a much lower decrease in the virus titer after 90 d of storage at pH 4.9 while the inactivity of the virus was completely lost after 120 d of storage. The high pH value 4.7 may be responsible for virus inactivation. Nearly similar findings were recorded by (15, 26) who reported complete inactivation of the virus in cheese with increased acidity. FMD virus did in fact survive in soft cheese at pH 5.7 - 4.9. The protein and milk fat in these products possibly serve as protective microenvironment for the virus. The current basis for microbial safety of cheese is to use pasteurized milk or aged cheese. However, the effectiveness of aging process in destroying the virus is questionable because cheese was found to contain virus even after 90 d of aging. This may be due to the protection of FMD virus by the acid precipitated milk protein. It may be vital importance in virus protection. This protective influence may be either due to physical protection of viral cell or due to other types of interaction between the virus and milk casein (27). In contrast, (28) could not detect FMD virus in cheese 2 weeks after processing. This may be due to different manufacturing conditions, pH and method used for virus detection.

Table 4: Stability of FMDV during processing and storage. of yoghurt

Storage period (d)	Virus titer	pH
0 (fresh)	ND	4.4
1	ND	4.3
2	ND	4.0

ND: not detected

The survival of FMD virus in yoghurt was recorded in Table 4. FMD virus was completely inhibited in yoghurt after processing (pH 4.3) and during 2 d of refrigerated storage (pH 4.0). These findings are likely to be due to the rapid increase in number of lactic acid bacteria and production of lactic acid that inactivate the virus throughout processing and storage period. Similar trend of FMD virus in yoghurt was found by (22, 29). The obtained results indicated that pH of yoghurt was of great importance for complete inactivation of FMD virus.

4. Conclusion

It could be concluded that young children should consume double set pasteurized milk. The use of commercially available UHT product and aging of soft cheese for 120 d before consumption are recommended. Strict hygienic measures should be imposed during milk production, handling and processing to reduce the risk of milk contamination with FMD virus

5. References

(1) AGGARWAL, N., BARNETT, V.: J. Virol. **83** (1) 775-782 (2005)
(2) GRUBMAN, M., BAXT, B.: Rev. Sci. Technol. **17** (2) 465-493 (2004)
(3) ANON.: In: Epidemic outbreaks of infectious disease in livestock (Ed. S. Chairman, F. Brian). Royal Soc. London, 8 (2002)
(4) KAKU, Y., CHARD, L, INQUE, I.: Lebensmittel **76** (11) 1721-1728 (2003)
(5) MARCO, S., JOHANNES, K., BARBARA, B.: Int. J. Food Microbiol. **74** (2) 73-78 (2002)
(6) FRY, E., STUART, D.: Cur. Microbiol. **88** (1) 71-101 (2005)
(7) MAHY, B. : Cur. Immunol. **288** (1) 1-9 (2004).
(8) SALGUERO,J., SANEL, M., DIAL, F.: J. Virol. **103** (1) 4-6 (2005)
(9) SCHIJVEN, J.; GERARD, J, MARIA, R: Risk analysis **25** (11) 1113-1118 (2005)
(10) SUTMOLLER, G., VASE, D.: Rev. Sci. Tech. **16** (1) 30-33 (1997)
(11) LEWIS, M.J.: Int. J. Dairy Technol. **12** (5) 901- 908 (1999)
(12) ABOU-DONIA, S.: J. Dairy Sci. Technol. **21** (2) 167-190 (1986)
(13) MEHANNA, S., HEFNAWY, A: Egypt. J. Dairy Sci. **18** (3) 425-434 (1990)
(14) KUMAR, R, SANY, A, HEMADI, D.: Arch. Viral. **149** (9) 1801- 1819 (2004)
(15) BLACKWELL, J.H.: J Dairy Sci. **70** (2) 200-210 (1997)
(16) REID, M., FERRIS, P., HUTCH, H.: Neth. Milk J. **10** (1) 67-80 (2001)
(17) ALEXANDERSEN, S., ZHANG, D., DONALDSON, I.: Milk Dairy J. **4** (12) 1099-1110 (2002)
(18) NIEBALSKI, W.: J. Vet. Sci. **6** (2) 331-339 (2003)
(19) HUGHES, G., MIOULETI, V., KITCHING, R: J. Dairy Sci. Technol. **4** (2) 724-772 (2004)
(20) MOORE, J.E.: J. Dairy Res. **69** (3) 483-490 (2002)
(21) DOEL, R, BACCARINI, J.: Arch. Virol. **70** (1) 21-32 (1999)
(22) TOMASULA, P., KONSTANCE, R: J. Dairy Sci. **87** (10) 1115-1121 (2004)
(23) THOM, L.: Am. Vet. J. **66** (8) 1119-1122 (2001)
(24) AMASS, S., MASON, P., PACHEO, J.: Vet Microbiol. **15** (3) 711-729 (2004)
(25) DONALDSON, AI: Am. Rev. Tech. **16** (1) 117-124 (1997)
(26) KHUKHOWROV, B., THOM, H., SAVAY, M.: Can. J. Med. **88** (1) 305-309 (2005)
(27) SONDER, E., ACKERMANN, M., KIHM, U.: Rev. Sci. Technol. **17** (2) 339- 353 (1999)
(28) SCHJERNING, A, THEISEN, J.: Vet. Rec. **87** (1) 178-190 (1999)
(29) BACHRACH, L., BREESE, S., CALL, S, J.: Bio. Med. J. **95** (2) 147-152 (1995)