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Effect of heat on foot-and-mouth disease virus (FMDV) in the components of milk from FMDV-infected cows

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SUMMARY

Foot-and-mouth disease virus (FMDV) survived in skim milk, cream and the pelleted cellular debris components of milk obtained from FMDV-infected cows after pasteurization at 72° C for 0·25 min. Virus was recovered from whole milk of infected cows after that milk was heated at 72° C. for 5 min. and from the skim milk component after it was heated at the same temperature for 2 min. Evaporation of the whole milk samples after they were heated at 72° C. for 3 min. did not inactivate the virus, but evaporation of infected skim milk samples after they were heated at 72° C. for 0·5 min. did inactivate the virus. FMDV survived in the cream component after it was heated at 93° C. for 0·25 min.

INTRODUCTION

In an earlier study, Hyde, Blackwell & Callis (1975) reported that FMDV in milk obtained from FMDV-infected cows survived minimum recommended high temperature-short time (HTST) pasteurization (71·7° C. for 0·25 min.; U.S. Food and Drug Administration, 1971). In the above report, the infected milk samples were collected before the onset of clinical signs of disease in the cows. The detection of FMDV in this milk after pasteurization further supports the contention of other researchers that a potential for the transmission of foot-and-mouth disease (FMD) exists in milk (Dawson, 1970; Hedger & Dawson, 1970; Terbruggen, 1932). This type of transmission was probably in evidence during a series of outbreaks of FMD in England during 1967–8 (Hedger & Dawson, 1970).

MATERIALS AND METHODS

Cattle

Three grade dairy cows obtained from commercial sources on Long Island, New York, USA, and varying in age from 3 to 7 years were used in these studies. Two ml (*ca.* $10^{7\cdot0}$ plaque forming units (p.f.u.)/ml.) of a suspension of FMDV type A, subtype 3 (A_3) strain Mecklenburg were inoculated into the milk sinuses of the

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right front and left rear teats of each cow. In addition, 2 ml. of the same virus suspension was inoculated intravenously into the jugular vein. All cows received in the animal holding unit had subclinical mastitis. Animal housing and maintenance have been previously described (Hyde *et al.* 1975). Serum samples from inoculated animals were assayed by the method described by Graves, McVicar, Sutmoller & Trautman (1971).

Preparation of component samples

Milk samples were collected at 24 hr. intervals and then centrifuged at 800 *g* for 10 min. The cream layer of the samples was aspirated and pooled. The skim milk part of the samples was removed without disturbing the pelleted debris and then pooled, and the pelleted debris was resuspended to the original volume with reconstituted powdered skim milk.

Procedures

(*a*) *Pasteurization.* Samples of whole milk, skim milk and pelleted cellular debris were pasteurized as described previously (Hyde *et al.* 1975) on the day of collection at the respective exposure periods. The cream samples were pasteurized after storage at −70° C.

(*b*) *Evaporation.* Milk samples were evaporated within 7 days after pasteurization and storage at −70° C. Before evaporation, the samples were thawed in a 37° C. water bath, then decanted aseptically into a sterile, 2 l., round-bottom evaporation flask with a 24/40 ground-glass tapered joint and then connected to a condensing flask of a flash rotary evaporator (Buchler Instruments, Fort Lee, New Jersey, USA). The evaporation flask rested in a water bath, preheated to 65° C., at a level such that the volume of milk would always be submerged below the water surface of the bath. The condensing flask was rotated in an ice bath containing NaCl (commercial rock salt) and 70% ethyl alcohol, which maintained the bath temperature at −12° C. The samples were reduced to 50% of their original volume by heating at 65° C. under a vacuum of 60 cm. of mercury for a maximum period of 1 hr.

Virus detection

One-tenth ml. of each sample was adsorbed onto each of three monolayer cultures of primary bovine kidney cells in 4 oz. bottles. If the monolayer cultures were negative for plaque production, the rest of each sample was inoculated into each of 2 steers, 2 ml. intradermally into the tongue and 35 ml. intramuscularly into either flank. The animals were observed daily for febrile response and the development of vesicular lesions. Upon detection of the latter, samples of vesicular material were harvested for serological typing (Cowan & Trautman, 1967). Steers that remained free of clinical signs of FMD for 14 days after inoculation were challenged subsequently by intradermal inoculation of the tongue with 10^6 p.f.u. of FMDV A_3 (Mecklenburg).

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Table 1. *Concentration of virus in the components of whole milk collected after intramammary and intravenous inoculation of cows with foot-and-mouth disease virus*

Days after inoculation	Milk component	$\log_{10}$ p.f.u./ml		
		Cow no. 1	Cow no. 2	Cow no. 3
1	Whole	6·38	4·95	5·29
	Skim	6·29	4·6	4·74
	Cream	6·9	5·8	5·83
	Pellet	2·9	2·7	4·2
2	Whole	4·0*	3·07	4·5*
	Skim	2·9	2·7	4·2
	Cream	2·94	4·02	4·19
	Pellet	NT	NT	NT
3	Whole	4·04	4·02	4·66
	Skim	3 75	2·9	4·36
	Cream	4·26	3·57	4·34
	Pellet	NT	NT	NT
4	Whole	5.·27	NT	5·5
	Skim	4·32	NT	5·76
	Cream	4·81	NT	5·65
	Pellet	NT	NT	NT

* Appearance of vesicular lesions. NT, not tested.

RESULTS

Recovery of virus

Untreated milk

The milk samples studied were collected 1 day after inoculation but before the onset of clinical signs of FMD. The cream and skim milk samples were prepared from these whole milk samples. Cows no. 1 and no. 3 developed vesicular lesions on the tongue, the dental pad and the interdigital spaces of the hoofs 2 days after inoculation (Table 1). Cow no. 2 did not show signs of clinical disease through 14 days after inoculation; however, a significant virolactia did occur. The serum of this animal contained specific neutralizing activity against FMDV A_3 (Mecklenburg) before challenge and the animal remained negative for 14 days for clinical signs of disease after challenge with $10^{6·3}$ p.f.u. of virus. As described previously (Hyde *et al.* 1975), subclinical mastitic infections did not prevent significant replication of virus in the mammary glands of the test cows. The highest titres of virus were recovered at 24 hr. after inoculation in whole milk, skim milk and cream (Table 1). Titres of virus recovered from the cream component were consistently higher than those recovered from the skim milk component. A second peak of virus infectivity was observed also in the 3 components.

Milk after heating

Whole milk

FMDV survived in all of the whole milk samples from FMDV-infected cows after the milk was heated at 72° C. for 5 min. Survival was evidenced by a clinical

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Table 2. *Survival of FMDV in milk from infected cows after heating*

Exposure period in min. at 72°C	Milk Sample	Virus concentration in cell culture, $\log_{10}$ p.f.u./ml. Cow no. 1 Before heating	Cow no. 1 After heating	Cow no. 2 Before heating	Cow no. 2 After heating	Cow no. 3 Before heating	Cow no. 3 After heating	Heated milk samples inoculated in cattle*
0·25	Whole	6·4	NT	4·95	<0·4†	5·3	NT	NT
	Skim	6·3	NT	4·60	<0·4	4·7	<0·4†	6/6
0·5	Whole	6·4	0·50	4·95	<0·4	5·3	<0·4	6/6
	Skim	6·3	0·80	4·6	<0·4	4·7	<0·4	6/6
1·0	Whole	6·4	0·50	4·95	<0·4	5·3	<0·4	6/6
	Skim	6·3	0·47	4·6	<0·4	4·7	<0·4	6/6
2·0	Whole	6·4	<0·4†	4·95	<0·4	5·3	<0·4	6/6
	Skim	6·3	<0·4	4·6	<0·4	4·7	<0·4	2/6‡
3·0	Whole	6·4	<0·4	4·95	<0·4	5·3	<0·4	6/6
	Skim	6·3	<0·4	4·6	<0·4	4·7	<0·4	NT
4·0	Whole	6·4	<0·4	4·95	1·2	5·3	<0·4	6/6
	Skim	6·3	<0·4	4·6	<<0·4	4·7	<0·4	NT
5·0	Whole	6·4	<0·4	4·95	<0·4	5·3	<0·4	6/6
	Skim	6·3	<0·4	4·6	<0·4	4·7	<0·4	NT

* Number steers positive/number steers inoculated.
† Plaques not observed in 0·3 ml. sample.
‡ Milk from cow no. 3 was infective for cattle.
NT = not tested.

response in cattle after inoculation with the milk samples. FMDV also survived in whole milk after it was heated at 85° C. for 0·25 min.

FMDV has been detected routinely in cell culture after milk from FMDV-infected cows has been heated at 72° C. for 0·25 min. In the present study, FMDV was detected in the milk of cow no. 1 (Table 2) after milk was heated at 72° C. for 1 min. and in the milk of cow no. 2 after milk was heated at the same temperature for 4 min.

Skim milk

FMDV survived in skim milk after samples from all 3 cows were heated at 72° C. for 1 min.; however, milk from only cow no. 3 was infective for steers when that milk was heated at 72° C. for 2 min. (Table 2).

Cream

All cattle became infected with FMD 48 hr. after inoculation with the cream component, which had been heated at 93° C. for 0·25 min. (Table 3).

Pelleted cellular debris

One of three reconstituted cellular debris samples heated at 72° C. for 0·25 min. produced FMD in cattle.

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Table 3. *Survival of FMDV in the cream component of milk from infected cows after heating*

Cow no.	Temperature of exposure (° C)	Virus concentration in cell culture $\log_{10}$ p.f.u./ml. Before heating	After heating*	Heated cream samples inoculated in cattle†
1	72	1·8	<0·4‡	2/2
	93	1·8	<0·4	2/2
2	72	5·6	0·51	2/2
	93	5·6	<0·4	2/2
3	72	6·3	1·0	2/2
	93	6·3	<0·4	2/2

* Exposure period 0·25 min.
† Number positive/number inoculated.
‡ Plaques not observed in 0·3 ml. sample.

Milk after evaporation

FMDV survived in whole milk after it was heated at 72° C. for 3 min. and then evaporated. The virus survived in 1 of 3 skim milk samples after they were heated at 72° C. for 0·25 min. and then evaporated. FMDV was inactivated in all of the skim milk samples after they were heated at 72° C. for 0·5 min. and then evaporated.

Thus, FMDV is not inactivated by HTST pasteurization of skim milk, cream or pelleted cellular debris components of whole milk from FMDV-infected cows. The virus also is neither inactivated in whole milk after it is heated at 72° C. for 5 min., nor after 3 min. and then evaporated. In addition, the virus survives in cream after it is heated at 93° C. for 0·25 min.

DISCUSSION

The thermal stability of FMDV in milk from FMDV-infected cows after pasteurization has been documented by Hyde *et al.* (1975). These authors reported that conventional HTST pasteurization was not adequate for the thermal inactivation of FMDV in the milk of infected cows. These findings were similar to those of other reports in the literature on the thermal stability of FMDV in non-milk systems (Bachrach *et al.* 1957; Bachrach, Patty & Pledger, 1960; Dimopoullos *et al.* 1959). For the report described herein, we studied the survival of FMDV in whole milk, skim milk, cream and reconstituted cellular debris after these components were heated at 72° C. for 0·25 min. or longer. Significant concentrations of virus in skim milk and cream were partly expected because of the reported recoveries of virus in whole milk by Burrows *et al.* (1971) and later by Hyde *et al.* (1975). The lowest concentration of virus recovered in each instance was obtained from the pelleted cellular debris. This material represents intact and fragmented somatic cells, leukocytes and environmental debris that normally are removed by filtration or clarification of the milk. Although the fact was not proved, virus

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recovered from these samples could possibly have had its origin as intracellular virus in cellular fragments of epithelial cells sloughed from the bovine udder or could have originated upon collection by contamination of the pelleted debris with residual cream layer. Therefore, attention also should be given, on farms and in processing plants, to filtration and clarification processes as being potential foci for the dissemination of FMDV.

As reported earlier by Hyde *et al.* (1975), cattle were more sensitive than primary bovine kidney cell culture in detecting small amounts of virus. Most of the heated samples in the study were negative by cell culture assay but positive when inoculated into steers.

Cream as a product itself or when present in other milk products as high fat content is treated commercially by heating over a range of 72–84·4° C. for 0·25 min. (Dahlberg, Adams & Held, 1953). On the other hand, cream that will be used in the manufacture of butter is pasteurized at a minimum temperature of 87·7° C. for 0·25 min. (U.S. Dept. Agr., 1967). These temperatures are not adequate for the inactivation of FMDV in cream and this points out the protection that cream would confer to virus present in dairy products prepared from whole milk from FMDV-infected cows (Table 3). The fact that FMDV survives the evaporation of whole milk heated for expanded exposure periods but not that of skim milk samples receiving the same treatment suggests that the butter fat in cream of whole milk confers an even higher degree of protectivity during heating than does the protein in skim milk. In addition, the survival of FMDV in each of 3 trials with whole milk but in only one of 5 trials in skim milk after heating at 85° C. for 0·25 min. also demonstrates the protectivity of butter fat.

As was reported earlier by Burrows *et al.* (1971) and by Hyde *et al.* (1975), milk secreted during the prodromal stage of FMD contains large amounts of virus. It is this milk that would be delivered to processing plants and placed into general distribution before the disease could be detected. Although milk and milk products are processed essentially for consumption in the human food chain, the feeding of livestock with surplus products is a common occurrence. Such feeding practices, as well as contamination of milking parlours, milking equipment and animal holding areas by infected milk, lend themselves to the rapid dissemination of FMD in livestock populations. Therefore, dairy products prepared from skim milk and especially from cream from milk of FMDV-infected cows are of utmost importance to countries either manufacturing or importing these products.

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