J. Hyg., Camb. (1978), **81**, 415
Printed in Great Britain

Excretion of foot-and-mouth disease virus in oesophageal-pharyngeal fluid and milk of cattle after intranasal infection

By P. W. de LEEUW, J. G. van BEKKUM and J. W. A. TIESSINK
Central Veterinary Institute, Virology Department, 39 Houtribweg, Lelystad, The Netherlands

(*Received* 28 *February* 1978)

SUMMARY

The virus growth in the pharyngeal area and the virus excretion in milk of susceptible and vaccinated dairy cows after intranasal instillation of foot-and-mouth disease (FMD) virus type O_1 were examined. Ten vaccinated cows were purchased through a market. Of these, nine had delivered their first calf. The cows were inoculated 2–9 months after having received the last dose of vaccine. All vaccinated cows resisted the intranasal challenge. The virus multiplied in the pharyngeal area but, compared with two susceptible controls, to a limited extent. No clear relation was found between virus growth and the titre of circulating neutralizing antibody at the time of challenge.

Virus was first detected in milk samples of the susceptible cows when generalized FMD lesions had developed on day four; the excretion lasted for 3–4 days.

Up to 19 days after inoculation untreated milk of the vaccinated cows was examined for the presence of infectious FMD virus. Samples were inoculated onto cell cultures, fed to susceptible pigs and calves and injected intramuscularly and/or intradermolingually into susceptible steers. No infectious FMD virus could be detected, either in cell cultures or in susceptible animals. The animals did not develop neutralizing antibody against FMD virus and were subsequently shown to be fully susceptible to challenge. The results are discussed with particular reference to current problems regarding the export of milk products from countries where vaccination against FMD is practised to countries free of the disease.

INTRODUCTION

McVicar & Sutmoller (1976), working with cattle vaccinated with an experimental oil-adjuvant vaccine, reported that after intranasal instillation of foot-and-mouth disease (FMD) virus, the virus growth in the upper respiratory tract was inversely related to the degree of immunity.

In this study virus growth in the oesophageal-pharyngeal area of cows vaccinated under field conditions with aluminium hydroxide vaccines is compared with that in susceptible controls. In addition FMD virus excretion in milk of both vaccinated and susceptible cows after intranasal infection was examined. Burrows (1968), Hedger & Dawson (1970) and Burrows *et al.* (1971) reported that susceptible dairy cows infected with FMD virus may excrete large quantities of virus in their milk

before symptoms of the disease are observed. Such infected milk could play a role in the spread of FMD, as shown in Great Britain during the 1967–8 epizootic (Dawson, 1970).

To our knowledge, no data have been published on FMD virus excretion in milk of vaccinated cattle after exposure by a natural route. Yet, such data are important, for instance to assess the risks involved in the importation of milk products from countries like those in western Europe where FMD vaccination is carried out yearly.

MATERIALS AND METHODS

Virus

FMD virus type O_1, strain Weerselo was used. This strain was isolated in The Netherlands from pigs in the 1966 epizootic, when it affected both cattle and pigs.

The virus used to infect cattle had received three intradermolingual passages in this species in the laboratory.

The preparation used for the challenge of pigs was passaged twice in pigs and once in primary pig kidney cells.

All virus pools were stored at −70 °C.

Neutralization tests were done with virus grown in surviving cattle tongue epithelium by the Frenkel method.

Cell cultures, plaque titration and neutralization test

In all tests primary or secondary pig kidney (PK_1 or PK_2) cells were used. Details of these tests will be described elsewhere (de Leeuw, Tiessink & Frenkel, 1978). Briefly, confluent monolayers of PK_2 cells were grown in 6 cm disposable plastic Petri dishes in a CO_2 incubator. After inoculation and a 1 h adsorption period, the monolayers were washed and overlaid with medium containing 1% methyl cellulose. Cultures were stained after 48 h. Virus titres are expressed as plaque forming units (p.f.u.) per ml.

Serum antibody assay was done in flat-bottomed microtitre trays. Volumes of 0·05 ml of serum were diluted in twofold steps and mixed with 0·05 ml volumes of a virus suspension containing approximately 100 TCID 50. Each serum was tested in duplicate. After 1 h at 37 °C, 0·15 ml of a PK_1 cell suspension, containing approximately 30000 viable cells, was added and the trays were returned to the CO_2 incubator. The cells were stained and the results were read on the third day. Serum neutralizing antibody titres (NT-titres) are expressed as the $\log_{10}$ of the reciprocal of the serum dilution endpoint, taken as the dilution that protected the cells in 50% of the wells inoculated.

Animals, inoculation and sampling procedures

The following FMD susceptible animals were used: five approximately 18-month-old steers and three 4-year-old dairy cows, all imported from Ireland; two 6-months-old colostrum-deprived calves and 16 pigs about 5 weeks of age.

Ten vaccinated Friesian dairy cows were purchased through a market. They

Table 1. *Specifications of the vaccinated cows used*

No. of cow	Age at the time of challenge (months)	No. of vaccinations received	Interval between last vaccination and challenge (months)
1	27	3	2
2	23	2	2
3	25	2	3·5
4	26	2	4·5
5	23	2	5
6	120	11	5
7	21	1	9·5
8	29	2	9
9	29	2	9
10	31	2	9

originated from eight different farms; their specifications are summarized in Table 1.

All animals were observed daily for febrile response and the development of vesicular lesions. If vesicles developed material was harvested for typing by complement-fixation.

Intranasal inoculation was done as described by McVicar & Sutmoller (1976), by slow instillation of 2·5 ml of a virus suspension containing 10^7 p.f.u. per ml, into the ventral part of each nasal passage. The day of inoculation with virus is always regarded as day 0.

Oesophageal-pharyngeal fluid (OPF) was obtained as described by means of a cup probang (van Bekkum *et al.* 1959). Samples were stored at −70 °C until tested. They were then treated with fluorocarbon* by blending in an Omnimixer† at 16000 rev./min for 3 min followed by centrifugation as described by Sutmoller & Cottral (1967). The supernatant was plated for assay of infectious FMD virus as described.

Milk samples were obtained after cleaning the mammary glands with warm water, rinsing with a citric acid solution and drying with sterile towels. The milk was collected in sterilized bottles using stainless steel funnels, cleaned and disinfected before each milking. Milk samples were examined for the presence of infectious FMD virus by inoculation of 10 ml volumes onto confluent PK_2 cell monolayers grown in Roux bottles. The cell sheets were washed twice with PBS before inoculation. After 1 h at 37 °C to allow adsorption, the monolayers were washed again and maintenance medium consisting of Hanks' MEM with 3% lamb serum was added. All lamb sera were previously shown to be free from antibody against FMD virus. After 3 days a blind passage was made and if no cytopathic effect developed in another 3 days the sample was considered not to contain FMD virus. Positive milk samples were titrated by plaque assay as described.

A FMD susceptible cow was inoculated intramammarily and intravenously by administering 10 ml of a virus suspension, containing 10^6 p.f.u./ml, into the milk

* Arklone P, ICI, Alderley Park, Macclesfield, Cheshire, United Kingdom.

† Omnimixer, Ivan Sorvall, Inc., Norwalk, Conn., U.S.A.

https://doi.org/10.1017/S0022172400025304 Published online by Cambridge University Press

Table 2. *Injection scheme of milk into susceptible recipients, experiment* 3

Steer no.	Milk from cows no. 8–10 injected i.m. and i.d.l. on days*	Milk from cow no. 7 on days	
		i.d.l.*	i.m.*
1	1, 4, 7, 13, 16	—	—
2	2, 5, 8, 14, 19	—	—
3	3, 6, 9, 15	—	—
4	—	3, 6, 9, 14	1, 2, 4, 5, 8, 13, 15, 19

* Quantity: i.d.l., 2 ml; i.m., 50 ml.

sinuses of four teats and the jugular vein. Milk samples were obtained before and 8 h after inoculation and stored in 20 ml volumes at −70 °C. After thawing the 8 h sample was diluted 1/10 and 1/100 in pre-exposure milk. It was then mixed with 9 or 99 volumes of pre-exposure milk from cows no. 1–6 and of milk taken from a tank on a farm 10 months after the last FMD vaccination of the cows. After 4 and 16 h at 4 °C the mixtures were titrated on PK_2 cell monolayers as described. Pre-exposure samples of the intramammarily infected cow were used as controls. Similar samples were also used to obtain 'contaminated' milk by addition of 10^4 p.f.u. of virus per ml. 'Contaminated' milk was diluted 1/10 in pre-exposure milk of cows no. 1 and 5 to 9, and farm milk. These mixtures were incubated and titrated as described above. Before titration several mixtures of antibody-containing and 'contaminated' or naturally infected milk were treated up to five times with fluorocarbon, as described for OPF samples.

Experiments

Experiment no. 1. Two susceptible cows and vaccinated cows no. 1 and 2 were housed individually in isolation units and inoculated intranasally. OPF samples were obtained from one of the susceptible cows and vaccinated cow no. 1 after 0·5, 1, 1·5, 2, 4, 6, 8, 14, 22 and 28 h and then twice daily for 15 consecutive days. The other susceptible cow was sampled during inoculation, after 0·25, 0·5, 1, 1·5, 2, 3, 4·5, 6·5 and 12 h, twice daily on days 1–10 and once daily on days 11–15. OPF samples from cow no. 2 were obtained twice daily on days 1–4 and 7–11, and once daily on the other days up to day 21. All cows were milked twice daily and each time a representative sample was frozen at −70 °C. After thawing, samples from the same day were mixed for each cow and 10 ml was assayed for infectious virus. Blood samples were obtained before exposure and 2 weeks later. After 3 weeks the animals were killed and autopsy was performed.

Experiment no. 2. Cows no. 3–6 were housed together in one isolated room, tied up at distances of about 3 m from each other. After intranasal exposure, OPF samples were taken on days 1–9, 11 and 14–18. Milk samples of each cow were taken daily, frozen at −70 °C and later pooled for each day for the whole group. Ten ml of each mixture was examined for infectious virus on cell cultures. In addition, fresh untreated milk was fed to or injected into FMD susceptible animals within 2 h after it had been obtained. Eight pigs received 14 l of pooled milk daily and 6 l were fed to two calves. One steer received 50 ml of the same pool intramuscularly (i.m.) on days 2–5, 8–10, 12 and 15–18.

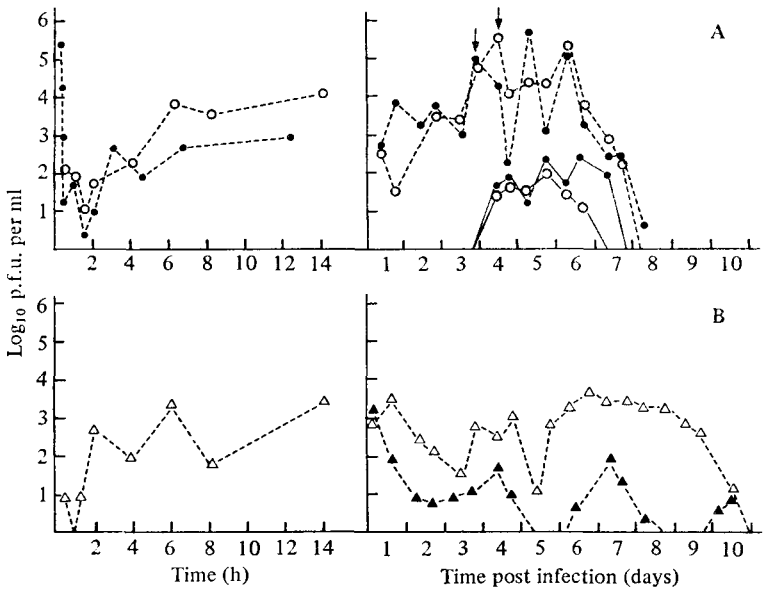

Fig. 1. Pharyngeal virus growth curves and virus excretion in milk after intranasal instillation of $10^{7\cdot7}$ p.f.u. of foot-and-mouth disease virus type O_1. A, Susceptible cows; B, vaccinated cows. ---, Pharyngeal virus; ——, milk virus; ↓, generalization.

Blood sampling and post-mortem examination of cows no. 3–6 were done as in the first experiment. Blood samples of the 'indicator' animals were taken before the first milk samples were given and prior to challenge. The pigs were challenged after 5 weeks by inoculating two pigs of the group intravenously with 10 ml of a virus suspension containing 10^5 p.f.u. per ml. The steer and the calves were challenged intradermolingually (i.d.l.) by injection of 0·1 ml of a virus suspension containing 10^4 p.f.u. per ml after 5 and 8 weeks, respectively.

Experiment no. 3. Cows no. 7–10 were housed and inoculated as in the previous experiment. OPF samples were obtained on days 1–10 and 13–18.

The yield of each milking of cows no. 8, 9 and 10 was mixed and 14 l were fed daily to eight FMD susceptible pigs. Samples of this mixture were also administered to three susceptible steers. Each time 50 ml was given i.m. and 2 ml i.d.l. The same quantity of milk from cow no. 7 was injected either i.d.l. or i.m. into a fourth steer, as indicated in Table 2.

Cows no. 7–10 were not killed at the end of the experiment since they had to be used for other purposes; blood samples were obtained before and 3 weeks after exposure. 'Indicator' animals were sampled and challenged as in the previous experiment. Challenge was performed after 6 weeks.

RESULTS

Experiment no. 1

The OPF sample collected from one of the susceptible cows during intranasal inoculation had a titre of $10^{5\cdot3}$. The first half hour after infection the virus titre

https://doi.org/10.1017/S0022172400025304 Published online by Cambridge University Press

Table 3. *Neutralizing antibody titres of sera from vaccinated dairy cattle before and 2–3 weeks after intranasal exposure to FMD virus*

Cow no.	Pre-exposure titre	Titre 2–3 weeks after exposure
1	2·55*	2·95
2	2·85	2·85
3	3·0	2·85
4	2·25	3·15
5	2·55	2·7
6	3·45	3·15
7	1·65	2·95
8	1·8	2·7
9	1·35	2·25
10	1·95	2·4

* Log_{10} of the reciprocal of the serum dilution endpoint.

in the OPF of both susceptible cattle dropped rapidly and then at a slower rate during the next hour. Thereafter titres of OPF rose abruptly, then slower and levelled off at 4–6 h. Titres of 10^2–10^4 were found at between 12 and 72 h (Fig. 1A). The highest titres were found the following 3 days, they dropped again on day seven. The ninth day OPF samples of both susceptible cows were negative. One cow had developed FMD lesions on the tongue and the feet after 80 h and the other after 96 h; febrile responses were first noted 8–16 h before. Virus was first detected in milk samples of both cows on the fourth day; the titre remained between $10^{1·2}$ and $10^{2·7}$ until the seventh day. The eighth day all milk samples were negative again (Fig. 1A).

The first 24–32 h the pharyngeal virus growth pattern of vaccinated cow no. 1 resembled that of the susceptible cattle (Fig. 1B). Thereafter titres found in OPF samples of the vaccinated cows were lower than in susceptible cattle; however, although irregularly, they continued to excrete traces of virus in their OPF up to days 17 and 21. Cows no. 1 and 2 remained healthy: all temperatures recorded were below 39·1 °C and no FMD lesions were observed during inspection of the live animal or at autopsy. There was no significant serological response 2 weeks after infection (Table 3).

No virus could be detected in milk samples of cows no. 1 and 2 by inoculation of cell cultures.

Experiment no. 2

Vaccinated cows no. 3–6 also remained healthy after intranasal exposure 3–5 months after vaccination; no febrile responses were noted and no FMD lesions were seen in the living animal or at autopsy.

In OPF samples collected the first 6 days, virus titres varied between $10^{2·5}$ and $10^{3·7}$. Samples of cows no. 5 and 6 became negative after the seventh day (Fig. 2B) and of cow no. 3 after the tenth day p.i. Cow no. 4 continued to excrete varying but small quantities of virus in its OPF up to day 18 (Fig. 2A).

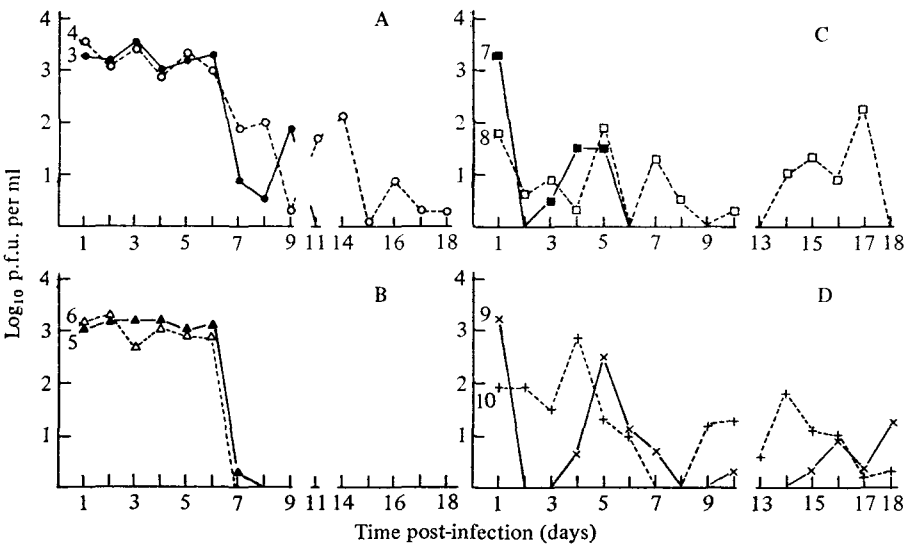

Fig. 2. Pharyngeal virus growth curves after intranasal inoculation of vaccinated cattle with FMD virus type O_1. A and B challenged 3–5 months after the last vaccination; C and D challenged 9 months after the last vaccination.

Only this cow showed a significant rise of NT-titre 2 weeks after exposure (Table 3). In milk samples no virus could be detected by inoculation of cell cultures.

All 'indicator' animals remained healthy and none developed a measurable neutralizing antibody titre. After challenge all animals developed generalized FMD within 72 h.

Experiment no. 3

The cows used in this experiment, no. 7–10, were challenged 9 months after they received their last vaccination. As expected their NT-titres were lower than those of the cows used in the preceding experiments that had been revaccinated 2–5 months before challenge (Table 3). All cows remained healthy after intranasal exposure. The first 6 days after infection the OPF titres in general were lower than those found in corresponding samples in the previous experiment (Fig. 2C, D). Titres found on successive days varied markedly. Cow no. 7 became negative on the sixth day, but the others excreted virus intermittently up till day 18. A serological response was detected in all four cows (Table 3).

The susceptible steers and pigs receiving the milk did not develop symptoms of FMD and no neutralizing antibody was detected in their sera 6 weeks after the beginning of the experiment. These animals were then challenged and all developed FMD within 72 h.

The milk sample obtained 8 h after intramammary and intravenous infection of a susceptible cow contained $10^{6\cdot1}$ p.f.u./ml. All milk samples from vaccinated cows that were tested caused a 10- to 100-fold reduction in the virus titre of this sample, regardless of whether the virus-containing sample had previously been diluted with pre-exposure milk of the same cow to contain $10^{5\cdot1}$ or $10^{4\cdot1}$ p.f.u./ml

https://doi.org/10.1017/S0022172400025304 Published online by Cambridge University Press

Table 4. *Effect of antibody-containing milk on the virus titre of milk from an infected susceptible cow and on virus added to pre-exposure milk from the same cow*

Antibody containing milk from	'Naturally infected' milk mixed* 1/10	1/100	'Contaminated' milk mixed† 1/10
Cow no. 1	1·5‡	1·3	1·8
3	1·1	1·0	—§
4	1·5	1·4	—
5	1·2	1·2	1·0
6	1·5	2·1	2·4
7	—	—	1·1
8	—	—	1·6
9	—	—	1·4
Farm milk ‖	1·3	1·5	1·6

* Infected milk diluted in pre-exposure milk of the same cow to contain $10^{5\cdot1}$ p.f.u. of virus/ml, was mixed with 9 or 99 volumes of antibody-containing milk.

† Milk of a susceptible cow to which 10^{4} p.f.u. of virus was added per ml was diluted 1/10 in antibody-containing milk. All virus-containing samples incubated with antibody-containing samples for 16 h at 4 °C.

‡ Neutralization index; log reduction in virus titre with respect to a control diluted and incubated in milk without antibody against FMD virus.

§ Not tested.

‖ Sample taken from a milk tank on a farm, 10 months after the last vaccination of the cows.

or whether one volume was mixed with 9 or 99 volumes of antibody-containing milk. Incubation of the mixtures for 4 h at 4 °C was as effective as for 16 h at 4 °C. Individual antibody-containing milk samples showed a similar activity after mixing with infected and 'contaminated' milk and incubation for 16 h at 4 °C (Table 4).

Fluorocarbon treatment of mixtures of antibody-containing and virus-containing milk, repeated up to five times, did not raise the amount of infectious virus that was subsequently recovered in cell cultures.

DISCUSSION

In The Netherlands more than 80% of the cows have at least been vaccinated twice before they deliver their first calf. Trivalent vaccine containing formaldehyde-inactivated Frenkel-type virus adsorbed onto aluminium hydroxide gel is used. One dose contains at least six cattle PD 50 of each virus type. The efficacy of this vaccination program under field conditions was confirmed in studies reported by van Bekkum, Fish & Dale (1963) and Fish *et al.* (1969). The immune status of the vaccinated dairy cows used in this study can be regarded as representative of that of the first-calf dairy cow population in the Netherlands. This was confirmed by the results of neutralizing antibody tests carried out with stock sera obtained from first-calf dairy cows the previous year (Table 5). The NT-titres of cows no. 7–10, that were sampled approximately the same time after vaccination, are in the middle region of the frequency distribution.

Intranasal challenge was chosen because it represents a natural route of infec-

Table 5. *Frequency distribution of neutralizing antibody titres against FMD type* O_1 *of* 71 *sera collected from first-calf dairy cows,* 8–10 *months after the last vaccination*

Titre ...	< 1·5	1·5–2·0	2·0–2·6	2·6–3·2	> 3·2
No. of sera	2*	20	26	22	1

* These sera had titres of 1·2 and 1·35.

tion and may provide a more rigorous challenge of immunity than i.d.l. inoculation (McVicar & Sutmoller, 1976). All OPF samples were stored at −70 °C since no significant difference was found in the titre before and after storage at this temperature. During intranasal inoculation approximately 5% of the virus infectivity was recovered from the anterior oesophagus, similar to the results reported by McVicar & Sutmoller (1976). Compared with the pharyngeal virus growth curves they presented, which were obtained after intranasal instillation of 10^7 p.f.u. O_1 CANEFA, the growth rates found in this study in general are lower and the curves are less smooth, both in susceptible and vaccinated cattle. Virus titres in the OPF samples of vaccinated cow no. 2 (Fig. 1B) and of the cows used in experiment no. 3 (Fig. 2C, D) were lower than those found in the other vaccinated cows. The pharyngeal virus growth in cow no. 6, that had the highest NT-titre, was not less than that of the other vaccinated cows. Apparently pharyngeal virus growth after intranasal instillation of FMD virus depends only to a limited extent on the titre of circulating neutralizing antibody in cows that are protected from disease. However, the serological response following inapparent infection is clearly correlated with a low NT-titre before exposure (Table 3).

Within 4 days after challenge both susceptible cows developed generalized FMD. Virus excretion in milk started on day four and lasted 3–4 days. The highest titre found was $10^{2\cdot7}$. These results do not completely confirm the observations of Burrows (1968) and Hedger & Dawson (1970) who reported that FMD virus may be excreted in milk before clinical signs are observed and that up to 10^5 p.f.u. of virus per ml may be excreted.

All ten vaccinated dairy cows resisted the intranasal challenge. Although the virus multiplied in their oro-pharyngeal tissues, no virus could be detected in their milk. It is unlikely that significant quantities of virus were excreted by way of the udder and were subsequently neutralized by antibodies present in the milk (Table 4). Persistence of FMD virus in the pharyngeal region is well documented (van Bekkum *et al.* 1959; Sutmoller & Gaggero, 1965; Burrows, 1966). Burrows *et al.* (1971) reported that FMD virus persisted up to 19 days in mammary tissue of susceptible cows exposed by contact and for periods of 3–7 weeks after instillation of virus in the mammary gland. Based on the similarity of the situations in this regard in the udder and the pharyngeal region, Callis *et al.* (1975) suggested that persistence of FMD virus in the mammary gland might constitute another mechanism for a continuing infection in endemic areas. However, since primary infection of the mammary gland is unlikely to be a common occurrence in the pathogenesis of FMD (Burrows *et al.* 1971), virus excretion in milk from vaccinated

cows normally would result from initial infection and multiplication in the upper and/or lower respiratory tract (Sutmoller & McVicar, 1976).

Recently considerable concern has arisen since it was shown that FMD virus in milk and milk products was remarkably heat resistant (Hyde, Blackwell & Callis; 1975; Blackwell, 1976; Blackwell & Hyde, 1976; Cunliffe & Blackwell, 1977; Dhennin & Labie, 1976). Based on these results, certain countries free of FMD have taken measures to protect themselves from the risk attached to the import of milk products from countries that are not considered free of the disease, including countries where vaccination against FMD of all cattle is carried out yearly, even if no outbreaks occurred in a number of years. A serious obstruction of a long established trade in milk products has been the result. The data on FMD virus excretion in milk of cows in the field were obtained during outbreaks in Great Britain where vaccination against FMD is not practised (Dawson, 1970; Hedger & Dawson, 1970). Subsequent laboratory studies of Burrows (1968) and Burrows *et al.* (1971) were also done with cattle susceptible to FMD. In addition, most studies cited above on virus survival in milk and milk products have been carried out with milk obtained after inoculation of the virus into the mammary gland of susceptible cows. In regions where FMD is controlled by annual vaccination of the cattle population and where infected herds are eliminated by slaughter, the disease occurs only sporadically. In such cases it nearly always affects susceptible young stock or pigs; clinical disease among dairy cattle is rare. As this study confirms, such animals may become infected but even young cows are highly unlikely to excrete virus in their milk. Even if this should happen the concentration of the virus in milk could not be expected to approach that recorded for susceptible cows infected by intramammary infusion. The heat treatment routinely applied in the preparation of various milk products, combined with the activity of the neutralizing antibodies present in pooled milk, should certainly be adequate to inactivate it.

The authors wish to thank Messrs G. Kreuger, G. van Engelen and J. Schaake for help with the animal experiments and Dr S. Frenkel and his staff for providing cell cultures.

REFERENCES

Bekkum, J. G. van, Frenkel, H. S., Frederiks, H. H. J. & Frenkel, S. (1959). Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Tijdschrift voor Diergeneeskunde* **84**, 1159.

Bekkum, J. G. van, Fish, R. C. & Dale, C. N. (1963). Immunogenic studies in Dutch cattle vaccinated with foot-and-mouth disease vaccines under field conditions. 1. Neutralizing antibody responses to O and A types. *American Journal of Veterinary Research* **24**, 77.

Blackwell, J. H. (1976). Survival of foot-and-mouth disease virus in cheese. *Journal of Dairy Science* **59**, 1974.

Blackwell, J. H. & Hyde, J. L. (1976). Effect of heat on foot-and-mouth disease virus (FMDV) in the components of milk from FMDV-infected cows. *Journal of Hygiene* **77**, 77.

Burrows, R. (1966). Studies on the carrier state of cattle exposed to foot-and-mouth disease virus. *Journal of Hygiene* **64**, 81.

Burrows, R. (1968). Excretion of foot-and-mouth disease virus prior to the development of lesions. *Veterinary Record* **82**, 387.

Burrows, R., Mann, J. A., Greig, A., Chapman, W. G. & Goodridge, D. (1971). The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. *Journal of Hygiene* **69**, 307.

Callis, J. J., Hyde, J. L., Blackwell, J. H. & Cunliffe, H. R. (1975). Survival of foot-and-mouth disease virus in milk and milk products. *Bulletin de l'Office international des Epizooties* **83**, 183.

Cunliffe, H. R. & Blackwell, J. H. (1977). Survival of foot-and-mouth disease virus in casein and sodium caseinate produced from the milk of infected cows. *Journal of Food Protection* **40**, 389.

Dawson, P. S. (1970). The involvement of milk in the spread of foot-and-mouth disease: an epidemiological study. *Veterinary Record* **87**, 543.

Dhennin, L. & Labie, J. (1976). Thermorésistance du virus de la fièvre aphteuse dans le lait de vaches infectées. *Bulletin Académie Vétérinaire de France* **49**, 243.

Fish, R. C., van Bekkum, J. G., Lehmann, R. P. & Richardson, G. V. (1969). Immunologic responses in Dutch cattle vaccinated with foot-and-mouth disease vaccines under field conditions: neutralizing antibody responses to A, O and C types. *American Journal of Veterinary Research* **30**, 2115.

Hedger, R. S. & Dawson, P. S. (1970). Foot-and-mouth disease virus in milk: An epidemiological study. *Veterinary Record* **87**, 186.

Hyde, J. L., Blackwell, J. H. & Callis, J. J. (1975). Effect of pasteurization and evaporation on foot-and-mouth disease virus in whole milk from infected cows. *Canadian Journal of Comparative Medicine* **39**, 305.

Leeuw, P. W. de, Tiessink, J. W. A. & Frenkel, S. (1978). Vaccination experiments in pigs with formaldehyde-inactivated aluminium hydroxide foot-and-mouth disease vaccines potentiated with diethylaminoethyldextran (DEAE-D). *Zentralblatt für Veterinärmedizin* B, in the press.

McVicar, J. W. & Sutmoller, P. (1976). Growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation. *Journal of Hygiene* **76**, 467.

Sutmoller, P. & Cottral, G. E. (1967). Improved techniques for the detection of foot-and-mouth disease virus in carrier cattle. *American Journal Veterinary Research* **28**, 101.

Sutmoller, P. & Gaggero, A. (1965). Foot-and-mouth disease carriers. *Veterinary Record* **77**, 968.

Sutmoller, P. & McVicar, J. W. (1976). Pathogenesis of foot-and-mouth disease: the lung as an additional portal of entry of the virus. *Journal of Hygiene* **77**, 235.