J. Hyg., Camb. (1971), **69**, 307
Printed in Great Britain

The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland

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(*Received* 4 *February* 1971)

SUMMARY

In animals exposed to foot-and-mouth disease virus by indirect contact, virus was recovered from the blood, milk, pharynx, vagina and rectum for variable periods of time before clinical disease was apparent. Virus instilled into the mammary gland multiplied rapidly and virus concentrations greater than 10^7 p.f.u./ml. were recorded within 8–32 hr., depending on the virus strain and dose inoculated. Virus multiplication was accompanied by clinical signs of mastitis but the classical signs of foot-and-mouth disease did not appear for 52–117 hr. Dissemination of virus from the mammary gland occurred within 4–24 hr. and in some animals samples taken from the pharynx, mouth, nose and vagina contained virus for periods up to 97 hr. before the appearance of vesicular lesions. Virus production in the udder declined with the appearance of virus neutralizing activity in the blood and the milk but persisted in some animals for periods of 3–7 weeks. The ability of foot-and-mouth disease virus to persist in mammary tissue was confirmed by the demonstration of virus multiplication in the udders of immune animals.

INTRODUCTION

The presence of foot-and-mouth disease virus (FMDV) in the milk of some cattle incubating or exhibiting signs of the disease has been demonstrated or inferred on many occasions since the original observations of Lebailly (1920), and those concerned with the control of the disease recognize the importance of milk in the dissemination of virus both within and beyond the immediate locality of an infected farm. Examples of the spread of disease associated with infected milk have been recorded in the Report of the Departmental Committee on Foot-and-Mouth Disease 1952–54 and by Dawson (1970).

During the series of outbreaks of FMD in England and Wales in 1967 and 1968, virus was recovered from milk which had been collected from farms before the disease was diagnosed; virus concentrations of 10^4 and $10^{5.5}$ ID 50/ml. (mouse) were measured in samples taken from a bulk milk tanker and from a churn respectively (Hedger & Dawson, 1970). Experimental infections of dairy cattle with the strain of virus concerned confirmed that high concentrations of virus could be found in the milk of some animals for several days before the appearance of clinical disease (Burrows, 1968).

This paper records the results of experiments which show that the bovine mammary gland is an important site of FMDV multiplication and possibly of the persistence of some strains of FMDV.

MATERIALS AND METHODS

Virus strains

1. O_1-BFS 1860: British field sample collected from cattle in the Wrexham area, 1 November 1967, and used after one passage in cattle at Pirbright.

2. A_{22}-Iraq 24/64: World Reference Laboratory sample used after two passages in cattle at Pirbright.

Cattle

A heterogeneous collection of commercial dairy cattle was used (ten Ayrshire, four Friesian and two Jersey). They varied in age from 3 to 8 years and, apart from one newly calved animal, were in middle to late lactation. Only two animals (supplied by the Institute for Research in Animal Diseases, Compton, through the courtesy of Professor W. M. Henderson) had a known history and could be regarded as mastitis-free at the beginning of the experiment.

The cattle were milked by hand twice daily during the early stages of experiment. During the later stages they were milked by machine once or twice a day, according to the requirements of the individual animal. Normal dairy hygiene was practised in respect of udder washing and disinfection between animals.

Infection, sampling and examination procedures

Animals exposed to infected 'donor' cattle, sheep and pigs

The animal accommodation consisted of 12 loose boxes opening on three sides of a central area which had stalls for ten cattle. The 'donor' animals were placed in three boxes and the 'recipient' dairy cattle were placed in the central area. The two groups were separated by at least 10 metres and were handled by different attendants.

Air movement in the animal area was from intake points in each loose box to extraction points above the stalls in the central area. The 'donor' animals were infected by the intradermal inoculation of the tongue (one steer), the coronary band (four sheep) and the bulb of the heel (four pigs). Under these conditions, trace amounts of virus have since been detected in the communal air of the shed within 24 hr. and considerable amounts within 48 hr. (Sellers & Parker, 1969).

The 'recipient' animals were examined each morning for clinical signs of disease and samples of blood and pooled quarter milk were collected. Swabs were taken from the vaginal and rectal cavities, and oesophageal/pharyngeal samples were collected as described by Burrows (1966). Rectal temperatures were recorded during the early morning and late afternoon.

Animals infected by udder inoculation

Two ml. of a virus suspension were introduced into the milk sinus of the right hind quarter, using a teat cannula. Oesophageal/pharyngeal samples and nasal, conjunctival, vaginal and salival swabs were taken each morning and afternoon. Clinical examinations were carried out, rectal temperatures recorded, and blood and individual quarter milk samples were collected every 4 hr. until clinical disease was apparent.

Some animals were retained on experiment for up to 12 weeks. During this period samples were collected at irregular intervals and examined for antibody content and for evidence of virus persistence.

Detection and assay of virus and neutralizing antibody

Oesophageal/pharyngeal specimens, blood and milk collected during the day were tested for infectivity within 1–3 hr. of collection. Samples taken during the night were stored at 4° C and examined the following morning. Blood samples were allowed to clot and only the serum screened for infectivity. Swabs were stored at − 20° C for 1–3 weeks before being processed in 3 ml. of diluent immediately before examination.

The majority of samples were examined in parallel in two culture systems. Trace amounts of virus were detected in primary monolayers of calf thyroid (BTY) cells in roller tubes (Snowdon, 1966). Larger amounts of virus were assayed by counts of plaque forming units (p.f.u.) in monolayers of the pig kidney cell line IB-RS-2 (Instituto Biologico-Renal Swine-2; de Castro, 1964). Because high concentrations of milk damaged the tissue cultures, it was found necessary to remove the inoculum and rinse the monolayer with diluent before adding the nutrient fluid or agar overlay.

The identity of virus strains was monitored periodically by complement fixation and neutralization tests, using type specific sera. Serum neutralizing antibody titres were determined by the cell metabolic inhibition test (Martin & Chapman, 1961). Comparisons of milk and serum neutralizing antibody content were carried out by means of a plaque reduction test on IB-RS-2 monolayers, using a constant virus/variable serum or milk dilution procedure similar to that described by Federer, Burrows & Brooksby (1967).

RESULTS

Cattle exposed to an indirect contact infection

Experiments were carried out with both the O_1 and A_{22} strains of virus. In each experiment the four dairy cattle formed part of a large group of recipient cattle, sheep and pigs (Burrows, 1968).

Clinical observations

The period of time which elapsed between infection of the donor animals and the appearance of disease in the recipient dairy cattle ranged from 5 to 8 days.

Above average rectal temperatures were recorded for four of the eight cattle during the 24 hr. preceding the appearance of vesicles and for six animals at the time when lesions were first seen. The first crop of vesicles was detected in the interdigital regions and the bulbs of the heels of one or more feet (six animals); four of these also had vesicles on the dental pad or lips. One animal had lesions on the dental pad only and one animal on one teat only. During the following 24–72 hr. further crops of vesicles appeared at the majority of susceptible sites in the mouth, on the feet and on the teats of all animals. A mucoid nasal discharge was seen in some animals and this resulted in some degree of excoriation of the outer nares and muzzle. Milk production dropped by 30–50 %, commencing on the day that lesions were first seen, and the milk of five of the cattle contained obvious clots. Bacteriological and cytological examinations of the milk were carried out in the A_{22} experiment. No significant changes in the bacterial flora took place and the cellular content of the milk did not increase more than three-fold. Milk pH values remained within the limits of 6·5 to 6·9.

Virological findings

The amounts of virus recovered from samples taken before the appearance of clinical disease are listed in Tables 1 and 2. Virus was recovered from the pharynx, blood and milk of 7 of the eight cattle, from the vagina (four cattle) and from the

Table 1. *Virus content of samples taken before the appearance of clinical lesions* (*indirect contact infection*, O_1)

Animal no.	Sample origin	Day of experiment 2	3	4	5	6	7	8
GI 27	Serum	—	—	—	4·0*			
	Pharynx	—	—	2·5	> 4·5			
	Milk	—	—	—	3·3	Lesions		
	Vagina	—	—	—	2·9			
	Rectum	—	—	—	1·6			
GI 28	Serum	—	—	—	—	1·7	2·1	
	Pharynx	—	2·8	2·5	3·1	4·0	5·5	
	Milk	—	—	1·4	3·5	4·6	4·5	Lesions
	Vagina	—	—	—	—	—	—	
	Rectum	—	—	—	—	—	—	
GI 29	Serum	—	—	1·1	4·1			
	Pharynx	—	—	3·5	> 4·5			
	Milk	—	—	1·0	5·2	Lesions		
	Vagina	—	—	—	> 3·3			
	Rectum	—	—	—	1·0			
GI 30	Serum	—	—	1·1	1·0			
	Pharynx	—	—	2·5	3·5			
	Milk	—	—	1·3	2·4	Lesions		
	Vagina	—	—	—	> 3·3			
	Rectum	—	—	—	—			

* Log_{10} p.f.u./ml. (serum, milk) or per sample (oesophageal/pharyngeal samples, vaginal/rectal swabs).

— No virus recovered.

https://doi.org/10.1017/S0022172400021537 Published online by Cambridge University Press

rectum (two cattle) for variable periods of time before disease was apparent. In the majority of animals, virus was first detected in those samples taken from the pharyngeal area. The geometric mean concentrations of virus ($\log_{10}$ p.f.u./sample) in the positive pharyngeal samples collected 5, 4, 3, 2 and 1 day before signs of disease were 1·0, 2·1, 3·5, 3·5 and $\geqslant$ 4·2. Although the presence and concentrations of pharyngeal virus during the pre-clinical stages of disease were similar in the two experimental groups, the occurrence and concentration of virus in other samples differed. In the A_{22} experiment virus was detected in only three samples

Table 2. *Virus content of samples taken before the appearance of clinical lesions (indirect contact infection,* A_{22})

Animal no.	Sample origin	Day of experiment 2	3	4	5	6	7	8
GQ 20	Serum	—	—	—	—	> 2·6*		
	Pharynx	—	T	2·9	2·7	> 3·8	Lesions	
	Milk	—	—	—	—	2·8		
GQ 21	Serum	—	—	—				
	Pharynx	—	—	—	Lesions			
	Milk	—	—	—				
GQ 22	Serum	—	—	—	1·8	—	—	
	Pharynx	—	T	3·3	4·5	> 3·8	4·2	Lesions
	Milk	—	—	—	—	—	1·2	
GQ 23	Serum	—	—	—	2·4			
	Pharynx	—	—	5·3	> 3·8	Lesions		
	Milk	—	—	—	1·7			

* Log_{10} p.f.u. per ml. (serum, milk) or per sample (oesophageal/pharyngeal).
T = trace amounts of virus detected in BTY cultures only.
— = no virus recovered.
Only one vaginal swab contained virus (GQ 22 Day 7–$10^{2\cdot6}$ p.f.u.).
No virus recovered from rectal swabs.

of serum (mean infectivity 2·2), three samples of milk (mean infectivity 1·9) and in one vaginal swab. In the O_1 experiment virus was found in seven samples of serum (mean infectivity 2·16) and nine samples of milk (mean infectivity 3·02), three vaginal swabs and two rectal swabs. This slight difference in the distribution of virus found in the two groups of milking cows before the appearance of disease was not seen in the larger group of steers which were included in each experiment.

During the first 4 days of clinical disease the majority of milk samples contained considerable amounts of virus (29/32 – mean infectivity 3·09, range 1·2–5·2). Neutralizing antibody appeared in the sera of the cattle on the 8th and 9th days of the experiment (5–7 days after virus was first detected in the pharynx) and this coincided with a reduction in both the frequency and the amounts of virus recovered from the milk. The O_1 cattle were not sampled after the 12th day of experiment but the A_{22} cattle continued to excrete virus in the milk up to the 19th day.

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Cattle infected by udder inoculation

Clinical findings

Although there was some individual variation in the thermal response of the animals to infection, the general pattern was the same. Sixteen to 24 hr. after instillation of virus into the udder, the rectal temperature increased over a period of 4–8 hr. to a maximum of 104·2 to 106·8° F. and then returned to near normal levels. A second increase in temperature up to 103–106·2° F. occurred 4–28 hr. before the appearance of vesicular lesions.

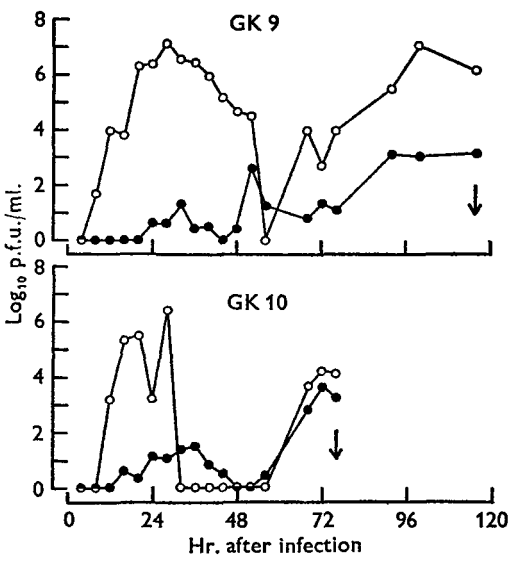

Fig. 1

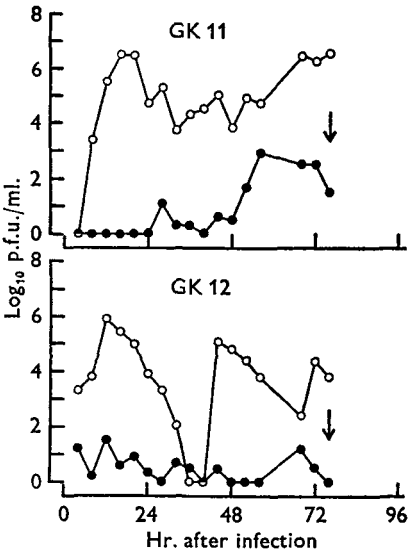

Fig. 2

Figs. 1–4. The occurrence and concentrations of virus in the milk before the appearance of generalized disease following udder inoculation. Fig. 1. O_1, 10^3. Fig. 2. O_1, 10^6. Fig. 3. A_{22}, 10^3. Fig. 4. A_{22}, 10^6. ○—○, Inoculated quarter; ●—●, geometric mean: non-inoculated quarters; ↓, appearance of generalized disease.

Fig. 2. For legend see Fig. 1.

All cows exhibited clinical signs of mastitis in the inoculated quarter within 28 hr. of infection. The affected quarter was slightly swollen, hot and tender and the milk was somewhat watery, slightly coloured, contained numerous clots and was considerably reduced in amount. Penicillin was given to reduce the possible activity of commensal bacteria and this resulted in an improvement to a less acute form of mastitis which progressed during the next 48 hr. to involve the non-inoculated quarters of the udder. The milk yield dropped to approximately 60% of the pre-infection volume.

Signs of generalized disease developed between 76 and 117 hr. in the O_1 group (Figs. 1, 2) and between 52 and 88 hr. in the A_{22} animals (Figs. 3, 4). The first crops of vesicles appeared on the feet and these were followed during the next 48 hr. by vesicles in the mouth and on the teats.

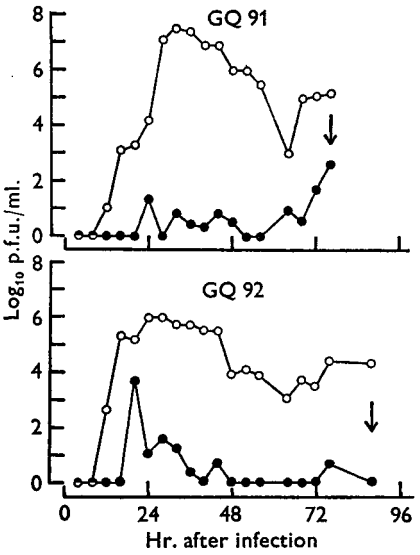

Fig. 3

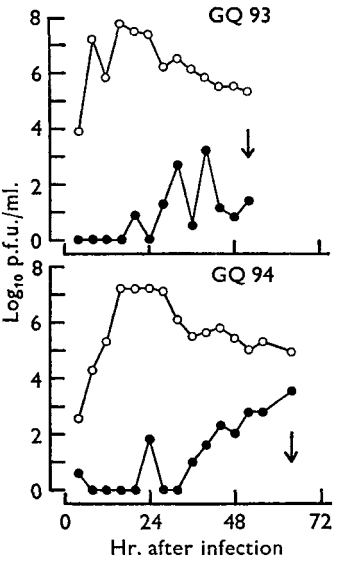

Fig. 4

Fig. 3. For legend see Fig. 1.
Fig. 4. For legend see Fig. 1.

Table 3. *Percentage frequency of virus recovery and mean infectivity of milk samples taken from different quarters prior to the development of generalized disease (udder inoculation)*

	O_1		A^{22}	
Quarter	Percentage frequency virus recovery	Mean infectivity of positive samples	Percentage frequency virus recovery	Mean infectivity of positive samples
Right hind	78	4·68*	93	5·36
Left hind	42	2·07	20	2·13
Right fore	37	2·18	34	2·46
Left fore	34	1·81	29	2·71

* Log_{10} p.f.u./ml.

Virological findings

The occurrence and concentration of virus in the milk before the appearance of generalized disease are detailed in Figs. 1–4. In animals given a small dose of virus (10^3 p.f.u.), the first indication of virus multiplication in the udder was obtained at the 8th and 12th hr. and peak virus concentrations were reached between 24 and 36 hr. (Figs. 1, 3). In the animals given the larger dose (10^6 p.f.u.), virus was present in the samples taken at 4 hr. in three cows and peak virus titres occurred between 12 and 20 hr. (Figs. 2, 4). In three of the O_1 cattle a complete loss of infectivity for various periods of time was found in samples collected between 32 and 56 hr. after infection. This feature was not evident in the A_{22} cattle, although some decrease in the virus content of the milk was found after the 32nd hr. Virus was recovered sporadically and at relatively low titre from the non-inoculated quarters

from 16 to 24 hr. after infection, although in two animals virus was recovered in the samples taken at 4 hr. No differences in the response of the non-inoculated quarters was apparent in respect of the frequency of virus recovery or of mean virus content (Table 3).

Table 4. *Virus content of samples taken before the appearance of clinical lesions (udder inoculation, O_1)*

Animal no.	Sample origin	Hours after infection: 4	20	28	44	52	68	76	117
GK 9	Serum	—	T	T	1·1*	1·6	2·1	> 2·7	
	Pharynx	—	T	1·0	2·6	2·3	2·0	3·7	
	Saliva	—	1·1	2·6	2·2	2·6	—	2·7	Lesions
	Nose	—	—	—	2·9	2·9	3·0	2·0	
	Vagina	—	—	—	—	1·0	2·5	2·3	
GK 10	Serum	—	T	T	1·9	> 2·7	2·4		
	Pharynx	1·6	2·6	3·6	3·6	3·2	4·0		
	Saliva	—	—	2·0	1·3	2·2	2·7	Lesions	
	Nose	—	—	1·0	3·2	2·7	2·7		
	Vagina	—	—	—	2·6	1·7	2·5		
GK 11	Serum	—	T	T	2·4	> 2·7	> 2·7		
	Pharynx	—	2·4	2·4	3·2	3·2	> 4·0		
	Saliva	—	—	1·4	1·7	> 2·7	> 2·7	Lesions	
	Nose	—	—	—	3·3	> 3·3	> 3·3		
	Vagina	—	2·3	2·2	2·5	2·5	2·5		
GK 12	Serum	—	—	T	0·7	1·0	0·4		
	Pharynx	—	—	—	3·0	2·0	3·5		
	Saliva	—	—	—	—	—	—	Lesions	
	Nose	—	0·3	1·7	2·4	2·5	2·8		
	Vagina	—	2·0	1·0	2·0	2·0	2·5		

* Log_{10} p.f.u./ml. or sample.
T = Trace amounts of virus detected in BTY cultures only.
— = No virus recovered.
Animals GK 9 and GK 10 each received 10^3 p.f.u. of virus O_1.
Animals GK 11 and GK 12 each received 10^6 p.f.u. of virus O_1.

A feature of these experiments was the rapid dissemination of virus from the inoculated quarter to other sites, both in the udder and the pharyngeal area. Tables 4 and 5 list the virus content of samples collected at various times after inoculation and before the appearance of generalized disease. In the O_1 cattle, virus was detected in the pharynx of one animal 4 hr. after infection, in the blood of the two high virus dose cattle at 8 hr. and in the low virus dose group at 12 and 20 hr. Virus was found in nasal and vaginal swabs and in saliva collected from the mouth from the 20th hour onwards in some animals. In general, the virus content of the samples increased with time (Table 4) and must represent multiplication of virus in the pharyngeal, oral, nasal and vaginal mucosae at a level below that required to produce gross vesiculation.

A similar but less marked pattern of virus recovery was obtained for the A_{22} cattle (Table 5), although virus was not recovered from vaginal or rectal swabs before the 88th hour, by which time all animals had developed generalized disease.

Virus was detected in the pharynx of one animal at 16 hr. and in the blood of the high virus dose animals at 16 and 20 hr., and in the low virus dose pair at 20 and 24 hr.

Table 5. *Virus content of samples collected before the appearance of clinical lesions (udder inoculation, A_{22})*

Animal no.	Sample origin	Hours after infection: 16	24	40	48	64	72	
GQ 91	Serum	—	1·5*	3·9	3·9	3·6	3·8	Lesions 76 hr.
	Pharynx	—	—	T	2·6	2·5	2·7	
	Saliva	—	—	—	T	—	—	
GQ 92	Serum	—	1·0	—	—	2·0	1·0	Lesions 88 hr.
	Pharynx	—	—	—	—	—	T	
	Saliva	—	—	—	—	T	3·6	
GQ 93	Serum	0·8	3·3	3·1	3·5	Lesions 52 hr.		
	Pharynx	T	2·7	—	3·1			
	Saliva	—	1·0	2·4	3·4			
GQ 94	Serum	—	2·3	3·0	3·4	Lesions		
	Pharynx	—	—	T	3·4			
	Saliva	—	—	2·7	2·8			

* Log_{10} p.f.u. per ml. or per sample.
T = Trace amounts of virus detected in BTY cultures only.
— = No virus recovered.
No virus detected in vaginal or rectal swabs. Animals GQ 91 and GQ 92 each received 10^3 p.f.u. of virus A_{22}. Animals GQ 93 and GQ 94 each received 10^6 p.f.u. of virus A_{22}.

The virus content of the milk during the clinical and convalescent stages of disease is detailed in summary form in Table 6 which lists the total number of quarter samples examined, the number of samples from which virus was recovered and the means and ranges of infectivities recorded. In general, the virus content of samples remained high ($\geqslant 3{\cdot}0$ log_{10} p.f.u./ml.) for the first 2 or 3 days of obvious disease and then, with the development of serum antibody, the frequency of recovery and the virus content of samples decreased. In the A_{22} experiment approximately 85 % of all milk samples collected between the 4th and 10th days contained virus, no virus was detected on the 11th and 16th days, but virus was recovered from three quarters of one animal on the 23rd day. In the O_1 experiment approximately 65 % of quarter samples collected between the 4th and 10th days, 40 % collected between the 11th and 20th days, 34 % collected between the 21st and 30th days, and 12 % collected between the 31st and 84th days contained virus. Virus was recovered intermittently from two animals for 30 days and from the other two cattle for 44 and 51 days, respectively.

The occurrence of virus in the milk of convalescent cattle was unexpected in view of the high neutralizing activity of the milk of these animals. The appearance and concentration of neutralizing antibody in the milk in relation to that of serum is tabulated in Table 7. Serum antibody was first detected on the 4th or 5th day after udder inoculation; trace amounts of neutralizing activity were detected in the milk on the 6th day and considerable amounts on the 7th day and this

Table 6. *Infectivity of milk during the clinical and convalescent stages of FMD initiated by udder inoculation*

Days after infection	Cow*	O_1 No. of samples positive/tested		O_1 Geometric mean infectivity and range		A_{22} No. of samples positive/tested		A_{22} Geometric mean infectivity and range	
4–10	1	25	36	3·26†	0·7–7·2	38	40	3·49	1·0–6·5
	2	22		3·60	1·0–5·9	30		2·67	0·7–4·9
	3	21		3·74	1·5–6·5	38		3·77	1·2–6·6
	4	25		2·47	0·7–3·5	32		3·38	1·0–5·8
11–20	1	9	32	1·40	0·7–2·0	0	8	.	.
	2	13		1·58	0·7–3·9	0			
	3	2		2·85	2·2–3·5	0			
	4	26		1·94	0·7–3·2	0			
21–30	1	11	28	1·59	0·7–3·5	0	8		
	2	2		1·45	0·7–2·2	0			
	3	7		1·21	0·7–2·0	3		1·33	1·0–2·0
	4	19		1·94	1·0–2·7	0			
31–84	1	2	32	2·75	2·5–3·0	0	24	.	.
	2	0		—	—	0			
	3	12		2·07	0·7–3·7	0			
	4	1		2·2	—	0			

* O_1: 1. GK 9 A_{22}: 1. GQ 91 † Log_{10} p.f.u./ml.
2. GK 10 2. GQ 92
3. GK 11 3. GQ 93
4. GK 12 4. GQ 94

coincided with a decrease in the virus content of the milk. From about the 9th day onwards the level of milk antibody parallelled that of the serum, being approximately 14-fold lower (range 9- to 22-fold) in the O_1 group of cattle and approximately 4-fold lower (range 2- to 11-fold) in the A_{22} cattle. No obvious differences in these relationships were measured in animals producing 2 litres or 10 litres of milk a day or in the milk from individual quarters of an animal.

Re-infection of convalescent cattle by udder inoculation

The response of convalescent cattle to re-infection by the instillation of virus into the udder was examined 12–15 weeks after their first experience of FMD. Details of the experiment are given in Table 8, which shows that despite considerable neutralizing activity of the milk (90 % neutralization of test virus at dilutions

Table 7. *Virus neutralizing activity of serum and milk following FMD initiated by udder inoculation*

Days after infection	O_1		A_{22}	
	Serum	Milk	Serum	Milk
3	⩽ 0·3*	⩽ 0·3	⩽ 0·3	⩽ 0·3
4	⩽ 0·3	⩽ 0·3	0·95	⩽ 0·3
5	1·85	⩽ 0·3	1·85	⩽ 0·3
6	3·15	0·95	2·90	0·70
7	3·75	2·65	3·90	2·10
8	4·35	3·20	—	—
9	—	—	3·75	3·40
14	4·35	3·30	—	—
16	4·40	3·30	4·00	2·95
21–30	4·15	3·00	3·60	3·35
31–40	4·30	2·95	3·80	2·95
41–50	4·30	—	4·05	3·25
51–60	4·00	—	3·30	3·10
61–70	—	2·90	3·80	3·10

* Log_{10} reciprocal of the initial serum or milk dilution which neutralized 90 % of test virus.
O_1, geometric means for cows GK 9 and GK 11.
A_{22}, geometric means for cows GQ 92 and GQ 94.

of 1/200 to 1/500) it was possible to demonstrate a virus growth cycle in the inoculated quarter over a period of at least 90 hr. Twenty-four to 42 hr. after re-infection, a transitory swelling of the inoculated quarter along with some thickening of the milk was noted. No virus was recovered from the blood or from the non-inoculated quarters during 7 days of observation. Three of the four cattle were carrying virus in the pharynx at the time of re-infection.

DISCUSSION

Afshar & Bannister (1970) have reviewed the literature concerning viral infections of the bovine mammary gland and have listed the viruses which have been reported to multiply in the udder. These include the viruses of human influenza (PR. 8), Newcastle disease, fowl plague, mumps, canine distemper, poliomyelitis,

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Table 8. *Virus multiplication in the mammary gland of convalescent cattle re-infected by udder inoculation*

Animal no.	History	Pre-infection antibody: Serum	Pre-infection antibody: Milk	Virus dose A_{22} (log_{10} p.f.u.)	Virus content of milk from inoculated quarter. Hours after infection: −1	18	24	42	66	90	168
GQ 92	84 days after infection (udder inoculation A_{22})	3·5*	2·3	5·0	0	4·2†	4·5	0	1·4	2·7	0
GQ 94		3·3	2·6	3·0	0	1·7	3·0	3·0	0	0	0
GQ 23	105 days after infection (in-direct contact A_{22})	3·5	2·5	5·0	0	3·3	3·5	0	0	0	0
GQ 20		3·0	2·7	3·0	0	0	3·5	3·2	1·4	0	0

* Log_{10} reciprocal of the serum or milk dilution which neutralized 90% of the test virus (A_{22}).
† Log_{10} p.f.u./ml.

vesicular stomatitis, foot-and-mouth disease, infectious bovine rhinotracheitis, pseudo-cowpox, African swine fever, a bovine enterovirus and vaccinia and parainfluenza 3 viruses. FMDV was included on the evidence that instillation of the virus into the udder was followed by generalized disease (Thomas & Leclerc, 1961). Some of these viruses have been reported to multiply without causing obvious clinical abnormalities, whilst others have been shown to produce changes in the mammary gland and its secretions which ranged from a transitory increase in the cellular content of the milk to the more obvious physical changes associated with acute mastitis.

The mastitis which has been described in some cattle experiencing FMD has usually been attributed to the activity of commensal bacteria following the appearance of vesicular lesions on the teats and udder. In the present series of experiments the mastitis was almost certainly due to virus proliferation within the mammary gland, as it occurred before the appearance of detectable lesions on the skin of the mammary regions and was accompanied by considerable amounts of virus in the milk.

Although, in the indirect contact experiments, the behaviour in steers of the O_1 and the A_{22} viruses was not noticeably different, small variations in the distribution and concentrations of virus were evident in the dairy cattle. These differences were more marked in animals infected by udder inoculation. The A_{22} virus appeared to be the more virulent virus in the milking cow in that it grew more readily in the udder, produced great virus concentrations in the blood, produced clinical lesions more quickly and did not appear to persist in the udder to the same extent as did the O_1 virus. During the period 20–48 hr. after infection, all the milk samples from the inoculated quarters of the A_{22} cattle and 39% of samples from the other quarters yielded virus (mean infectivity in $\log_{10}$ p.f.u./ml.–6·03 and 2·33, respectively). The comparable figures for the O_1 cattle were 78% (mean infectivity 4·96) for the inoculated quarters and 32% (mean infectivity 1·40) for the other quarters. The reason for the disappearance of virus from the milk of some of the O_1 cattle for varying periods of time 30 or more hours after udder inoculation is not understood. A retrospective examination of the sera of these animals showed a transitory increase in virus inhibitory activity (up to initial serum dilutions of 1/64 measured by the cell metabolic inhibition test) during this period but the nature of this inhibition was not investigated, nor were the milk samples examined for inhibitory activity. The virus neutralizing activity of the milk appeared soon after that of the serum and was associated with a decline in the infectivity of the milk, which was more marked in the A_{22} cattle than in the O_1 animals.

This difference in virus growth in the udder was reflected in the concentrations of virus which were present in the blood during the pre-clinical period. In the A_{22} cattle, blood virus levels of 3·0 $\log_{10}$ p.f.u./ml. were measured in two cattle 20 hr. after infection and the mean infectivity levels of samples taken every 4 hr. from three of the four cattle were 3·43, 3·16 and 2·71 for the period between 20 hr. after infection and the time of appearance of disease. Although virus was detected in the blood of two of the O_1 cattle 8 hr. after udder inoculation and in the blood of the other two animals at 12 and 20 hr. and in all subsequent samples, the concentra-

tions of virus remained low and did not exceed 1·0 $\log_{10}$ p.f.u./ml. during the first 40 hr. Despite this low level of viraemia, the O_1 virus succeeded in localizing and multiplying at other sites more readily than did the A_{22} virus. Virus was recovered from 65 of the 100 samples taken from the pharynx, the mouth, the nose and the vagina of the O_1 cattle before the development of disease, whereas only 18 of the 80 samples taken from the pharynx, the mouth, the vagina and the rectum of the A_{22} cattle contained virus.

The appearance of virus in the pharynx of 'recipient' animals 3 or 4 days after the inoculation of 'donor' animals is not now surprising, in the light of present knowledge. Sellers & Parker (1969) have now shown that the virus content of the communal air in this animal compound is greatest at the time of appearance of generalized disease and this usually occurs 40–72 hr. after inoculation. Whether this 'pharyngeal virus' in the recipient animal is the result of air sampling by the upper or by the lower respiratory tract has not yet been resolved but the results of sequential post-mortem studies of animals infected by either natural or simulated natural means have indicated that the pharynx is the primary site of virus localization and multiplication in the ruminant (R. Burrows, J. A. Mann, A. J. M. Garland & D. Goodridge – unpublished work). The susceptibility of the pharynx to haematogenous infection as well as to surface infection was confirmed in the cattle infected by udder inoculation. Virus was detected in the pharynx of one animal 4 hr. after udder infection and in four of the eight cattle within 24 hr. Although the possibility of the pharynx having been infected by either the oral or respiratory route cannot be completely excluded, this is most unlikely as the four animals were fastened by neck chains at least 10 metres apart and precautions were taken to limit aerosol formation during milking procedures.

Although primary infection of the mammary gland is unlikely to be a common occurrence in the pathogenesis of FMD, the results of experimental inoculation of the udder show that it is a highly susceptible organ which is capable of producing large amounts of virus. This susceptibility is of some significance; the mammary gland of the lactating cow is richly supplied with blood and so may well be one of the first organs to become infected from the transitory viraemia which follows primary multiplication of virus in the pharyngeal area. Once the mammary tissue is infected, virus could be expected to appear in the milk fairly quickly, whereas virus localizing in the epithelium of the mouth or feet might require 24–30 hr. to produce recognizable vesicles. The susceptibility of the mammary gland tissue to FMDV may also explain the ability of virus to persist in this region. The recovery of virus from the milk of the O_1 cattle for periods up to 51 days and the A_{22} cattle for 23 days, and the susceptibility of convalescent A_{22} cattle to re-infection by udder inoculation, indicate that these strains of FMDV continued to multiply in the mammary tissue of the immune animal. The persistence of FMDV in the pharyngeal region of cattle for long periods is well documented (van Bekkum, Frenkel, Frederiks & Frenkel, 1959; Sutmöller & Gaggero, 1965; Burrows, 1966) but until now no evidence for the possible persistence of FMDV in other regions has been obtained.

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