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The persistence of foot-and-mouth disease virus in sheep

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van Bekkum *et al.* (1959), Sutmöller & Gaggero (1965) and Burrows (1966) showed that foot-and-mouth disease virus (FMDV) persisted in the majority of cattle for periods of several months after clinical disease. The possibility that FMDV also persisted in convalescent sheep and pigs was examined and this paper records the results of the experiments with sheep.

MATERIALS AND METHODS

Virus strains

(i) A–119 — Pirbright stock cattle strain used at the 26th cattle passage

(ii) A–Iraq 24/64
(iii) O–Israel 1/63
(iv) SAT 1–S.A. 13/61
— World Reference Laboratory samples received from the field and used at the 1st to the 3rd cattle passage.

Sheep

Commercial crossbred Southdown ewes, 8–15 months old, were used for the majority of experiments. The animals were housed in groups of four to eight in cattle boxes.

Infection was produced by either (i) the inoculation of the coronary band area of both main digits of one foot with $10^{4\cdot0}$ ID 50 (cattle tongue), or (ii) contact exposure to the inoculated sheep.

Examination for clinical lesions was carried out daily for 10 days. Three weeks after infection the animals were washed thoroughly and moved to a clean isolation unit.

Collection and handling of samples

Blood

Animals were bled daily for 4 days after inoculation, in-contact animals were bled on the 4th, 5th and 6th or 7th day. Further samples were taken periodically throughout the period of the experiment.

Oesophageal/pharyngeal samples

A modified version of the cattle sampling instrument referred to by van Bekkum *et al.* (1959) and described by Sutmöller & Gaggero (1965) was developed (Fig. 1). The volume of the sample obtained from the sheep varied between 0·1 and 0·3 ml. and this was collected from the instrument by rinsing in 2 ml. of diluent.

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Post-mortem specimens

The regions sampled, the method of collection, and the preparation of specimens for infectivity assay have been described in connection with similar investigations in cattle (Burrows, 1966). The contents of the tonsillar sinuses and the tonsillar tissue were collected together by vigorous scraping from the pharyngeal surface. The rumen was examined and scrapings made from those areas which showed evidence of previous damage.

Isolation and infectivity assay of virus

All specimens were examined for infectivity within 1–3 hr. of collection. Blood samples were allowed to clot and only the serum was tested (previous work had established that the virus content of the serum did not differ significantly from that of whole blood during the viraemic stage).

All samples were assayed by counts of plaque-forming units (pfu) in baby hamster cell strain monolayers (BHK strain 21, clone 13, MacPherson & Stoker, 1962). Some samples were also examined for infectivity for primary monolayers prepared from calf thyroid (Snowdon, 1966). The identity of virus isolates was established 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).

RESULTS

Clinical observations

A detailed description of FMD following experimental infection of housed sheep has been given by Dellers & Hyde (1964), and of natural infection in pastured sheep by Zaikin (1959). Although classical lesions involving the mouth and feet may be seen following infection most workers have stressed that clinical manifestations of disease are frequently vague and difficult to demonstrate (Stockman & Minett, 1927; Viviano, 1957; Rivensen, Segura & Zakin, 1964; Cardassis *et al.* 1966; P. G. Howell & W. A. Geering, personal communications).

In the series of experiments recorded here only four of the 39 sheep developed obvious lesions in the mouth, and although the majority of animals displayed lesions on one or more feet, in general these lesions were mild and transitory in nature. A biphasic febrile response was recorded for the inoculated animals, but this was masked to some extent in those animals which acquired infection by contact.

Viraemia and antibody studies

The indeterminate nature of clinical lesions displayed by many infected animals has resulted in the use of other criteria to establish the presence of infection in sheep. The demonstration of circulating virus or a specific antibody response has been used for this purpose (Viviano, 1957; Dellers & Hyde, 1964; Rivensen *et al.* 1964; Cardassis *et al.* 1966; Fontaine, Dubouclard & Bornarel, 1966). Based on these criteria 38 out of 39 sheep, inoculated or exposed to virus, were shown to

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have acquired infection. Details of the viraemia studies are presented in Table 1. Virus was recovered from the blood of the majority of the inoculated animals on each of the 3 days after infection, and from the in-contact animals on the 4th to the 7th day after initial exposure.

Neutralizing antibody appeared in the sera of the experimentally infected sheep 4 days after inoculation and reached peak levels after 7–10 days. Ten of the 11 sheep, exposed to virus by contact, acquired infection and developed significant levels of antibody within 11 days. The mean antibody levels of three of the four groups of sheep are recorded in Table 2.

Table 1. *Infectivity of blood (serum) after infection by or exposure to FMDV*

		Virus					
		A-119	A-Iraq		O-Israel		SAT 1
Method of exposure		CB*	CB	Contact	CB	Contact	CB
No. of animals		8	4	4	8	7	8
Days after exposure	1	8/2·90†	3/3·06	–	8/2·99	–	8/4·03
	2	6/4·31	4/2·70	–	8/3·59	–	8/5·04
	3	7/2·98	4/1·75	–	8/2·31	–	8/2·20
	4	2/0·85	0	3/2·00	0	4/1·80	0
	5	–	–	3/1·63	–	–	–
	6	–	–	0	–	5/2·35	–
	7	–	–	–	–	1/2·50	–

* Coronary band inoculation.
† Number of sheep with viraemia/mean infectivity of positive sera as $\log_{10}$ pfu/ml.
– Not tested.

Table 2. *Geometric mean neutralizing antibody titres of sheep used for 'carrier' studies*

		Virus				
		A-Iraq		O-Israel		SAT 1
No. of animals		4	8	8	14	8
Days after infection	0–3	0·8*	.	0·8	.	0·8
	4	–	.	1·7	.	1·7
	7	–	.	2·87	.	2·75
	11	2·4	.	2·90	.	2·79
	21	2·4	.	2·80	.	–
	28	.	2·47	.	2·63	3·08
	40–50	.	2·47	.	2·47	2·89
	70–90	.	2·33	.	–	3·00
	120–150	.	2·32	.	2·47	2·73

* Log_{10} reciprocal serum dilution (final).
– Not tested.

The infectivity of oesophageal/pharyngeal samples

Details of the frequency of recovery of virus and the mean titres measured in samples taken from the groups of sheep are shown in Table 3. Virus was recovered from approximately 80 % of animals 4 weeks after infection, from 45 % after 8

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weeks, from 25% after 12 weeks and from one animal after 20 weeks. The mean infectivity of samples declined gradually from approximately 150 pfu/sample at 4 weeks (29 samples) to approximately 50 pfu/sample at 12 weeks (9 samples). The highest infectivity recorded for individual animals was greater than 1000 pfu/sample.

The results indicate that the duration of the carrier stage in sheep is much shorter than that observed in cattle infected with the same virus strains. The A-119 virus was recovered from five of 10 cattle for up to 7 months after infection and from one animal for more than 2 years. Two of four cattle infected with the A-Iraq strain yielded virus for a period of 14 months after infection.

Table 3. *The frequency of recovery of virus and the infectivity of pharyngeal samples from sheep after infection with FMDV*

		Virus			
		A-119	A-Iraq	O-Israel	SAT 1
No. of animals		7	8	14	8
Weeks after infection	2	7/1·71*	7/2·74	–	6/1·83
	3	7/2·48	8/2·54	8/2·16	–
	4	7/2·47	7/2·76	9/1·74	6/1·83
	6	6/1·87	7/2·1	8/2·20	5/1·86
	8	3/1·53	4/1·27	4/1·60	6/2·00
	12	1/2·2	4/1·90	1/1·40	3/1·50
	15	1/1·4	2/2·2	0	1/1·30
	20	0	1/0·3	0	0

* Number of sheep from which virus was recovered/mean infectivity of positive samples as $\log_{10}$ pfu per sample (BHK).
– = not tested.

Table 4. *Details of sheep examined* post mortem

Virus	No. of animals	History prior to infection	No. of weeks after killed	No. of animals from which virus recovered
A-119	1	None	3	1
	7		34	0
A-Iraq	8	Inactivated vaccine*	5–10	4
O-Israel	14	None	26	0
SAT 1	15	Inactivated vaccine†	3–6	4
	8	None	21	0
Total	53			9

* Challenged 21 days post-vaccination by coronary band inoculation.
† Challenged 21 days post-vaccination by tongue inoculation.

Sites of virus persistence

Information concerning the sites of virus persistence was obtained from two *groups of vaccinated sheep which were killed several weeks after challenge with virulent virus. Three of the four groups of sheep which had* been used for studies on the duration of the carrier stage were also sampled *post mortem* 5–8 months after

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Table 5. *Virus content of specimens taken from sheep* post mortem

Virus	Animal identification	Specimen: DPI*	Soft palate	Pharynx	Tonsil
A-119	FK 76	21	0·6†	0·0	2·3
A-Iraq	FP 20	49	0·0	0·0	1·2
	FP 21		0·0	0·0	1·7
	FP 17	71	0·0	1·5	0·3
	FP 23		0·6	0·0	2·3
SAT 1	FM 6	30	2·7	2·5	3·0
	FL 95	36	0·0	0·0	2·0
	FL 97	36	0·0	0·6	0·3
	FL 98	37	2·6	1·2	4·0

* Days after infection.
† Log_{10} pfu/specimen (BHK).

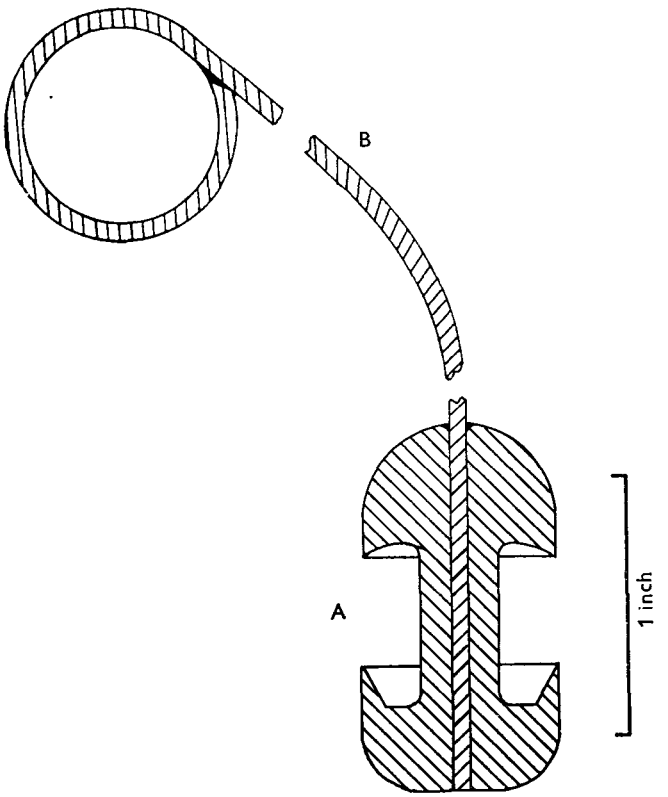

Fig. 1. Instrument for collecting pharyngeal samples from sheep. (A) Body machined from brass rod 0·75 in. O.D.; (B) stainless steel spring wire 16 SWG (0·064 in.), 12 in. long with finger loop.

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infection, but no virus was recovered from these animals. Details of the numbers and history of the sheep used for this work are given in Table 4.

Virus was recovered from nine of the 53 animals examined. The distribution and concentration of virus found in these animals are given in Table 5. Virus was recovered most frequently and in highest titre from the tonsillar area and less frequently from the pharynx and the dorsal surface of the soft palate. No virus was found in samples taken from the nasal passages, the trachea or the rumen.

The distribution of virus in the carrier sheep differed slightly from that found in cattle in that the main concentration of virus in the sheep was in the tonsillar area, and in the cow the dorsal surface of the soft palate and the pharynx. This difference might be due to the fact that in some sheep the tonsils project into the *isthmus faucium* to a greater extent than they do in the bovine.

DISCUSSION

The clinical signs of disease in the sheep used in these experiments were mild and in many animals inapparent. This contrasts to some extent with the description of experimental infection in housed sheep reported by Dellers & Hyde (1964). However, Zaikin (1959) has observed that the frequency and character of lesions in sheep can be altered by environmental and climatic conditions. In our laboratory investigations the A-Iraq virus produced the most obvious signs of disease in sheep and the O-Israel virus the least obvious. However, the records of the World Reference Laboratory (J. Davie, personal communication) show that disease was reported frequently in sheep during the 1963 'O' outbreak but not during the 1964/1965 'A' epizootic.

In these and subsequent experiments no attempt was made to adapt virus to sheep. It was found that the inoculation of the coronary band area of susceptible sheep with cattle virus resulted in infection and the subsequent development of viraemia in all animals. This would appear to be a suitable challenge procedure for the evaluation of FMDV vaccines in sheep. Tongue inoculation of sheep with cattle virus (SAT 1–S.A. 13/61) did not initiate infection in all animals based on viraemia and serum antibody studies. This agrees with the findings of Fontaine *et al.* (1966), who demonstrated the necessity for passaging cattle virus in sheep in order to obtain regularity of infection by tongue inoculation.

The existence of a 'carrier state' in sheep following infection with FMDV was not unexpected in view of the findings for cattle and it would appear likely that FMDV persists in the majority of susceptible ruminants for variable periods of time after infection. The ability of the virus to persist in the pharyngeal area of the ruminant must signify a special virus/host relationship. No evidence for a 'carrier state' in either the pig or the guinea-pig has been obtained in a number of experiments, and repeated examinations of cattle following infection with strains of vesicular stomatitis virus (Federer, Burrows & Brooksby, 1967) showed no evidence of persistence of this virus in the pharyngeal area (unpublished work).

The difference in the sites of virus localization in cattle and sheep may be related to the virulence or attenuation of the virus strain for the particular species.

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The original observations (Burrows, 1966) that cattle strains of virus could be recovered most regularly and in highest titre from the dorsal surface of the soft palate and from the pharyngeal walls have been confirmed in subsequent work with other cattle strains of virus. However, studies of the carrier state in cattle following vaccination with virus strains attenuated for cattle (to be published) have shown that in those animals in which virus persists localization of virus occurs mainly in the tonsillar region of the pharynx. It could well be that persistence of virus in the tonsillar region of sheep signifies that these strains are not particularly virulent for sheep. The clinical response of the animals to the viruses used in this study would support this hypothesis.

SUMMARY

Sheep infected with FMDV strains of different epizootiological origin developed a carrier state which persisted in the majority of animals for 1–5 months.

The sites of virus persistence and multiplication in the convalescent animal were identified by titration of suspensions of mucosae and epithelia taken *post mortem*. Virus was recovered most frequently and in highest titre from the tonsillar area and less frequently from the pharynx and dorsal surface of the soft palate. No virus was found in samples taken from the nasal passages, the trachea or the rumen.

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