J. Hyg., Camb. (1980), **84**, 355
Printed in Great Britain

Swine vesicular disease: pathways of infection

By J. A. MANN and G. H. HUTCHINGS
The Animal Virus Research Institute, Pirbright, Surrey

(*Received* 4 *July* 1979)

SUMMARY

The pathways of infection in swine vesicular disease have been studied by (i) an estimation of the amounts of virus required to produce infection by different artificial inoculation procedures; (ii) the distribution and amounts of virus in various tissues of pigs killed at intervals after contact infection; (iii) an investigation of the susceptibility to virus infection of pig tissue explants.

The results show that pigs can be infected by a number of pathways and that the skin, as the most susceptible tissue, is probably the most frequent route of infection.

INTRODUCTION

Swine vesicular disease is an enterovirus classified provisionally as porcine enterovirus sero-group 9 (Huang & Dunne, 1974). Graves (1973), however, observed a distinct serological relationship between swine vesicular disease virus and Coxsackie B5 virus, which has since been confirmed by Brown & Wild (1974), Harris & Brown (1975), Brown *et al.* (1976) and Harris, Doel & Brown (1977). More recent studies on the classification of porcine enteroviruses by Knowles, Buckley & Pereira (1979) have led to the identification of sero-groups 9, 10 and 11 of porcine enteroviruses, and suggest that swine vesicular disease virus should be considered a porcine strain of Coxsackie B5 on the basis of serological and biochemical properties. Enteroviruses have been isolated from a wide range of tissues and associated with a variety of clinical conditions of pigs: polioencephalomyelitis (Mayr & Hecke, 1960; Singh, Bohl & Sauger, 1964; Yamanouchi *et al.* 1964; Long, Koestner & Kasza, 1966; Alexander & Betts, 1967), pneumonia, myocarditis and pericarditis (Sibalin & Lannek, 1960), diarrhoea (Hancock, Bohl & Birkeland, 1959; Lamont & Betts, 1960) and embryonic and fetal deaths and abnormalities (Dunne *et al.* 1965; Cartwright & Huck, 1967; Wang *et al.* 1973).

Viruses in this group usually infect through mouth and nose (Betts & Jennings, 1960; Mayr & Hecke, 1960; Sibalin & Lannek, 1960), and they have a strong affinity for intestinal tissues (Gois, 1971) especially the spiral colon and ileum, and for the tonsil. Swine vesicular disease virus causes a vesicular condition clinically indistinguishable from foot-and-mouth disease (Nardelli *et al.* 1968; Mowat, Darbyshire & Huntley, 1972). The virus has a marked tropism for epithelial tissues, and early studies by Burrows, Mann & Goodridge (1974) indicated that infection most likely gains entry through the skin or the mucosa of the digestive tract.

0022–1724/80/0060–1979 $01.00

Attempts to determine more clearly the pathways of infection were made by: (i) an estimation of the amounts of virus required to produce infection by different artificial inoculation procedures; (ii) establishing the distribution and amounts of virus in various tissues of pigs killed two to four days after contact with diseased animals and (iii) an investigation of the susceptibility to virus infection of organ cultures prepared from various tissues.

MATERIALS AND METHODS

Virus and virus assays

Swine vesicular disease virus England/72 (Dawe, Forman & Smale, 1973) was used at the second to fourth passage level in the pig kidney cell line IB-RS-2 (de Castro, 1964). Samples were assayed for virus content by counts of plaque forming units (p.f.u.) on IB-RS-2 cell monolayers after 48 h incubation at 37 °C.

Studies of clinical responses

Groups of six Large White cross Landrace pigs weighing from 20 to 40 kg were exposed to increasing amounts of virus which was either instilled into the mouth, nostril or eye or painted on to the tonsils with a large cotton swab. Pairs of pigs were exposed by the application of virus suspensions to areas of skin on the abdomen or the coronary bands which had been scarified by a scalpel blade or abraded with coarse emery paper. Increases in the amounts of virus administered were made at approximately weekly intervals. The pigs were examined daily and rectal temperatures recorded.

It is appreciated that repeated exposure to amounts of virus which fail to infect could induce a degree of resistance which might raise the infective threshold. Several serological examinations during this experiment gave negative results and suggest that these pigs were not being immunized by small doses of virus. Furthermore, the results obtained are similar to those reported by Burrows *et al.* (1974) when recording the approximate 50% end-point in a titration of swine vesicular disease virus in fully susceptible pigs.

Sequential slaughter experiments

Experimental pigs: infecting and sampling procedures

Groups of pigs were exposed to infection by contact with inoculated donors. The recipients were killed 1–4 days later by the intravenous administration of thiopentone sodium (B.P.C.). Following exsanguination, samples of tissues were collected for assay. No attempt was made to collect the samples aseptically but each tissue was washed in cold running water to remove traces of environmental virus.

Tissues sampled

Paired lymph nodes: parotid, mandibular, medial retropharyngeal, lateral retropharyngeal, prescapular, brachial or sternal, iliac and popliteal. Other lymph nodes: inguinal, bronchial, mesenteric (at 3 levels) and colonic. Other tissues: tonsil, pharyngeal wall, soft palate, glosso-pharyngeal wall, tongue, oesophagus, striated and cardiac muscle, ileum, jejunum, caecum, spiral colon, terminal colon, thyroid, thymus, bronchus, lungs, liver, spleen, kidney, pancreas, mandibular and parotid salivary glands, brain, spinal cord, coronary band skin and thoracic skin.

Processing and assay of samples

Samples of each tissue were prepared as a 1/10 (w/v) suspension in phosphate buffered saline by mincing with scissors and grinding with sterile sand (with a pestle and mortar). Virus content was determined in tissue culture as described above.

Organ culture method

Tissues collected within one hour of death from piglets one week old were washed in phosphate buffered saline containing 100 i.u. penicillin/ml; 100 i.u. polymixin/ml; 70 i.u. neomycin/ml and 50 i.u. mycostatin/ml (P.B.S.+), and cut into approximately 2 mm cubes. Three portions of each tissue were placed in narrow-necked screw-capped 15 ml. bottles with 10 ml of maintenance medium (Eagle's basal medium incorporating similar antibiotics to those described for P.B.S+ above) and incubated at 37 °C in a water bath. The following tissues were cultured: hairy skin, tongue, tonsil, pharyngeal wall, glosso-pharyngeal wall, parotid lymph node, triceps muscle, cardiac muscle, ileum, caecum, spiral colon, terminal colon, rectum, salivary glands, spleen and lung.

Infection and sampling procedures

Approximately 10^6 p.f.u. of virus were added to each culture and allowed to adsorb for 1 h. The cultures were then washed in warm P.B.S.+ and fresh maintenance medium added which was sampled immediately.

Subsequently samples of medium were taken at 24 h intervals, at which time the medium was renewed. Suspensions of virus in maintenance medium containing no tissue and suspensions of virus in maintenance medium containing equine tissue were incubated and sampled as virus and tissue controls.

RESULTS

Amount of virus required to produce clinical disease

The amounts of virus required to produce disease by various methods of exposure are listed in Table 1. No sign (or serological evidence) of disease resulted when amounts of virus up to $10^{5\cdot3}$ p.f.u. were instilled into the mouth, nose and conjunctiva or painted on the tonsils. When pigs were exposed in similar

Table 1. *The amounts of swine vesicular disease virus required to produce disease by different methods of exposure*

Method of exposure ...	Mouth	Nose	Eye	Tonsil	Skin
Amounts of virus which did not produce disease	4·6* 5·3	4·6 5·0	4·1 5·3	4·1 5·3	1·3 3·0
Amounts of virus which produced disease	6·8	6·8	6·8	6·8	3·6
Number of pigs developing disease / Number of pigs exposed	3/6	4/6	4/6	4/6	3/4
Days after last application when disease was first observed	2	7	2	7	2–7

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

fashion to $10^{6·8}$ p.f.u., disease was produced in 3–4 animals in each group within periods of 2–7 days. $10^{3·6}$ p.f.u. produced infection when applied to abraded skin surfaces.

Sequential slaughter experiments

Of the 18 pigs killed 2–4 days after exposure to infected animals, 4 had not apparently acquired infection and 4 were viraemic and thus unsuitable for this particular study. In 9 pigs, virus was found in one or both tonsils (mean infectivity of positive samples of $10^{4·19}$ p.f.u./g, range $10^{2·3}$ to $10^{5·8}$ (Table 2)). In 7 pigs virus was detected either in samples of epithelium or the lymph nodes of the head or, in some cases, both. Lymph nodes draining the hind limbs in 3 pigs were found to contain virus. Although moderate amounts of virus were found in the intestinal contents of several pigs, virus was found in the intestinal lymph nodes of only 2.

Organ cultures

Three patterns of virus growth were found in organ cultures (Table 3). Epithelial tissues produced significant amounts of virus ($> 10^{5·0}$ p.f.u./ml) from the 3rd to the 7th days. Explants of salivary gland produced similar amounts from the 6th to the 9th days. Little or no growth of virus was detected in muscle, lymph nodes or various parts of the digestive tract for 4–5 days, although after this period some growth occurred in cultures of colon, spleen and lung. No virus was found in the controls after the 4th day of incubation.

DISCUSSION

Burrows *et al.* (1974) found that some lymph nodes of pigs affected with swine vesicular disease contained large amounts of virus before the appearance of clinical signs. Furthermore it has been shown that virus could be detected in the popliteal lymph node shortly after the inoculation of the coronary band of that foot and before virus could be recovered from other regions of the body (Mann and Hutchings, unpublished work). It is likely that virus found in a lymph node, although not multiplying there, indicates a site of infection within the drainage

Table 2. *Recovery of swine vesicular disease virus from the tissues of pigs killed two to four days after contact with infected donors*

Pig number ...	1		2		3		4		5		6		7		8		9		10	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Tonsil	—	2·3*	4·1	—	—	4·7	4·6	4·2	3·5	3·1	5·2	—	3·7	—	5·8	ND	4·9	ND	—	—
Mandibular lymph node (LN)	—	—	—	—	—	—	—	—	—	—	2·5	—	—	—	2·7	3·1	2·9	—	—	—
Lateral retropharyngeal LN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·7	—	2·9	—	1·7	2·0
Parotid LN	—	—	—	—	—	—	—	—	—	—	—	—	2·8	—	—	—	—	—	—	—
Popliteal LN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·6	—	3·0	—	4·0	1·7
Iliac LN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3·3	2·8	3·8	—	—	—
Mesenteric LN	—		—		—		—		—		—		—		1·7		—		1·7	
Colonic LN	—		—		—		—		—		—		—		1·2		—		—	
Pharyngeal wall	—		—		—		2·0		—		—		—		—		—		—	
Tongue epithelium	—		—		—		—		2·6		—		—		—		—		—	
Foot epithelium – hind feet	ND		—		—		—		ND		—		—		—		2·0		—	

* Log_{10} p.f.u./g —, < 0·7 p.f.u./g L, Left R, Right ND, Not done.

No virus recovery from the following:

Paired lymph nodes: medial retropharyngeal, prescapular, brachial or sternal.

Other lymph nodes: inguinal, bronchial.

Other tissues: soft palate, glosso-pharyngeal wall, oesophagus, striated and cardiac muscle, ileum, jejunum, caecum, colon, large intestine, thyroid, thymus, bronchus, lungs, liver, spleen, kidney, pancreas, mandibular and parotid salivary glands, brain, spinal cord and thoracic skin.

Table 3. *The growth of swine vesicular disease virus in organ cultures prepared from seven-day-old piglets*

Hours after infection	Epithelial tissues*	Salivary gland	Lymph node,† muscle, intestine	Colon, spleen, lung	Control medium	Control medium + equine tissue
1	4·2‡	4·3	3·9	3·9	6·4	4·2
24	3·7	4·2	3·5	3·8	5·6	3·0
48	4·7	2·9	1·9	2·1	3·0	1·8
72	5·7	2·6	0·7	1·2	1·2	0·6
96	5·8	4·6	0·4	1·1	0·5	< 0·3
120	6·0	4·8	< 0·3	1·7	< 0·3	< 0·3
144	5·5	5·8	0·8	2·1	< 0·3	< 0·3
168	5·2	5·6	0·4	2·5	< 0·3	< 0·3
192	4·5	5·3	< 0·3	2·1	< 0·3	< 0·3
216	4·7	5·3	< 0·3	2·5	< 0·3	< 0·3

* Hairy skin, tongue, tonsil, pharyngeal wall, glosso-pharyngeal wall.
† Parotid lymph node, triceps muscle, ileum, caecum, large intestine, rectum.
‡ Log_{10} p.f.u/ml of culture fluids. Geometric means of duplicate or triplicate cultures of each tissue.

https://doi.org/10.1017/S0022172400026887 Published online by Cambridge University Press

area. Thus a wide range of lymph nodes as well as other tissues were examined in these experiments.

The pigs used for the sequential slaughter studies were exposed to very large amounts of virus by being in contact with previously infected animals. In nine of the ten infected pigs which were killed before the onset of viraemia, evidence of tonsillar infection was obtained, and in five of these animals it was the only tissue to be identified as infected. In the other animals virus was also recovered from lymph nodes in the head and the neck, indicating infection at other sites which could have been the skin of the face, rostrum of the snout, lips, buccal mucosae and upper respiratory and digestive tract epithelia (Sarr & Getty, 1964). Similarly the presence of virus in the popliteal lymph nodes of pigs 8, 9 and 10 and in the iliac nodes of pigs 8 and 9 indicated sites of infection in the lower hind limbs. This was confirmed in pig 9 by the recovery of virus from the skin of a hind foot. Evidence of active infection in the lower digestive tract was rare, although virus was found in the bowel contents of several pigs. However, the recovery of virus from the colonic and mesenteric nodes of pigs 8 and 9 could indicate localized growth in the intestinal mucosa.

The response of pigs exposed to varying amounts of virus by methods simulating natural modes of infection indicated, as in earlier studies (Burrows *et al.* 1974), that the skin is the most sensitive route. Relatively large amounts of virus were required to produce clinical disease when instilled into the mouth, nose or conjunctiva, or applied to the tonsil.

Studies in organ culture indicated that after a prolonged period many porcine tissues will support virus multiplication. However, it is likely that, as with foot-and-mouth disease virus (Williams & Burrows, 1972), the true susceptibility of tissues is related to their ability to support virus growth in the first few days of culture. Thus it would appear that cultures of skin and epithelium of the upper digestive tract are more susceptible to swine vesicular disease virus than those prepared from lymph nodes, intestinal mucosa and other organs. Whilst the low susceptibility of some of these latter tissues may have been due to the difficulty in maintaining them intact in culture, especially the bowel elements, it is consistent with unsuccessful attempts to produce clinical disease by feeding virus mixed with food (Mann, Burrows & Goodridge, 1975).

It is clear from these results that, when pigs are exposed to large amounts of virus, e.g. in the presence of active disease, infection can take place by a number of routes simultaneously. In natural outbreaks, one or two pigs become infected first following exposure to relatively small amounts of virus, e.g. in uncooked swill or contaminated fomites. In such situations infection probably takes place by the most sensitive route, i.e. the skin. Since virus entry can only occur through damaged epithelium, it is most likely to take place around the coronary bands and lower parts of limbs which are most prone to minor injuries.

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