J. Hyg., Camb. (1974), **72**, 135
Printed in Great Britain

Swine vesicular disease: virological studies of experimental infections produced by the England/72 virus

By R. BURROWS, J. A. MANN and D. GOODRIDGE
The Animal Virus Research Institute, Pirbright, Woking, Surrey, GU24 0NF

(*Received* 2 *July* 1973)

SUMMARY

Pigs exposed to relatively small amounts of virus by intradermal inoculation of the feet or by skin scarification developed clinical disease. Large amounts of virus were recovered from samples taken from the nose, mouth, pharynx, rectum and the prepuce or vagina during the first week of infection and smaller amounts during the second week. Virus was recovered from the faeces of most animals 16 days after infection and from one animal for 23 days. Pigs in contact with inoculated animals were killed at intervals before the appearance of clinical disease. The distribution and amounts of virus in various tissues indicated that infection had most likely gained entry through the skin or the epithelia and mucosae of the digestive tract. Some pigs acquired subclinical infections in which no virus excretion was detected and no transmission of infection to susceptible pigs took place over a period of 5 weeks.

INTRODUCTION

The outbreak of swine vesicular disease (SVD) which began in the West Midlands of England in December 1972 (Dawe, Forman & Smale, 1973) was the fourth to be identified. Previously the disease had been recognized in Italy in 1966 (Nardelli *et al.* 1968), in Hong Kong in 1971 (Mowat, Darbyshire & Huntley, 1972) and in Italy in 1972 (L. Nardelli, personal communication). In January 1973 further cases of disease were reported in Austria and Poland (Draft report of the *ad hoc* committee consultation on swine vesicular disease and the foot-and-mouth disease position in Eastern and Southeastern Europe, F.A.O. Rome, 9 January, 1973) and in France (Dhennin & Dhennin, 1973).

Epidemiological investigations of outbreaks of disease in Great Britain (R. S. Hedger, R. F. Sellers & G. N. Mowat, personal communications) have indicated that, although infection spreads rapidly within groups of pigs housed in the same pen, transmission of infection from pen to pen or from building to building occurred less readily than in many outbreaks of foot-and-mouth disease (FMD). This difference in epidemiological behaviour could be due to differences in the susceptibility of pigs to particular routes of infection, to differences in virus excretion from diseased animals or to differences in the resistance of the virus to environmental conditions.

These questions are studied in this paper, which is concerned with the responses

of pigs exposed to virus by different routes, and with the identification of regions of virus multiplication and excretion during the course of disease.

MATERIALS AND METHODS

Virus

The England/72 virus (Dawe *et al.* 1973) was used as a suspension of infected pig foot epithelium or as a tissue culture harvest from the first or second passage in the pig kidney cell line IB-RS-2 (de Castro, 1964).

Experimental pigs – inoculation and sampling procedures

Groups of large white pigs ranging in weight from 30–40 kg. were exposed to virus by one of the following methods: the intradermal inoculation of approximately 0·1 ml. of a virus suspension in the bulbs of the heel, the coronary band or the skin of the thorax and abdomen; the application of approximately 0·1 ml. of virus suspension to areas of scarification on the rostrum of the snout or on the skin of the thorax and abdomen; the instillation of approximately 0·5 ml. of virus suspension into the nasal or oral cavities, or by direct contact with inoculated pigs. All animals were examined daily and rectal temperatures recorded. In some experiments nasal, oral, rectal and preputial or vaginal swabs, blood and pharyngeal/tonsillar samples (Burrows, Greig & Goodridge, 1973) were taken daily for varying periods after exposure. Seven pigs exposed to infection by contact with inoculated pigs were killed 2–5 days later by the intravenous inoculation of thiopentone sodium (BPC) and a range of tissues were taken for virus assay. No attempt was made to collect the tissues aseptically but each tissue or mucous membrane was washed thoroughly in running water after collection.

Assay of virus and neutralizing antibody

All swabs and tissue samples were stored over liquid nitrogen before examination. Swabs were processed by immersion and shaking in 3 ml. diluent and tissues were prepared as 1/10 (w/v) suspensions. The virus content was estimated by counts of plaque forming units (p.f.u.) after 48 hr. incubation on IB-RS-2 monolayer cultures. The identity of the plaque forming virus was monitored periodically by neutralization tests using an antiserum prepared in guinea pigs against the Italy/66 virus. Serum neutralization tests were performed as described by Burrows *et al.* 1973, using a plaque reduction procedure in which residual virus was determined after 48 hr. incubation.

RESULTS

Response to inoculation by different routes

Table 1 lists the concentrations of virus used for each method of inoculation or exposure and the numbers and responses of pigs used in the different experiments. The clinical signs of natural and experimental SVD have been described by Nardelli *et al.* (1968), Mowat *et al.* (1972) and by Dawe *et al.* (1973).

Foot inoculation. Inoculation of the bulbs of the heel with $10^{5·9}$ p.f.u./site resulted in the development of vesicles within 48 hr. at 22 of 36 sites in nine pigs.

Table 1. *Numbers and responses of pigs exposed to various concentrations of virus by different routes*

	Intradermal inoculation			Skin scarification		Instillation	
Virus dose*	Heel	Coronary band	Abdominal skin	Snout	Abdomen	Oral	Nasal
1·5	—	—	0/8†	—	1/8	0/4	0/4
2·5	2/8	5/8	0/8	0/2	1/8	0/4	1/4‡
3·5	5/8	3/8	0/8	0/2	2/8	0/4	2/4‡
4·5	5/8	6/8	0/8	0/2	3/8	—	—
5·5	—	—	0/8	0/2	2/8	—	—
5·9	22/36	—	—	—	—	—	—
6·5	—	—	0/8	—	1/8	—	—
No. of pigs	13	4	4	8	4	12	12

* Log_{10} p.f.u./site or pig.
† $\frac{\text{No. of sites or pigs reacting}}{\text{No. of sites or pigs exposed}}$.
‡ Subclinical infection.
—, Not tested.

Table 2. *Virus content of samples taken from pigs after heel inoculation – England/72 virus*

Days after inoculation	Nasal swabs	Mouth swabs	Pharyngeal samples	Rectal swabs	Preputial/ vaginal swabs	Serum
1	0*	0	0·74 (0–5·9)	1·25 (0–2·5)	1·05 (0–2·1)	2·56 (1·0–5·2)
2	3·85 (3·2–4·5)	4·0 (3·5–4·5)	4·88 (3·3–5·9)	4·75 (4·5–5·0)	3·05 (2·2–3·9)	4·61 (2·7–6·0)
3	4·3†	6·3 (6·1–6·5)	5·73 (4·7–6·3)	5·1†	3·5†	3·84 (2·9–5·3)
4	5·22 (3·5–6·5)	6·0 (4·5–7·0)	5·40 (4·1–6·5)	4·59 (3·0–5·7)	4·24 (2·3–4·8)	0·68 (0–3·2)
5	5·06 (2·5–6·7)	5·14 (4·0–7·3)	5·07 (4·3–6·8)	4·85 (4·3–6·3)	3·41 (2·5–5·0)	0
6	3·55 (0–5·0)	4·19 (1·5–6·3)	3·72 (2·3–4·7)	3·75 (2·7–4·7)	2·50 (1·7–4·4)	0
7	2·52 (0–3·9)	0·99 (0–2·7)	2·97 (1·5–4·5)	2·29 (0–4·0)	2·34 (0–5·7)	0
8	0·71 (0–2·2)	0	1·59 (0–2·5)	1·15 (0–3·0)	0·70 (0–1·7)	—
9	0·45 (0–2·1)	0	0·69 (0–3·1)	1·24 (0–2·9)	0·89 (0–3·1)	—
10	0	0	0·21 (0–1·7)	0	0	—
11	0	0	0	0·87 (0–2·5)	0	—
12	0	0	0·27 (0–2·2)	0·19 (0–1·5)	0	—
14	0	0	0	0	0	—

* Log_{10} p.f.u./swab/sample/ml. (serum) – geometric mean and range of 8 pigs.
† Result for one pig.
0 No virus recovered i.e. < 1·0/swab/sample, <0·7/ml serum.
—, Not tested.

Secondary lesions were evident in six of the nine pigs within 72 hr. Comparative titrations by the bulb of the heel and the coronary band procedures in groups of four pigs (Burrows, 1966) indicated that both regions were of similar sensitivity to virus. Approximate concentrations of virus estimated to produce lesions at 50% of sites inoculated were $10^{3·4}$ p.f.u. (heel) and $10^{3·3}$ p.f.u. (coronary band).

Skin scarification and inoculation. Eight pigs were exposed by the application of single virus concentrations ranging from $10^{2·5}$ to $10^{5·5}$ p.f.u. to scarified areas of the

https://doi.org/10.1017/S0022172400023305 Published online by Cambridge University Press

Table 3. *Virus content of samples taken from pigs exposed to contact infection*

	Days after inoculation of donor pigs				
	1	2	3	4	5
No. of pigs ...	7	7	6	4	2
Nasal swab	0·3* (0–2·0)	2·4 (0–3·3)	4·0 (3·3–5·0)	4·7 (3·3–5·8)	3·9 (3·8–4·0)
Oral swab	0	2·8 (1·7–3·5)	3·7 (3·0–4·7)	5·3 (4·0–7·0)	4·5 (4·5)
Pharyngeal swab	*0*	*2·4 (1·7–3·1)*	*3·5 (2·9–4·5)*	*5·0 (3·3–5·8)*	*3·9 (3·5–4·3)*
Rectal swab	0	2·6 (0–3·7)	4·3 (3·7–5·0)	4·0 (3·9–4·2)	4·0 (3·8–4·2)
Preputial/vaginal swab	0	1·0 (0–2·2)	3·0 (1·5–5·0)	3·3 (2·3–4·2)	4·1 (3·4–4·8)
Serum	0	0	0·8 (0–2·8)	2·2 (0–4·6)	2·0 (1·0–3·0)

* Log_{10} p.f.u./swab/sample/ml (serum) – geometric mean and range.

rostrum of the snout. No lesions developed at the sites of virus application but 2 of the 8 pigs developed signs of generalized disease 6 days later. Four pigs were exposed to multiple concentrations of virus ($10^{1·5}$–$10^{6·5}$ p.f.u.) applied to scarified areas of the abdominal skin lateral to each teat. Erythematous plaques approximately 1·5 cm. in diameter and 2–3 mm. in height appeared within 48 hr. at 10 of the 48 sites exposed but these lesions were distributed in random fashion and were not obviously related to the concentrations of virus applied to the area. Biopsy material from one lesion contained $10^{1·8}$ p.f.u./g. whereas only traces of virus were recovered from an adjacent area of normal skin. Two of the four pigs developed signs of generalized disease 4 days after exposure. Intradermal inoculation of the abdominal skin with the same concentrations of virus produced no obvious lesions and no signs of disease appeared within a 4-day period.

Oral and nasal exposure. No clinical signs were seen in pigs given virus concentrations up to $10^{3·5}$ p.f.u. as a single dose. However, serological studies showed that one of the four pigs given $10^{2·5}$ p.f.u. and two of the four pigs given $10^{3·5}$ p.f.u. by intranasal instillation had acquired a subclinical infection.

Virus concentrations in the secretions and excretions of inoculated pigs

Table 2 lists the geometric mean amounts and ranges of virus measured in nasal, oral, rectal and preputial or vaginal swabs, and from pharyngeal/tonsillar and serum samples collected from eight pigs infected by heel inoculation (four sites at $10^{5·9}$ p.f.u./site). Peak concentrations of virus were found in the serum on the second day and in other samples on the third to the fifth day after inoculation. These periods coincided with the development of primary and secondary lesions. The infectivity of samples declined after the fifth day and no virus was recovered from the mouth after the seventh day or from swabs taken from the nose and the urogenital orifices after the ninth day. Although only 5 of 32 rectal swabs taken between the 10th and 14th days yielded virus, no difficulty was experienced in demonstrating virus in the faeces for longer periods. Twenty-eight of 30 samples of faeces collected from pigs 14–16 days after infection contained virus (mean infectivity

Table 4. *Virus content* of samples and tissues taken from pigs exposed to contact infection.*

	Pig identification						
KE:	3	4	5	6	7	8	9
Days after inoculation of donors	2	3	3	4	4	5	5
Ante-mortem samples							
Nasal swab	2·5	3·7	3·9	4·9	5·1	3·8	4·0
Oral swab	3·3	4·0	3·0	7·0	6·5	4·5	4·5
Pharyngeal sample	2·8	4·5	3·3	5·8	4·7	3·5	4·3
Rectal swab	2·5	5·0	4·0	4·1	4·2	4·2	3·8
Serum	0	0	0	4·6	0	1·0	3·0
Post-mortem samples							
Nasal septum	0	0	0	3·3	0	2·2	—
Turbinate mucosae	0	1·7	0	4·0	0	2·5	—
Dorsal surface of soft palate	0	0	0	3·5	0	0	—
Tracheal mucosa	0	0	0	3·9	0	0	—
Lung: Apical lobe	0	0	0	5·2	0	2·2	—
Cardiac lobe	0	0	0	5·3	0	0	—
Intermediate lobe	0	1·7	0	5·2	2·7	3·9	—
Diaphragmatic lobe	0	2·0	1·7	5·0	2·2	0	—
Tongue	0	4·0	2·2	3·9	1·7	1·7	—
Glosso-pharyngeal area	0	0	0	3·0	2·2	2·5	—
Tonsils (2)	0	1·5	0·7	5·2	0·9	3·2	—
Pharynx	0	3·0	1·7	4·4	2·2	3·5	—
Mandibular salivary gland	0	0	1·4	2·5	0	2·8	—
Parotid salivary gland	0	1·7	2·3	2·8	2·7	4·2	—
Duodenum	1·7	0	0	3·0	2·9	0	—
Small intestine	0	0	0	3·7	3·8	2·7	—
Caecum	0	2·0	0	3·0	3·7	1·7	—
Small colon	0	1·4	0	2·0	3·9	3·2	—
Large colon	0	2·0	0	2·5	3·0	0	—
Rectum	0	2·8	2·1	3·5	—	2·3	—
Spleen	0	0	0	4·5	2·3	2·7	—
Liver	0	0	0	5·0	2·3	2·2	—
Pancreas	0	0	0	3·5	2·3	2·0	—
Kidney	0	2·1	0	3·7	1·7	0	—
Cerebrum	0	0	—	2·9	0	0	—
Medulla	0	1·4	—	2·8	1·7	2·0	—
Spinal cord	0	2·0	0	2·8	0	0	—
Leg muscle	0	2·3	2·4	3·0	2·5	2·9	4·3
Heart muscle	0	0	0	4·2	3·9	2·3	—
Bone marrow	0	0	—	3·6	0	1·7	—
Skin: Rostrum of snout	0	2·8	4·0	2·7	2·7	7·3	—
Interdigital	3·4	2·5	2·6	4·0	2·4	2·7	8·6
Hairy (thorax)	0	2·3	3·0	4·2	3·2	3·5	3·8
Lymph nodes: Pharyngeal (2)	0	0	0	3·0	0	2·5	3·2
Mandibular	0	2·0	0	5·1	0	3·3	4·7
Parotid	0	0	0	2·8	2·7	3·8	—
Bronchial	0	2·1	0	4·9	0	2·2	3·8
Mediastinal	0	0	0	4·3	2·2	0	—
Mesenteric	0	0	0	5·0	3·0	2·6	—
Prescapular	0	0	0	3·9	0	2·3	—
Popliteal	0	1·4	2·0	2·6	3·0	0	6·7
Inguinal	0	2·8	0	5·7	3·0	—	7·0

* Log_{10} p.f.u./g/ml. or sample.

0 = < 0·7 per ml. of serum. < 1·0 per specimen (ante-mortem). < 1·4 per specimen (post-mortem). — = Not tested.

https://doi.org/10.1017/S0022172400023305 Published online by Cambridge University Press

Table 5. *Serum neutralizing-antibody responses of pigs following inoculation or exposure to England/72 virus*

No. of pigs	Method of exposure	Disease	Days after exposure				
			0	7	14	28–35	42–49
12	Inoculation or contact	Clinical	< 0·5*	1·8	—	4·1	3·9
3	Intranasal	Subclinical	< 0·5	0·8	2·0	2·7†	2·5†

* Geometric mean of the log reciprocal of the initial serum dilution which neutralized 90 % of the test virus.
† Two pigs.

$10^{2·85}$ p.f.u./g., range $10^{1·7}$–$10^{3·6}$) and one of five samples collected 23 days after infection contained $10^{2·5}$ p.f.u./g.

Virus concentrations in the secretions, excretions and tissues of pigs exposed to contact infection

The mean amounts of virus in samples taken daily from recipient pigs after the inoculation of two donor pigs are detailed in Table 3. Virus was recovered from the nose of only one of seven recipient pigs 24 hr. after inoculation of the donors, which indicated that little virus was excreted by the donor animals during this period. Virus was recovered from most of the samples (except serum) collected from the recipients from 48 hr. onwards. Comparisons of the virus content of samples taken before and after death (Table 4) from pigs killed on the second and third day indicated that most of the virus in the samples taken before slaughter was in the surface film overlying the epithelia and mucosae and was not related to virus growth in those regions. It was apparent that most of this virus was derived from the donor animals and had been acquired passively from the environment. However, some evidence of active infection and dissemination of virus was obtained in all recipient pigs (Table 4). Pig KE 3, killed on the second day had considerable amounts of virus in a sample of interdigital skin. In pigs killed on the third and subsequent days there was consistent evidence of virus growth in the epithelia and mucosae of the upper and lower digestive tracts, in salivary glands and in all regions of skin examined. Variable concentrations of virus were found in other tissues, in some pigs these were obviously related to amounts of virus in the blood or to drainage from regions of virus growth or to virus passively acquired from the environment. Only one of seven pigs (KE 9) had developed clinical signs of disease (an early foot lesion) by the time of killing.

Subclinical infection following a limited exposure to virus

Twenty-four pigs were exposed to infection by the instillation of small amounts of virus into the nose or into the mouth. Oral and rectal swabs were taken daily for a period of 14 days from all pigs but no evidence of virus growth or excretion was obtained. Serological tests revealed that 3 of the 24 pigs acquired a subclinical infection and in Table 5 the neutralizing antibody response of these pigs is com-

pared with that of pigs which had experienced clinical disease as a result of inoculation or contact infection. No transmission of infection took place from subclinically infected pigs to susceptible pigs housed in the same room over a period of 5 weeks.

DISCUSSION

The response of pigs to graded amounts of virus was irregular and did not permit accurate determinations of the 50 % infective doses. However, the results showed that pigs were highly susceptible to small amounts of England/72 virus by foot inoculation or by skin scarification. With FMDV the coronary band route of inoculation is approximately 300 fold less sensitive than the bulb of the heel route (Burrows, 1966), but with SVDV the coronary band was found to be of equal or greater sensitivity. This may be a reflexion of general skin susceptibility to virus as it has been observed that vesicles on the coronary band are not restricted to the skin/horn junction as in FMD, but may extend for 1 or 2 cm. up the limb. Skin lesions may also appear on the lower and upper regions of the limb and occasionally on the thorax and abdomen.

A number of outbreaks in the field were attributed to the movement of pigs in contaminated lorries (Ministry of Agriculture, Fisheries and Food, 1973). The probable route of infection in these circumstances would be by viral contamination of the minor wounds and abrasions which occur frequently during transport.

In inoculated pigs it was found that the amounts of virus in the secretions, excretions and tissues differed to some degree from those measured in comparable experiments with FMDV. In FMD maximum concentrations of virus were found in samples collected 2–4 days after inoculation; the amounts of virus were less than those found in SVD and rarely was virus recovered from samples (other than vesicular epithelium) for longer than 5 days (unpublished data). In SVD peak virus concentrations were measured in samples collected 2–5 days after inoculation and virus was recovered from swabs and pharyngeal samples for periods of 7–12 days. Faeces collected 16 days after infection contained considerable quantities of virus and evidence of faecal excretion was obtained for 23 days from one pig. An important source of virus is vesicular fluid and epithelium; in FMD infective virus is no longer detectable in lesions over 10 days old (unpublished data) whereas in SVD remnants of vesicular epithelium collected from a 10 day old lesion contained $10^{6.9}$ p.f.u./g. (unpublished data).

Susceptible pigs housed with inoculated pigs acquired virus rapidly and evidence of active infection and dissemination of virus was found in all pigs killed after the second day. Unfortunately the results do not identify any particular region as the initial site of virus entry and multiplication. No consistent evidence was obtained of early virus growth in respiratory tract mucosae and it would seem likely that infection took place through areas of damaged skin or through the epithelia or mucosae of the digestive tract.

Observations in the laboratory and the field indicate that SVD spreads less readily than FMD between groups of pigs and this has been related to the amounts of airborne virus produced in the 2 diseases. In SVD Sellers & Herniman (1974)

recovered smaller amounts of airborne virus for shorter periods of time than in comparable experiments with FMD. In addition a greater proportion of airborne SVDV was associated with a large particle aerosol which would tend to remain airborne for short periods only.

These studies of SVD show that large amounts of virus are present in the immediate vicinity of infected pigs for a considerable period of time. Airborne transmission of virus is unlikely (Sellers & Herniman, 1974) and the spread of disease depends mainly on the movement of infected pigs and their products. The stability of the virus is such that it is not inactivated by the acid changes which occur in the musculature after death, and the virus can be expected to withstand the various processes used in the production of sausages and salami. Little or no reduction in infectivity occurs in cold storage and so uncooked pork and pork products will remain a hazard indefinitely. Several outbreaks of disease in Great Britain were attributed to this recycling of virus during the spring and summer of 1973.

The numbers of outbreaks reported in France and Italy have been considerably fewer than those reported in Britain. This apparent difference in epidemiology may be due to regional differences in the methods of husbandry and marketing of pigs or it may be that cases of infection have gone unrecognized or have been subclinical in nature. In both the present and earlier experiments with SVD some pigs exposed to small amounts of virus acquired subclinical infections. An interesting finding was that these infections were not accompanied by virus excretion which was detectable by the methods employed or by the transmission of infection to susceptible animals over periods of 7 weeks (Italy/66 – Burrows *et al.* 1973) or 5 weeks (England/72). The pigs which acquired subclinical infection and had no contact with clinical disease developed lower titres of neutralizing antibody than did pigs which experienced or were in contact with clinical disease. This fact should be borne in mind in the interpretation of the results of serological surveys for evidence of subclinical and past infection.

We should like to thank Mrs Jean Huntley and Mr G. H. Hutchings for valuable assistance in the laboratory and in the Isolation Unit, and Messrs I. Hughes, M. Fortune and M. Tyrell, large animal attendants, for their meticulous attention to handling, cleansing and disinfection procedures.

REFERENCES

Burrows, R. (1966). The infectivity assay of foot-and-mouth disease virus in pigs. *Journal of Hygiene* **64**, 419.

Burrows, R., Greig, A. & Goodridge, D. (1973). Swine vesicular disease. *Research in Veterinary Science* **15**, 141.

De Castro, M. P. (1964). Behaviour of the foot-and-mouth disease virus in cell cultures: susceptibility of the IB-RS-2 cell line. *Archivos do Instituto biologico, São Paulo* **31**, 63.

Dawe, P. S., Forman, A. J. & Smale, C. J. (1973). A preliminary investigation of the swine vesicular disease epidemic in Britain. *Nature, London* **241**, 540.

Dhennin, L. & Dhennin, L. (1973). La maladie vésiculeuse du porc. Son apparition en France. *Bulletin de l'Académie vétérinaire de France* **46**, 47.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1973). Swine vesicular disease. *Veterinary Record* **92**, 234.

MOWAT, G. N., DARBYSHIRE, J. H. & HUNTLEY, J. F. (1972). Differentiation of a vesicular disease of pigs in Hong Kong from foot-and-mouth disease. *Veterinary Record* **90**, 618.

NARDELLI, L., LODETTI, E., GUALANDI, G. L., BURROWS, R., GOODRIDGE, D., BROWN, F. & CARTWRIGHT, B. (1968). A foot-and-mouth disease syndrome in pigs caused by an entero-virus. *Nature, London* **219**, 1275.

SELLERS, R. F. & HERNIMAN, K. A. J (1974). The airborne excretion by pigs of swine vesicular disease virus. *Journal of Hygiene* **72**, 61.