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Swine vesicular disease: comparative studies of viruses isolated from different countries

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SUMMARY

Seven viruses isolated from outbreaks of swine vesicular disease in various countries between 1966 and 1973 were compared in pigs and infant mice. All produced a similar disease and virus excretion pattern in the pig, although the Italy/66 virus was considerably less virulent than the other viruses. The results of cross neutralization tests of convalescent pig sera and the response of 5-day-old mice to intraperitoneal inoculation indicated minor differences between some viruses. The Italy/66, Hong Kong/71 and France/73 viruses differed from each other and also from the Italy/72, England/72, Austria/73 and Poland/73 group of viruses.

INTRODUCTION

Outbreaks of swine vesicular disease (SVD) were recognized in a number of European countries during the autumn and winter of 1972/1973. Previous outbreaks of disease had been identified in Italy in 1966 and in Hong Kong in 1971. This sudden appearance of a relatively new disease simultaneously in several countries suggested that infection had been introduced from a common source and that the same agent was responsible. However, obvious differences in the nature and the spread of the disease were reported from different countries. These apparent differences in epizootiology may have been due to regional differences in the methods of husbandry and marketing of pigs or to differences in the virus strains isolated in the various outbreaks. This paper is concerned with some comparative studies of SVD viruses isolated from different countries.

MATERIALS AND METHODS

Viruses

The following viruses: Italy/66 (Nardelli *et al.* 1968), Hong Kong/71 (Mowat, Darbyshire & Huntley, 1972), England/72 (Dawe, Forman & Smale, 1973), Italy/72, Austria/73, Poland/73 and France/73 – were used as suspensions of infected pig foot epithelium or as tissue culture harvests from an early passage in either primary pig kidney monolayers or in the pig kidney cell line, IB-RS-2 (de Castro, 1964).

Experimental animals

Pigs: inoculation, examination and sampling procedures

Experiments with Hong Kong/71, Italy/72, Austria/73, Poland/73 and France/73 viruses. Groups of four Large White pigs (30–40 kg.) were housed in different rooms in an isolation unit and exposed to a range of virus concentrations by heel inoculation (Burrows, 1966). A group of four uninoculated pigs were included as controls. All animals were examined daily, rectal temperatures recorded and oral swabs collected for virus excretion studies. The severity of the clinical disease was assessed periodically by computing a lesion score based on the appearance and severity of lesions at predilection sites on the coronary bands, heels, accessory digits, skin, snout and mouth. A pig exhibiting extensive lesions involving all susceptible sites qualified for a lesion score of 100.

Experiments with the Italy/66 and England/72 viruses. Details of these experiments have been recorded (Burrows, Greig & Goodridge, 1973; Burrows, Mann & Goodridge, 1974).

Mice

Litters of 5-day-old mice were inoculated by the intraperitoneal route with 0·1 ml. of various concentrations of each virus and observed daily for 3 weeks. Deaths and paralysis in mice given small amounts of virus were regarded as specific only if large amounts of virus were recovered from the carcase. Survivors were killed after 6 weeks and subclinical infections were identified by serum neutralization tests.

Assay of virus and neutralizing antibody

Virus was assayed by counts of plaque-forming units (p.f.u.) after 48 hr. incubation on IB-RS-2 monolayer cultures. 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. Cross-neutralization products were calculated as described by Federer, Burrows & Brooksby (1967).

RESULTS

Pigs

Response to heel inoculation

There was little correlation between the dose of virus inoculated into the heel and the frequency of lesions produced and it was not possible to determine the amounts of virus necessary to produce a 50% lesion endpoint (Table 1). The total number of sites reacting to the inoculation of $10^{3\cdot5}$ to $10^{6\cdot4}$ p.f.u. ranged from approximately 12% with the Italy/66, Hong Kong/71 and Italy/72 viruses and 20% with the France/73 virus to between 45 and 63% with the England/72, Austria/73 and Poland/73 viruses. With these last three viruses a 100-fold increase in the amounts of virus inoculated resulted in only a mean 18% increase in the number of sites reacting.

Table 1. *Response of pigs to heel-inoculation of various concentrations of SVD virus strains*

Virus dose*	Italy/66	Hong Kong/71	Italy/72	England/72	Austria/73	Poland/73	France/73
6.5	11/16†	—	—	—	—	—	—
5.5–6.4	3/8	1/8	0/8	22/36	6/8	5/8	3/8
4.5–5.4	1/28	1/8	1/8	5/8	7/8	2/8	0/8
3.5–4.4	—	1/8	2/8	6/10	2/8	4/8	2/8
No. of pigs	17	4	4	15	4	4	4

* Log_{10} p.f.u./site.
† Number of sites reacting at 72 hr./number of sites inoculated.
— Not tested.

Table 2. *Lesion scores of pigs following heel-inoculation of SVD virus strains*

Days after inoculation	Hong Kong/71	Italy/72	Austria/73	Poland/73	France/73
8	47 (25–68)*	45 (21–70)	55 (49–60)	39 (17–52)	52 (39–63)
14	37 (21–46)	32 (15–62)	56 (46–66)	33 (16–43)	52 (39–77)
21	33 (18–47)	26 (11–45)	49 (37–58)	33 (15–35)	53 (38–73)
28	28 (11–40)	24 (8–39)	40 (27–51)	18 (3–27)	37 (22–65)

* Mean and range of four pigs.

Table 3. *Virus content of buccal swabs taken from pigs after heel inoculation of SVD virus strains*

Days after inoculation	Hong Kong/71	Italy/72	England/72	Austria/73	Poland/73	France/73
2	5·57*	4·17	4·0	3·62	5·52	3·07
3	5·17	6·00	6·30	5·95	5·92	5·22
4	5·60	6·10	6·0	7·40	5·85	6·95
5	5·25	5·72	5·14	5·65	4·67	4·81
6	3·77	3·15	4·19	3·47	3·60	3·30
7	2·20	2·55	0·99	1·32	1·80	1·57
8	2·10	0·75	—	1·92	0·37	0·87
9	1·22	1·02	—	1·37	1·35	—
10	0·67	—	—	0·67	—	0·55
11	0·85	0·30	—	1·46	—	—
12	0·42	0·37	—	—	0·37	0·37
13	—	—	NT	—	—	—
14	NT	NT	—	NT	NT	NT
19	—	—	NT	—	—	—
22	—	—	NT	—	—	—

* Log_{10} p.f.u./swab – geometric mean of four pigs (England/72 = geometric mean of eight pigs).
– = $< 0{\cdot}25$ ($< 0{\cdot}12$ for England/72 group). $10^{1{\cdot}0}$ p.f.u./swab is the smallest amount of virus which could be detected by the method used in a single swab, but the figures in the table are means of four or eight animals.
NT = Not tested.

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Table 4. *Neutralizing antibody titres of convalescent pig sera (28 day) to homologous and heterologous strains of SVD virus*

	Virus strain						
Serum	Italy/66	Hong Kong/71	Italy/72	England/72	Austria/73	Poland/73	France/73
Italy/66	3·76*	2·92	3·30	3·30	3·10	3·36	3·30
Hong Kong/71	3·04	3·13	3·30	3·30	2·97	3·10	2·94
Italy/72	3·31	3·02	3·36	3·53	3·08	3·30	3·20
England/72	3·94	3·54	3·87	3·94	3·94	3·53	3·59
Austria/73	3·52	2·70	3·64	3·36	3·19	3·70	3·30
Poland/73	2·97	2·86	3·10	3·21	2·64	3·10	3·04
France/73	3·47	2·94	3·47	3·21	3·02	3·47	3·64

* Log_{10} reciprocal of initial serum dilution which neutralized 90 % of test virus – geometric mean of three tests.

All seven viruses produced a similar disease which was characterized by a febrile episode of 3–7 days' duration with group mean peak temperatures of 39·1° C to 41·1° C. on the fourth to sixth day after inoculation. The severity of the generalized disease was not necessarily related to the initial response to inoculation. Some pigs did not develop vesicles at the sites of injection but subsequently developed secondary lesions. Table 2 lists the means and ranges of lesion scores over a period of 28 days for the pigs used in the comparative examination of five of the seven viruses. Differences were seen in the distribution and severity of vesicular lesions but the variations between individuals within a group were as great as those seen between groups. The clinical records for earlier experiments with the Italy/66 and England/72 viruses were not detailed sufficiently to compute comparable lesion scores but they do confirm that, when tested in pigs, the England/72 virus was of similar virulence to, and the Italy/66 virus was considerably less virulent than, the viruses listed in Table 2.

Virus content of buccal swabs

Table 3 lists the geometric mean amounts of virus recovered from buccal swabs taken from six of the seven groups of pigs. In general, similar amounts of virus were found in each group, the greatest amounts being found on the third or fourth day after inoculation. Virus was not recovered from the England/72 group after the seventh day but small amounts were recovered from individual pigs of the other groups on the 10th to the 12th day.

Serological studies

Table 4 presents the neutralizing antibody titres of pooled 28-day convalescent pig sera to the homologous and heterologous viruses. Each result is the mean of three separate tests and the standard error of these means, calculated on the 147 paired results, is $\pm 0{\cdot}16$. The cross neutralization products derived from these results are listed in Table 5. The differences found are not sufficiently great to

Table 5. *Inter-relationships of SVD virus strains – cross-neutralization products of convalescent pig sera*

Italy/66						
0·93	Hong Kong/71					
0·51	0·17	Italy/72				
0·46	0·23	−0·10	England/72			
0·33	0·65	−0·17	−0·17	Austria/73		
0·53	0·27	0·06	0·30	−0·05	Poland/73	
0·63	0·89	0·33	0·78	0·51	0·23	France/73

Cross-neutralization product = (A − B) + (C − D), where A and C are antibody titres (Log reciprocal) against the homologous virus strains and B and D are antibody titres against the heterologous virus strains.

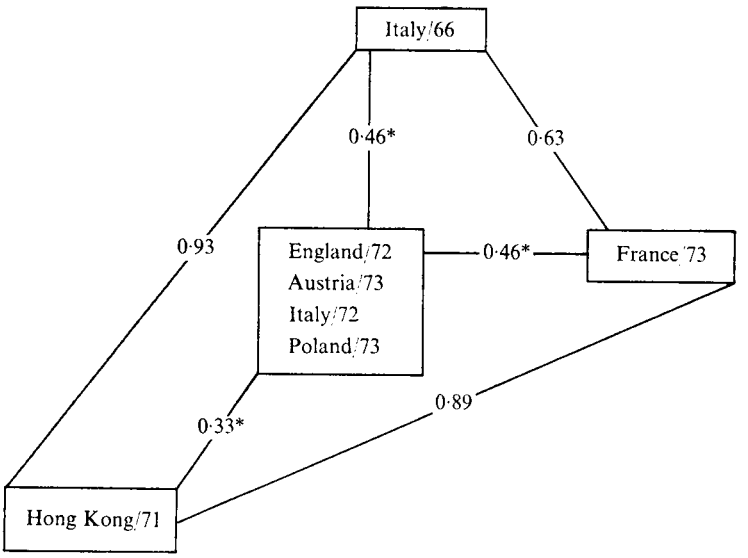

Fig. 1. Inter-relationships between SVD virus strains – cross neutralization products of convalescent pig sera. * Geometric means. Geometric mean of relationships between England/72, Austria/73, Italy/72 and Poland/73 = 0·14.

warrant a subtype classification but they do indicate minor antigenic differences between certain viruses. The England/72, Italy/72, Austria/73 and Poland/73 strains appear to be very closely related and slightly different from the other three strains, which also differ slightly from each other. A semi-diagrammatic representation of the serological inter-relationships between these viruses is shown in Fig. 1.

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Table 6. *The infectivity of Italy 66 virus for one-day old mice*

Route of inoculation	Number of tests	Geometric mean infectivity	Range
Intracerebral	5	3·10*	1·7–3·8
Intraperitoneal	2	4·05	3·3–4·8

* Log_{10} p.f.u. producing paralysis or death in 50 % of mice.

Table 7. *Response of 5-day-old mice to the intraperitoneal inoculation of various concentrations of SVD virus strains*

Virus dose*	Italy/66	Hong Kong/71	Italy/72	England/72	Austria/73	Poland/73	France/73
5·5–6·5	0/10†	—	2/10	1/10	9/10	6/10	0/10
4·5–5·4	8/18	0/10	6/19	11/19	5/19	8/18	1/18
3·5–4·4	0/19	0/18	4/19	7/18	3/19	14/20	4/19
2·5–3·4	2/18	0/19	7/20	9/18	5/20	10/18	0/20
1·5–2·4	0/19	0/20	1/19	3/20	9/20	5/19	0/20
0·5–1·4	0/20	0/19	0/20	4/19	1/20	1/18	0/20
Totals:	10/104	0/86	20/107	35/104	32/108	44/103	5/107

* Log_{10} p.f.u.
† Number of mice dead or paralysed/number of mice inoculated.

Infectivity for mice

In earlier studies of the Italy/66 virus, it had been found that one-day-old mice were susceptible to both intracerebral and intraperitoneal inoculation of virus and that 50 % end-points could be calculated on the numbers of mice which developed paralysis or died (Table 6). No apparent disease was seen in 7-day-old mice given similar amounts of Italy/66, but Dawe *et al.* (1973) reported that the England/72 virus produced paralysis and death in 6-day-old mice. The results of two experiments comparing the response of 5-day-old mice to the intraperitoneal inoculation of the seven viruses are listed in Table 7. The numbers of mice showing signs of disease differed considerably; less than 10 % of mice given the Italy/66, Hong Kong/71 and France/73 viruses were apparently infected, whereas between 20 and 40 % of mice inoculated with the Italy/72, England/72, Austria/73 and Poland/73 viruses showed signs of infection. The mean dose response curve in mice given this latter group of viruses was extremely flat, ranging from approximately 9 % reactors in mice given $10^{0·5}$ to $10^{1·4}$ p.f.u. to approximately 47 % reactors in mice given $10^{5·5}$ to $10^{6·5}$ p.f.u. The clinical signs shown by affected mice included tremors, hypersensitivity to stimuli, paralysis of one or more limbs and, if death was delayed, considerable wasting. Virus was widely distributed throughout the body, with the greatest amounts of virus being found in the brain (Table 8). Neutralization tests of the sera of some of the mice surviving after 6 weeks confirmed that over 70 % of mice given $10^{0·8}$ to $10^{2·0}$ p.f.u. of six of the seven viruses had suffered inapparent infections (Table 9). The Italy/66 virus proved less infective for this age group and inapparent infections occurred in only four of fourteen mice given $10^{2·9}$ p.f.u.

Table 8. *Distribution of virus in mice dying 7 days after intraperitoneal inoculation of England/72 virus*

Tissue	Infectivity*
Brain	7·36 (6·3–8·2)
Heart	4·10 (3·3–5·1)
Lungs	3·50 (2·8–4·3)
Alimentary tract	4·45 (1·6–5·8)
Carcase	5·00 (2·6–6·4)

* Log_{10} p.f.u./specimen – geometric mean and range of five mice.

Table 9. *The infectivity of SVD virus strains for 5-day-old mice – subclinical infections*

Virus	Inoculum	Number of subclinical infections†/ Number tested
Italy/66	2·9*	4/14
Hong Kong/71	2·0	17/19
Italy/72	1·3	5/15
England/72	1·5	14/15
Austria/73	1·0	15/15
Poland/73	1·4	7/13
France/73	0·8	7/13

* Log_{10} p.f.u.
† Number of mice with neutralizing antibody titres $> 2{\cdot}0$ (log reciprocal).

DISCUSSION

These comparative studies have shown that, although there were some differences in the ability of the viruses to produce primary vesicles at the sites of inoculation, six of the seven viruses produced diseases which were similar in severity, distribution of secondary lesions, febrile response and virus excretion. The seventh virus, Italy/66, was considerably less virulent for the pig and many pigs inoculated with virus concentrations up to $10^{5{\cdot}0}$ p.f.u. failed to acquire infection (Burrows *et al.* 1973). Neutralization tests of convalescent pig sera confirmed that such differences as existed between strains were of a minor degree. The reproducibility of the neutralization test was not good enough to distinguish each virus on the results of a single test but the mean results of three tests confirmed that the Italy/66, Hong Kong/71 and France/73 viruses differed from each other and from the Italy/72, England/72, Austria/73 and Poland/73 viruses. Unequivocal antigenic differences between these four groups of viruses using the same pools of convalescent pig sera have already been demonstrated by agar gel precipitation tests using purified viral antigens (Brown, Talbot & Burrows, 1973) and by complement fixation tests (A. Arrowsmith, unpublished). Differences between the viruses were also seen in the response of 5-day-old mice to intraperitoneal inoculation. The Italy/72, England/72, Austria/72 and Poland/73 group produced signs of disease in a greater proportion of mice than did the other three viruses. The

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Italy/66 virus could be differentiated from the Hong Kong/71 and France/73 viruses in that much larger amounts of virus were required to initiate inapparent infections.

The appearance of SVD in widely separated countries after intervals of several years indicates that a reservoir of infection probably exists in some part of the world. Persistence of infection in a semi-immune population over a long period may lead to slight changes in the biological and antigenic properties of the virus. Thus, it was not surprising to find slight differences in virulence and antigenic composition between the Italy/66, Hong Kong/71 and European 1972/73 viruses. The close similarity between the Italy/72, England/72, Austria/73 and Poland/73 viruses suggests that these outbreaks may have had a common origin. A direct link between the Austrian and Polish outbreaks of disease was established (Kubin, 1973) but the original source of these outbreaks or links with other outbreaks has not yet been established.

The France/73 virus differs slightly from the other viruses recovered during 1972/73 and it is possible that the disease in France may not be directly related to the other occurrences of disease in Europe. However, the differences between the experimental and natural diseases produced by the viruses prevalent in Europe in 1972/73 were not sufficient to explain the differences in the spread of disease in the various countries and it is concluded that the apparent differences in epizootiology may be due to other factors relating to the husbandry and marketing of pigs or to the attention paid to what on clinical grounds is a relatively mild disease.

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REFERENCES

Brown, F., Talbot, P. & Burrows, R. (1973). Antigenic differences between isolates of swine vesicular disease virus and their relationship to Coxsackie B5 virus. *Nature, London* **245,** 315.

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.

Burrows, R., Mann, J. A. & Goodridge, D. (1974). Swine vesicular disease – virological studies of experimental infections produced by the England/72 virus. *Journal of Hygiene* **72,** 135.

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.

Federer, K. E., Burrows, R. & Brooksby, J. B. (1967). Vesicular stomatitis virus – the relationship between some strains of the Indiana serotype. *Research in Veterinary Science* **8,** 103.

KUBIN, G. (1973). Auftreten der vesikulären Virusseuche der Schweine. *Wiener Tierärztliche Monatsschrift* **60,** 283.

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 enterovirus. *Nature, London* **219,** 1275.

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