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# Swine vesicular disease: attempts to transmit infection to cattle and sheep

By R. BURROWS, J. A. MANN, D. GOODRIDGE and W. G. CHAPMAN
*Animal Virus Research Institute, Pirbright, Woking, Surrey*

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## SUMMARY

Cattle and sheep were housed with infected pigs for 11 days. Small amounts of virus were recovered intermittently from the pharynx, milk and rectal swabs of the cattle, but no evidence of subclinical infection was found. Some indication of virus growth in the sheep was obtained in that large amounts of virus were recovered from the pharyngeal region 4 to 7 days after exposure and six of the eight sheep developed significant titres of neutralizing antibody which were maintained in four animals for at least 6 weeks.

## INTRODUCTION

The host range of swine vesicular disease virus (SVDV) appears to be limited to pigs and infant mice (Nardelli *et al.* 1968) and man (Brown, Talbot & Burrows, 1973). Nardelli *et al.* (1968) failed to produce signs of infection in cattle, donkeys, rabbits, guinea-pigs and hamsters with the Italy/66 strain and Dawe, Forman & Smale (1973) and Dhennin & Dhennin (1973) confirmed that the UK/72 and France/73 strains of virus did not produce lesions following intradermal tongue inoculation of cattle and calves. During the 1972/73 outbreak of swine vesicular disease (SVD), cattle and sheep were in close contact with large numbers of infected pigs on several farms. Although observations in the field (R. S. Hedger, personal communication) indicated that cattle and sheep were unlikely to be of importance in the epizootiology of the disease, experiments were carried out to study the response of these species to a prolonged and intimate exposure to infected pigs.

## MATERIALS AND METHODS

### *Virus*

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

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*Experimental animals*

*Pigs – inoculation and sampling procedures.* Eight Large White pigs (30 to 40 kg) were inoculated in both heels of both fore feet with $10^{5.9}$ p.f.u. of virus at each site. Forty-eight hours after inoculation the pigs were moved into animal rooms containing cattle or sheep and left there for 11 days. Two pigs were placed with each of three cattle and two pigs with eight sheep. Nasal, oral, rectal, preputial or vaginal swabs, blood and pharyngeal/tonsillar samples were taken daily for 12 days from the donor pigs (Burrows, Mann & Goodridge, 1974).

*Cattle.* Two aged Friesian cows, one in late lactation and one in middle lactation, and one 2-year-old cross-bred Devon steer were housed in separate boxes. The cows were milked by hand once or twice daily and samples of pooled fore milk and bulk milk were taken from each cow at each milking session. Oesophageal/pharyngeal samples (Burrows, 1966) and rectal swabs were taken daily. Five days after the removal of the pigs the milking cows were re-exposed to infection by the instillation of $10^{7.0}$ p.f.u. of virus into one quarter of the mammary gland (Burrows *et al.* 1971).

*Sheep.* Eight cross-bred sheep were housed in one room. Rectal swabs and pharyngeal samples (Burrows, 1968) were taken daily from four of the sheep.

*Assay of virus and neutralizing antibody*

Samples were stored, prepared and assayed for virus and neutralizing antibody as described by Burrows *et al.* 1974.

## RESULTS

*Pigs*

All pigs developed primary lesions within 48 hours and secondary lesions within 3 to 4 days. Details of the amounts of virus found in the daily samples collected from these animals have been recorded (Burrows *et al.* 1974). Peak concentrations of virus were found in samples from the third to the fifth day after inoculation. The infectivity declined after the fifth day and relatively few isolations of virus were made from the swabs after the eighth day. However, virus was excreted in the faeces for longer periods. The mean virus content of faeces collected 6 days after inoculation was $10^{4.8}$ p.f.u./g. and $10^{2.8}$ p.f.u./g. in samples collected on the 14th day.

*Cattle*

No clinical evidence of disease was seen. Table 1 lists the amount of virus found in the oesophageal/pharyngeal samples, in rectal swabs and in milk during the period that the cattle were exposed to the infected pigs. These amounts were small in relation to those found in similar samples from infected pigs and the variations in the appearance and amounts of virus were not indicative of virus multiplication in the cattle. Virus inoculated into the mammary gland disappeared rapidly. Approximately $10^{4.0}$ p.f.u./ml. were found in milk collected 6 hr.

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Table 1. *Recovery of virus from cattle housed with infected pigs*

| Days after exposure | Steer KE 50 | | Cow KE 52 | | | Cow KE 53 | | |
|---|---|---|---|---|---|---|---|---|
| | Pharynx | Rectum | Pharynx | Rectum | Milk | Pharynx | Rectum | Milk |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 2·0* | 1·7 | 2·2 | 2·5 | 0 | 0 | 2·3 | 0 |
| 2 | 2·6 | 2·6 | 2·0 | 2·0 | 0·9 | 0 | 1·9 | 0 |
| 3 | 2·4 | 1·2 | 1·7 | 2·5 | 0·8 | 0 | 1·7 | 0 |
| 4 | 2·0 | 1·5 | 2·3 | 3·2 | 0 | 0 | 1·8 | 0 |
| 5 | 0 | 2·4 | 0 | 1·2 | 0 | 0 | 1·9 | 1·0 |
| 6 | 2·0 | 0 | 0 | 1·8 | 0 | 0 | 0 | 0 |
| 7 | 1·7 | 0 | 0 | 1·7 | 0 | 0 | 0 | 0 |
| 8 | 0 | 2·8 | 0 | 2·1 | 0 | 0 | 0 | 1·2 |
| 9 | 2·6 | 1·7 | 0 | 0 | 0 | 0 | 0 | 0·7 |
| 10 | 0 | 2·0 | 0 | 0 | 0 | 0 | 1·2 | 0 |
| 11 | 0 | 1·5 | 0 | 0 | 0 | 0 | 1·2 | 0 |

* $Log_{10}$ p.f.u./sample or swab/ml. milk.
0 = < 0·3 p.f.u./ml. (milk), < 1·7 p.f.u./sample (pharynx), < 1·2 p.f.u./swab (rectum).

after instillation of $10^{7·0}$ p.f.u., $10^{1·5}$ p.f.u./ml. after 24 hr. and $10^{0·9}$ p.f.u./ml. (one cow only) after 48 hr. No virus was recovered in the milk collected 56 and 72 hr. after instillation. Slight increases in the virus-neutralizing activity of sera were detected during the course of the experiment but these increases were not as great as those found in subclinical infections of pigs (Burrows *et al.* 1974).

*Sheep*

No obvious signs of disease attributable to SVDV were seen. Table 2 lists the amounts of virus recovered from each sample taken from four of the eight sheep, the daily geometric mean virus content of these samples and, for comparison, the daily geometric mean amounts of virus found for all samples taken from the two donor pigs. Although the amounts of virus recovered from these pigs decreased from the first day of contact onwards, the amounts of virus recovered from the sheep increased. Maximum concentrations of virus were recovered from the pharyngeal region of three sheep on the fourth day and from one animal on the seventh day. The amounts of virus in rectal swabs varied but the highest mean amount was recorded on the sixth day of contact. Virus was not recovered from the pharynx after 8 days or from rectal swabs after 9 days.

Eleven days after their first exposure to infected pigs, the sheep were moved to a clean room and held there for a further 5 weeks. Samples of fresh faeces were examined on four occasions and virus was recovered from one animal 8 days and from three sheep 15 days after their last contact with the infected pigs (Table 3). The serum neutralizing antibody responses of the individual sheep are listed in Table 4. Six of the eight sheep developed significant antibody titres (> 1·5) and these were maintained in four animals for at least 6 weeks.

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Table 2. *Recovery of virus from sheep housed with infected pigs*

| Days after exposure | KE 42 | | KE 43 | | KE 44 | | KE 45 | | Geometric means | | Donor pigs |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pharynx | Rectum | Pharynx | Rectum | Pharynx | Rectum | Pharynx | Rectum | Pharynx | Rectum | |
| 1 | 2·4* | 1·8 | 3·2 | 1·9 | 2·2 | 2·5 | 2·8 | 2·7 | 2·6 | 2·2 | 5·7† |
| 2 | — | 3·9 | 5·5 | 3·3 | 2·3 | 2·9 | 2·8 | 2·8 | 2·6 | 3·2 | 4·9 |
| 3 | 1·7 | 3·0 | 5·9 | 2·4 | 3·4 | 2·7 | 3·5 | 3·5 | 3·6 | 2·9 | 4·4 |
| 4 | 5·0 | 2·4 | 6·1 | 2·5 | 2·8 | 2·7 | 3·7 | 2·5 | 4·4 | 2·5 | 3·5 |
| 5 | 3·8 | 2·8 | 4·5 | 3·0 | 3·7 | 2·1 | 2·9 | 2·1 | 3·7 | 2·5 | 2·3 |
| 6 | 3·1 | 3·9 | 3·1 | 2·8 | 4·8 | 3·8 | 1·7 | 2·7 | 3·2 | 3·4 | 0·6 |
| 7 | 2·0 | 3·5 | 3·3 | 3·7 | 5·0 | 4·7 | 2·0 | — | 3·1 | 3·0 | 0·5 |
| 8 | — | 1·7 | — | — | 1·7 | — | — | 1·5 | 0·4 | 0·8 | — |
| 9 | — | 1·7 | — | — | — | — | — | — | — | 0·4 | — |
| 10 | — | — | — | — | — | — | — | — | — | — | Not tested |
| 12 | — | — | — | — | — | — | — | — | — | — | — |

* $Log_{10}$ p.f.u./sample (Pharynx) or swab (rectum).

† Geometric mean amounts of virus ($log_{10}$ p.f.u.) recovered from oral, nasal, rectal and preputial swabs and pharyngeal samples.

— = < 1·2 p.f.u./per sample or swab.

Table 3. *The recovery of swine vesicular disease virus from the faeces of* 8 *sheep after their removal from an infected environment*

| Days after last exposure to infected pigs | Number of samples containing virus | Virus content |
|---|---|---|
| 8 | 1/5* | 2·8† |
| 14 | 0/8 | |
| 15 | 3/8 | 1·0, 1·0, 1·8 |
| 22 | 0/8 | |

* Number of samples from which virus was recovered/number of samples collected.
† $Log_{10}$ p.f.u./g.

Table 4. *Neutralizing antibody response of cattle and sheep exposed to infected pigs*

| Species | Identification | Exposure | Days after exposure: 0 | 11 | 22 | 31 | 44 |
|---|---|---|---|---|---|---|---|
| Steer | KD 50 | Contact | — | 1·5* | 1·5 | Experiment discontinued | |
| Cow | KD 52 | Contact and intramammary instillation after 16 days | — | 1·2 | 1·0 | Experiment discontinued | |
| Cow | KD 53 | | — | 1·5 | 1·0 | | |
| Sheep | KD 42 | Contact with daily sampling | — | 2·0 | 1·5 | 1·3 | 1·3 |
| | KD 43 | | — | 2·0 | 1·8 | 1·8 | 2·2 |
| | KD 44 | | — | 1·8 | 1·8 | 1·5 | 1·8 |
| | KD 45 | | — | 3·0 | 2·0 | 3·0 | 2·5 |
| Sheep | KD 46 | Contact only | — | 1·3 | 1·8 | 1·8 | 1·5 |
| | KD 47 | | — | 1·0 | 1·1 | 1·3 | 1·3 |
| | KD 48 | | — | 1·8 | 2·0 | 1·8 | 2·5 |
| | KD 49 | | — | 0·8 | 1·3 | 1·5 | 1·0 |

* Log reciprocal of the serum dilution which neutralized 90 % of test virus.
— = < 0·7.

## DISCUSSION

Although the three cattle acquired considerable amounts of virus from the infected environment, no evidence of active infection was obtained. The individual variations in the frequency and amounts of virus found in the samples collected from the cattle were believed to be due to differences in cleaning procedures adopted in the animal rooms and to differences in behavioural patterns exhibited by each group of animals. No bedding was provided for steer KE 50 and the floor of the room was washed and brushed each morning immediately before examination and sampling. This procedure is likely to produce aerosols of virus present on the floor or in pig faeces and this could explain the consistent pattern of virus recovery from this animal. Straw bedding was provided for the two milking cows and cleaning was restricted to the removal of faeces and soiled straw. Cow KE 53 accepted the pigs and showed little interest in them; no virus was recovered from pharyngeal samples and consequently only small amounts of virus were found in the rectal swabs. Cow KE 52 objected to the presence of the pigs and a corner of the room was fenced to enable the pigs to escape from her attentions. This interest

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in the pigs may be the explanation for the greater amounts of virus found in the pharyngeal samples and the rectal swabs from this cow. Virus was recovered sporadically and in small amounts from the milk of both cattle and this was almost certainly due to contamination during the milking procedures, as no virological or serological evidence of virus growth was obtained following the instillation of virus into the mammary gland.

In contrast to the findings for cattle, evidence was obtained of virus growth in the sheep. The virus content of pharyngeal samples increased over a period of 4 to 7 days, although during this period the amounts of virus excreted by the donor pigs declined. Virus concentrations of $10^{5\cdot0}$ to $10^{6\cdot1}$ p.f.u. were found in samples from three of the four sheep. These amounts were as large as those found in pharyngeal samples taken from contact pigs 2 to 5 days after a similar exposure (Burrows *et al.* 1974).

Significant titres of neutralizing antibody ($> 1\cdot5$) were found in five of the eight sheep within 11 days and these titres were maintained in four of the animals for at least 6 weeks. It had been appreciated in the design of the experiment that repeated sampling from the pharynx might introduce passively acquired virus into the epithelium of the area and so mimic vaccination. Lower antibody titres were found in the group which had not been subjected to pharyngeal sampling but significant titres developed in two animals.

The appearance of small quantities of virus in the faeces of sheep some time after they had been removed from an infected environment was unlikely to have been due to continued virus growth in the animal. No attempt had been made to wash or disinfect the sheep and it is likely that the fleece was heavily contaminated with virus. Self-grooming activities could explain the intermittent appearance of small quantities of virus in the faeces.

These findings confirm that cattle are unlikely to be of importance in the epizootiology of SVD apart from acting as mechanical transporters of virus. The role of sheep, however, is less clear. Although the results indicate that some sheep can acquire subclinical infections when exposed to large amounts of virus for prolonged periods, this situation is unlikely to arise under normal farming conditions. The importance of sheep in the epizootiology of SVD may depend on whether or not they can acquire infection from grazing contaminated pastures, and whether or not infection can spread from sheep to sheep or from sheep to pig under natural conditions.

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