SEROLOGICAL AND PATHOGENICITY STUDIES WITH SOME UNCLASSIFIED PORCINE ADENOVIRUSES

By

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INTRODUCTION

Some of the characteristics of porcine adenovirus types 1 to 3 were described in an earlier paper (Clarke, Sharpe and Derbyshire, 1967), while the adenovirus isolated by Kasza (1966) has subsequently been proposed as porcine adenovirus type 4 (Bibrack, 1969). In the course of studies previously reported (Derbyshire, Clarke and Jessett, 1966; 1969), additional strains of porcine adenovirus were isolated in this laboratory. Eight of these viruses have been compared serologically with types 1 to 4, and they have each been tested for pathogenicity in colostrum-deprived piglets. The results obtained are recorded in this paper.

MATERIALS AND METHODS

Viruses. The porcine adenovirus strains 6618 and A47 were described in a previous paper (Clarke et al., 1967), in which these viruses were proposed as the prototype strains of porcine adenovirus type 2 and 3 respectively. In subsequent papers, however, other workers (Christofinis, Edington, Betts and Prydie, 1972; Anon, 1973), including those involved in the WHO/FAO programme on comparative virology, have referred to strain A47 as type 2 and strain 6618 as type 3. In the hope of avoiding further confusion, we will adopt the latter designations. The clone of the 25R strain of porcine adenovirus type 1 (Clarke et al., 1967) was subsequently found to be contaminated with a parvovirus, and for the present study a fresh clone of this virus was prepared from the original stock of Haig, Clarke and Pereira (1964) by three passages at terminal dilution in pig kidney (PK) cell cultures as described by Clarke et al. (1967). The 8 new strains of porcine adenovirus, designated 81, 82, 100, 6585, A13, 79, 5715 and 6597, all of which were isolated either from faeces or directly from the intestinal tracts of swine in the course of earlier investigations (Derbyshire et al., 1966, 1969), were cloned by the same procedure. Stocks of each cloned virus were prepared in PK cells, and stored at −60 °C. Titrations of the virus stocks were made by infectivity assay in PK cells.

Virus neutralization tests. Antisera were prepared in rabbits (Clarke et al., 1967) and virus neutralization tests were performed by a method based on "procedure 2" of Rowe, Huebner, Hartley, Ward and Parrott (1955) for typing human adenoviruses. When the neutralizing antibody titre against the homologous virus was adequate, cross neutralization tests were conducted in stages, because it was impractical to carry out a complete chequerboard test with all the 12 viruses on the same occasion. The first stage was to test each virus in turn against serial dilutions of all the antisera. Secondly, dilutions of each antiserum in turn were tested against all the viruses.

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Finally, any cross reactions which were demonstrated in the above series of tests were further investigated by fully reciprocal cross neutralization tests with pairs of viruses and their corresponding antisera, each pair being tested in the same cultures on the same occasion. This procedure was intended to minimize variation associated with different batches of cells.

Pathogenicity tests. Each of the 8 unclassified adenoviruses was tested for pathogenicity in hysterotomy-derived, colostrum-deprived (HDCD) piglets, which were obtained as described by Sharpe and Jessett (1967). After delivery, the piglets were housed in strict isolation and fed a diet consisting of commercial ultra high temperature treated cows' milk,* supplemented with a mineral mixture (Young and Underdahl, 1953). Two piglets from the same sow were used for each virus. Piglets from different litters were used for different viruses, except that strains 6585 and A13, and 79 and 5715 were administered to piglets from the same sows. At 7 days of age, the piglets were lightly anaesthetized with ether and each was inoculated in each nostril with 1.0 ml. of infected PK cell culture fluid of the virus under test. Two pairs of piglets, used for controls, were inoculated with fluid from a non-infected PK cell culture. A blood sample was collected from each piglet immediately before inoculation and a second sample was collected when the piglets were killed. The sera were tested for virus neutralizing activity against the homologous virus. One piglet of each pair was killed 6 days and the second 8 days after inoculation, by the i.v. administration of an overdose of sodium pentobarbitoue. Immediately after death, samples of the following tissues were collected aseptically from each piglet: liver, spleen, kidney, pancreas, brain, lung, bronchial and mediastinal lymph node, tonsil, parotid salivary gland, mesenteric lymph node, jejunum, ileum, colon and rectum. Each sample was processed for virus isolation on PK cell cultures, as described by Sharpe and Jessett (1967), with a single blind passage in PK cells in the case of negative cultures. Viruses which were isolated were identified as the homologous adenovirus by virus neutralization tests (Sharpe and Jessett, 1967) with rabbit antisera.

RESULTS

Virus Neutralization Tests

In the first stage of the cross neutralization tests, in which each virus was tested in turn against all the antisera, numerous cross reactions occurred. However, many of these reactions were produced only by high serum concentrations containing greater than 20 units of the homologous antibody. Significant neutralization, occurring with 20 units or less of homologous antibody, was invariably unidirectional. In the second series of tests, in which each serum was tested in turn against the panel of 12 viruses, some of these one way cross reactions failed to occur, and more were eliminated when fully reciprocal cross neutralization tests were performed with pairs of viruses and their corresponding antisera in the same batches of cell cultures. The cross neutralization test results are summarized in Table 1 in which representative homologous titres are given for each antiserum, together with those cross neutralization reactions which were detected in fully reciprocal tests. These results confirmed that 25R, A47, 6618 and F618, the prototype strains of porcine adenovirus types 1 to 4, were antigenically distinct from each other, the only cross reactivity being unidirectional neutralization of 6618 virus by 25R antiserum, at low titre. None of the 8 new adenovirus strains reacted with F618, but reactions did occur between 7 of these viruses and one or other of 25R, A47 and 6618. The 25R antiserum neutralized 82 virus, but there was no reciprocal neutralization, and

* Longlife milk—Express Dairies.
TABLE 1

RECIPROCAL CROSS NEUTRALIZATION TESTS WITH PORCINE ADENOVIRUSES

<table>
<thead>
<tr>
<th>Porcine adenovirus strain</th>
<th>25R</th>
<th>A47</th>
<th>6618</th>
<th>F618</th>
<th>81</th>
<th>82</th>
<th>100</th>
<th>6585</th>
<th>A13</th>
<th>79</th>
<th>5715</th>
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<td>25R</td>
<td>226</td>
<td>226</td>
<td></td>
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</tr>
<tr>
<td>A47</td>
<td>14</td>
<td>537</td>
<td>57</td>
<td>226</td>
<td>452</td>
<td>113</td>
<td>452</td>
<td>1280</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>81</td>
<td>160</td>
<td>2260</td>
<td>640</td>
<td>638</td>
<td>160</td>
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<tr>
<td>82</td>
<td>57</td>
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<td>100</td>
<td>57</td>
<td>85</td>
<td>113</td>
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<td>A13</td>
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<td>14</td>
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<tr>
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<td>56</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>638</td>
</tr>
</tbody>
</table>

* Expressed as the reciprocal of the serum dilution which gave 50 per cent. neutralization.

82 showed no reactivity with A47 or 6618. The 25R virus was neutralized by 6597 antiserum, and A47 virus by 79 antiserum. Of the 4 prototype viruses, greatest reactivity was found with 6618, which was neutralized by the antiserum prepared against strains 81, 6586, A13, 79, 5715 and 6597. It will be noted from Table 1 that all these reactions were one way crosses. Strain 100 showed no reactivity with 25R, A47, 6618 or F618. In addition, the 6597 antiserum neutralized each of the 8 new viruses except for 81 and A13, and 82 virus was neutralized by antiserum prepared against A13, 79, 5715 and 6597. Again, all of these reactions were unidirectional.

Pathogenicity Tests

The infectivity titres of the virus stocks used for piglet inoculation are given in Table 2. No virus neutralizing antibodies were detected in any of the pre-inoculation serum samples. Low levels of neutralizing antibodies against the homologous viruses were found in some of the post-inoculation sera (Table 2), but many of these samples were negative, particularly those collected 6 days after infection. The only clinical abnormality detected was mild diarrhoea in the piglets inoculated with strain 82 and some gelatinous oedema of the mesocolon was found at post mortem examination. Slight oedema of the mesocolon was also seen in the piglets examined 8 days after infection with strain 81, 6 days with 6585 and 6 days with 6597. No other gross lesions were observed at necropsy. The control piglets were clinically normal and contained no gross lesions. Isolations of virus were made only from the tonsil, ileum, colon and rectum, and these results are recorded in Table 2. Each virus which was isolated from these tissues was identified in neutralization tests as the strain which was inoculated. No other viruses were isolated from these piglets, the other tissues examined failed to yield virus and the control piglets were virologically negative.
### TABLE 2

**TITRES OF VIRUS USED FOR INOCULATION, SEROLOGICAL RESPONSE TO INFECTION AND ISOLATION OF HOMOLOGOUS VIRUS IN PIGLETS INOCULATED WITH EIGHT PORCINE ADENOVIRUSES**

<table>
<thead>
<tr>
<th>Adenovirus strain no.</th>
<th>Litter no.</th>
<th>Titre of inoculum (Log TCD&lt;sub&gt;50&lt;/sub&gt;/ml.)</th>
<th>Neutralizing antibody titre of post-inoculation sera</th>
<th>Isolation* of virus at both 6 and 8 days after-inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>1</td>
<td>4.9</td>
<td>&gt;5</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>82</td>
<td>2</td>
<td>3.8</td>
<td>&gt;5</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>4.7</td>
<td>&lt;5</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>6585</td>
<td>4</td>
<td>4.6</td>
<td>26</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>A13</td>
<td>4</td>
<td>4.8</td>
<td>&gt;5</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>579</td>
<td>5</td>
<td>4.6</td>
<td>&gt;5</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>6597</td>
<td>5</td>
<td>5.3</td>
<td>&gt;5</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>3.9</td>
<td>&lt;5</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Piglets</td>
<td></td>
<td>0.0</td>
<td>&lt;5</td>
<td>--- --- --- ---</td>
</tr>
</tbody>
</table>

* + denotes isolation of homologous adenovirus.

--- no virus isolated.

† Virus was isolated 8 days but not at 6 days after-inoculation.

### DISCUSSION

The results of the cross neutralization tests confirmed that the 4 established serotypes of porcine adenovirus are antigenically distinct. Low titre neutralization of 6618 virus by 25R antiserum had been reported previously (Clarke et al., 1967). No relationship was found between any of the 11 porcine adenoviruses which were isolated in England and the porcine adenovirus type 4 (F618) which was isolated in the United States (Kasza, 1966). Conversely, Bibrack (1969) found that the adenoviruses which she isolated in Bavaria were mainly type 4 strains. However, there is serological evidence for the occurrence of porcine adenovirus type 4 in England (Kasza, Hodges, Betts and Trexler, 1969).

All the unclassified porcine adenoviruses which we tested serologically showed some crossing with either porcine adenovirus types 1 to 3, or among themselves. The antiserum prepared against strain 6597 was particularly broadly reactive, as were the 6618 and 82 viruses, although the cross reactions were invariably unidirectional. The significance of one way cross reactivity of this kind is not clear at the present time. The possibility exists that the broadly reactive 6597 virus might represent a prime strain of the kind discussed by the Committee on the Enteroviruses (1957). Further antigenic analysis is required to clarify the antigenic relationships among these porcine adenoviruses. While the evidence which we have presented does not justify the inclusion of these strains within the existing serotypes, neither does it justify the establishment of additional serotypes, except perhaps for strain 100, which showed no cross reactivity in either direction with types 1 to 4.

In the pathogenicity tests which we conducted in HDCD piglets, infection was restricted to the alimentary tract, as judged by our virus isolation results. This finding corresponded fairly well with that of Sharpe and Jessett (1967),...
who recovered porcine adenovirus types 2 and 3 from the alimentary tracts of experimentally infected HDCD piglets, but those workers occasionally isolated these viruses from the respiratory tract as well. On the other hand, Brack, Bernhardt, Liess, Bahr, Rohde and Amtsberg (1969) were able to isolate 25R, 6618 and an unclassified adenovirus from a variety of organs of gnotobiotic piglets which were inoculated with these viruses, although these workers used the intramuscular route of inoculation in addition to oral dosing of the piglets. Similarly, in gnotobiotic piglets which were inoculated by the respiratory route with porcine adenovirus type 4, Kasza et al. (1969) were able to reisolate the virus from lung, kidney and brain tissue, in addition to the intestinal tract.

We found no consistent clinical signs or gross lesions in our experimentally infected piglets. The occurrence of diarrhoea in piglets infected with strain 82 and the occasional presence of oedema of the mesocolon at autopsy require confirmation in experiments on a larger scale. The occurrence of diarrhoea following experimental infection of piglets with porcine adenoviruses has not been regularly reported in the literature, although Harkness, Chapman and Darbyshire (1971) referred to clinical enteritis in 1-day-old colostrum-deprived piglets inoculated with 25R virus, and histological lesions have been described in the intestines of pig foetuses inoculated with 25R (Sharpe, 1967) and of an HDCD piglet exposed to 6618 virus (Jericho, Derbyshire and Jones, 1971). Evidence of pneumonia was not apparent in our piglets, although macroscopic consolidation of the lung was a consistent finding in piglets experimentally infected with porcine adenovirus type 4 (Shadduck, Koestner and Kasza, 1967; Kasza et al., 1969; Smith, Betts, Watt and Hayward, 1973). Histological changes in the central nervous system and in the kidneys of gnotobiotic piglets infected with porcine adenovirus type 4 were described by Edington, Kasza and Christofinis (1972), and histological lesions were also described in these sites by Brack et al. (1969). We did not examine these tissues histologically, but we consistently failed to isolate adenovirus from brain and kidney, and from the respiratory tract.

Little evidence of a serological response to infection was found in our piglets, but the latter were killed either 6 or 8 days after infection, which allowed little time for the development of circulating antibodies. Sharpe and Jessett (1967) found that neutralizing antibodies against porcine adenovirus types 2 and 3 did not usually appear in the serum until longer than 8 days after experimental infection of HDCD piglets.

Although adenoviruses can be fairly regularly isolated from the gastro-intestinal tracts or faeces of weaned piglets (Derbyshire et al., 1966, 1969), it appears that many of these viruses may be of relatively low pathogenicity. Indeed, the published evidence at the present time suggests that, among the porcine adenoviruses, only type 4 is a clear-cut pathogen.

**SUMMARY**

Eight unclassified porcine adenoviruses were compared serologically with each other and with prototype strains of porcine adenovirus types 1 to 4 by means of reciprocal virus neutralization tests. There was no relationship
between any of the unclassified viruses and porcine adenovirus type 4, but unidirectional cross reactions did occur between 7 of the new viruses and one or other of porcine adenovirus types 1 to 3. In addition, cross reactions occurred among all the unclassified viruses, but all were unidirectional. Each of the unclassified viruses was inoculated intranasally into 2 hysterotomy-derived, colostrum-deprived, 7-days-old piglets. One piglet of each pair was killed 6 days, and the other 8 days, after inoculation. Diarrhoea occurred in one pair of piglets and oedema of the mesocolon was seen at post mortem examination of 5 piglets. Virus was isolated from the tonsil, ileum, colon and rectum of most of the inoculated piglets, but not from the liver, spleen, kidney, pancreas, brain, lung, bronchial or mediastinal lymph node, parotid salivary gland, mesenteric lymph or jejunum.

ACKNOWLEDGMENTS

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