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The isolation of parainfluenza 4 subtypes A and B in England and serological studies of their prevalence

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The term parainfluenza viruses was proposed for certain members of the myxovirus group which resembled the influenza viruses but which were not related to them antigenically (Andrewes, Bang, Chanock & Zhdanov, 1959). Included in this group were parainfluenza virus types 1, 2 and 3. Parainfluenza 2 (Croup associated or CA virus) was first described by Chanock in 1956, and parainfluenza 1 and 3 (Haemadsorption types 2 and 1 or HA-2 and HA-1) by Chanock and his colleagues in 1958.

In 1960 Johnson, Chanock, Cook & Huebner described a new haemadsorption virus which they called M-25. This strain was isolated from a college student and also from 30 infants in an orphanage nursery and was shown to be antigenically distinct from parainfluenza 1, 2 and 3 viruses. They proposed that M-25 should be the prototype strain for parainfluenza type 4.

In 1964 Canchola and his colleagues described a new strain which could not be identified with an antiserum prepared against the prototype parainfluenza type 4 (M-25) virus. They had isolated 25 strains but only two of these resembled M-25. The remaining 23 strains had a common complement fixing antigen but could be differentiated in neutralization tests. The two groups of strains were called parainfluenza 4 subtypes A and B respectively. For the first time also these workers were able to report an association of type 4 virus with respiratory disease in children.

In 1966 Zilisteanu and his colleagues reported the isolation of eight further strains of parainfluenza 4 subtype A. These were obtained from children in a nursery in Bucharest. Following an outbreak of measles, 12 of the children had mild respiratory signs, nasal obstruction, nasal catarrh, tracheitis and pharyngitis, but were afebrile and well, apart from these manifestations.

From these reports it appears that isolation of parainfluenza 4 virus is difficult because of the slow growth in cell culture and weak haemadsorption pattern and that infection with these strains produces a very mild respiratory illness mainly in young children.

Since 1962 in this laboratory all haemadsorbing viruses which could not be immediately identified have been stored at $-70°$ C. For the most part these strains came from other laboratories situated in different parts of the country.

This report is based on: (1) the identification of 18 strains of parainfluenza 4 virus, 15 being subtype A and 3 subtype B. (2) the clinical information available from these cases. (3) a serological survey to assess the prevalence of neutralizing antibody and these viruses in England.

MATERIALS AND METHODS

Prototype viruses

Prototype strains of parainfluenza 4 subtypes A and B were received from the National Institutes of Health, Bethesda, and were passaged in human embryo kidney and primary rhesus monkey kidney cultures. Customarily titres of 10^5 per 0·1 ml. were obtained.

Newly isolated strains

Only one of the parainfluenza 4 strains described here was isolated in this laboratory. It came from pooled nose and throat swab material of a child in a special respiratory survey. Seventeen strains were submitted for further study as unidentified haemadsorbing viruses in rhesus monkey kidney cell cultures. They were originally unidentifiable with the antisera then available, and were stored at −70° C. for later investigation.

All strains were passaged in primary rhesus monkey kidney cells maintained in Eagle's medium without serum. They were identified as parainfluenza 4 viruses by neutralization with antisera prepared against the prototype strains.

Preparation of specific immune sera

The sera were prepared in adult guinea-pigs which were bled before inoculation. An average of four guinea-pigs was used for each strain. Under light ether anaesthesia 0·2 ml. of virus suspension was inoculated intranasally. Ten days later a further 1 ml. of suspension was inoculated intraperitoneally and after a further 10 days the animals were bled out. Pre and post inoculation sera were tested simultaneously for antibody to the known human myxoviruses by cross neutralization tests. The titre of each serum to parainfluenza 4 A or B respectively was calculated.

Infectivity titrations

Serial tenfold dilutions of the virus suspensions were made in Earle's balanced salt solution (BSS) and 0·1 ml. of each dilution was inoculated into each of two tubes of primary rhesus monkey kidney cells. The cultures were rolled at 33° C. for 6 days and titres determined by the haemadsorption technique.

Identification of strains

The guinea-pig antisera were inactivated at 56° C. for 30 min. Doubling dilutions of each serum were then made in Earle's BSS and an equal volume of serum dilution was mixed with an equal volume of virus suspension calculated to contain 100 TCD_{50}/0·1 ml. Serum-virus mixtures were held at room temperature for 1 hr. and then 0·2 ml. of the mixture was inoculated into duplicate tubes of primary rhesus monkey kidney cell cultures. The tubes were rolled at 33° C. for 6 days and titres determined by the haemadsorption technique.

Neutralization tests on selected sera

Human sera from several sources were obtained as follows: (a) Sera from the London area submitted primarily for antistreptolysin (ASO) estimation. (b) Sera

from all age groups received from the Public Health Laboratory, Liverpool. (c) Sera from infants and young children submitted for rubella and cytomegalovirus studies.

Sera inactivated at 56° C. for 30 min. were screened for neutralizing antibody to parainfluenza 4 subtypes A and B at a dilution of 1/10.

In addition antibody titrations were done on paired sera from one of the patients from whom parainfluenza 4 subtype A was isolated.

RESULTS

Eighteen strains of parainfluenza virus type 4 have been identified; 15 resemble the prototype M-25 virus and are referred to as subtype A and three are strains of subtype B.

Table 1. *Strains of haemadsorbing virus received for identification*

Strain no.	From	Age	Admitted to hospital	Diagnosis
1/62	Manchester	12/12	Yes	Febrile cold
2/62	Manchester	3	Yes	NK
3/63	Bristol	18/12	Yes	Bronchitis
4/64	Prague	NK	NK	NK
5/64	Bristol	4	NK	Bronchitis
6/64	Oxford	21	No	URTI
7/64*	Stafford	7·6/12	No	Laryngitis
8/64	Bristol	3·4/12	Yes	Bronchopneumonia
9/65	Bristol	10/12	Yes	Bronchitis
10/65*	Manchester	4/12	Yes	Febrile convulsions Pleural effusion
11/67†	Manchester	12/12	NK	Rubella
12/67†	Manchester	5	No	Infectious erythema
13/67	Manchester	5	No	Contact of 12/67
14/67†	London	10/12	Yes	Bronchopneumonia Roseola infantum
15/67	London	2	No	Bronchitis
16/67	Leicester	10/12	NK	Febrile convulsions Tonsillitis
17/68*	London	5	No	Bronchitis
18/68	Oxford	3/52	Yes	Convulsions

* Parainfluenza 4 subtype *B* † Rash reported NK = not known

As shown in Table 1, these strains came from six different laboratories in England. The first strain to be identified in this country was from Czechoslovakia and was isolated in 1964. However, the first strain to be isolated in England was in February 1962 in Manchester.

Fifteen of the 17 English strains came from children under 6 years and nine were from children under 3 years. Clinical information was not available in all instances but Table 1 shows that eight out of 14 children were sufficiently ill to be admitted to hospital and nine out of 14 children had an infection of the lower respiratory tract, bronchitis or bronchopneumonia.

Strains 6, 7, 8 and 16 were from cases with pharyngitis and marked cervical

lymphadenopathy and strains 10, 16 and 18 from children with convulsions. One interesting clinical feature was that three strains, 11, 12 and 14 were isolated from children with rashes. They were diagnosed as having rubella, infectious erythema and roseola infantum respectively.

Paired sera were available from the patient from whom strain 6/64 was isolated. These sera showed a rise in antibody titre to parainfluenza 4 subtype A from < 10 to 80.

Four hundred sera were screened for neutralizing antibody to parainfluenza 4 viruses subtypes A and B and the results are shown in Tables 2 and 3.

Table 2. *Distribution of neutralizing antibody to parainfluenza* 4 *subtype A by age in* 399 *selected sera*

Age in years	No. tested	% with antibody
0–8/52	32	84
9/52–2	77	9
3–5	51	57
6–10	73	82
11–25	116	84
26–50	37	95
51–80	13	92

Table 3. *Distribution of neutralizing antibody to parainfluenza* 4 *subtype B by age in* 372 *selected sera*

Age in years	No. tested	% with antibody
0–8/52	29	65
9/52–2	75	7
3–5	50	52
6–10	74	65
11–25	94	64
26–50	37	76
51–80	13	70

From the distribution of antibody it would appear that, as with the other parainfluenza viruses, infection with both subtypes A and B is common in the pre-school child particularly in the age group 3–5 years, 57 % of the population having antibody to subtype A and 52 % to subtype B by the age of 5 years. After this age the distribution of antibody to type A and B varies somewhat with over 80 % of primary school children and over 90 % of young adults having antibody to type A, whereas with type B only 65–70 % of the adult population had antibody present.

DISCUSSION

Although strains of parainfluenza 4 virus have been found in England since 1962 they are infrequently isolated, a reason for this being the difficulty with which they are recovered from natural infections (Canchola *et al.* 1964). They require a

long incubation period for growth and on primary isolation little if any cytopathic change is seen. On passage these strains induce a distinctive cytopathic effect consisting of a granular rounding of cells, loss of cell outline, vacuolation of cells and in some cases a marked syncytial formation. Haemadsorption will often be demonstrable only at room temperature or 37° C, and because strains can best be recognized by haemadsorption some may be missed if the haemadsorption test is performed only at 4° C. The difficulties of virus recovery became apparent in a special respiratory survey by this laboratory when strains of parainfluenza 4 virus were specifically sought in a large children's home, where all age groups were represented and both severe and minor respiratory disease seen, but only one strain was recovered in 3 years from the 450 nose and throat specimens examined. It is therefore possible that other factors such as the presence of antibody or inhibitors may increase the difficulties of isolation of these viruses.

To see whether or not infection with parainfluenza 4 viruses really is infrequent a serological survey was done. This clearly established that antibody to parainfluenza 4 viruses was acquired early in life by a substantial proportion of the population.

Previous reports from America and Rumania suggested that infection with parainfluenza 4 viruses mainly causes a mild respiratory illness. In this country 9 out of 14 children from whom these viruses were isolated had involvement of the lower respiratory tract. Although the considerable immunity shown points to minor infections for the most part it seems probable that as with parainfluenza viruses types 1, 2 and 3, type 4 viruses may cause a range of illnesses from febrile colds to the more serious bronchitis and bronchopneumonia.

From the information presented parainfluenza 4 viruses could be important pathogens of respiratory disease in young children.

SUMMARY

The identification of 18 strains of haemadsorbing viruses as parainfluenza type 4 viruses subtypes A and B is reported, as is a serological survey to show the distribution of antibody to these viruses in various age groups. The results suggest that although isolation of the virus is infrequent, infection in early childhood is common and the viruses may be important respiratory pathogens.

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