Mem. Inst. Oswaldo Cruz, Rio de Janeiro,
Vol. 82 (1) :133-135, jan./mar. 1987

RESEARCH NOTE

STUDIES ON TYPE 1 POLIOVIRUS ISOLATED IN BRAZIL BY ELECTROPHORESIS AND MONOCLONAL ANALYSIS

EDSON E. DA SILVA, HERMANN G. SCHATZMAYR, MITIKO FUJITA & HELIO GELLI PEREIRA

Instituto Oswaldo Cruz, Departamento de Virologia, Centro Nacional de Referências para Enteroviroses, Caixa Postal 926, 20001, Rio de Janeiro, RJ, Brasil

In spite of the drastic decrease of paralytic poliomyelitis infections in Brazil following nationwide campaigns of vaccination carried out since 1980, the incidence of this disease still remain high, mainly in the northeastern region of the country as described by Risi (1984, Rev. infect. Dis., 6: 5400-5403). Even in developed countries specially where oral vaccines are used, a low level of cases appear every year as observed by Melnick (1982, Medical Virology, Elsevier Biomedical, 262-299).

The persistence of the disease in many countries, brought new interest to the study of poliomyelitis. This interest is due mainly to the incomplete immunity of population reached by the oral vaccines especially in tropical areas and to the report of clinical cases with vaccinal association as described by Schatzmayr (1985, Adel. Microbiol. Enf. Infecc., 4: 39-55). This last aspect is directly related to the high rate of viral mutation during its replication in the intestinal tract. For the above reasons we believe that the virus strains isolated from epidemiologically relevant cases should be throughly characterized.

Field isolates of poliovirus included in this study are listed in Table I. All were isolated from stools of patients with clinical symptoms of poliomyelitis. The strains were sent from different parts of the country for characterization at our National Reference Center for Enteroviruses. After isolation the viruses were cultivated for not more than three passages in GMK_2 cells. P1 Mahoney (reference strain) was supplied by the Institut Merieux (Lyon, France) and did not show genetic variation when analyzed by RNA fingerprinting (not shown). Poliovirus type 1 vaccine strain was supplied by the National Institute of Health (Japan). Virus titers were assayed by the microtiter method in GMK_2 cells.

Radiolabeled cytoplasmic extracts were obtained in the following way: monolayer cultures of GMK_2 cells in 35mm petridishes were infected with 150-200 viral particles/cell and allowed to adsorb for 30 min. at 35°C. The inoculum was withdrawn and the cells maintained for 3 hrs. in 1,5ml Dulbecco's MEM, lacking methionine. After this period, the medium was replaced by 0,5ml of the same medium but containing 10uCi [^{35}s] methionine (supplied by Dr. Moacyr A. Rebello of the Instituto de Biofísica – Universidade Federal do Rio de Janeiro). The period of pulse was 3 hrs. after which the labeling medium was removed and the cells solubilized in 250ul of a buffer consisting of 10mM tris-HCl pH 7,2, 100mM NaCl; 2mM EDTA; 1% NP40; 0,5mM Phenylmethyl sulfonyl fluoride. All the experiments were conducted at 35°C.

Electrophoresis was performed as described by Laemmli (1970, Nature, 227:680-685) slightly modified as follows: separating gels contained 12% acrylamide, 0.3% bys-acrilamide and the stacking gel contained 4% acrylamide 0.1% bys-acrylamide. Both stacking and separation gels contained 0.1% SDS and 0.5M urea. The tris-glycine upper reservoir contained 0,5M urea. Slab gels were 1,0mm thick, 16cm wide and 14cm high. Samples were boiled with equal parts of sample-Buffer (10M urea; 10% 2-mercaptoethanol; 0.3% SDS) and electrophoresed at 150v for 5hrs. at room temperature. Gels were fixed in 10% trichloroacetic acid (w/v) 10% acetic acid and 30% methanol for 30 min, stained in 0.06% coomassie blue, 10% acetic acid and 30% methanol and destained in this same solution without coomasssie blue. The gels were stained to visualize the structural proteins of the purified Mahoney strain which was not labeled. After drying, gels were exposed to Sakura x-ray film for 4 days.

The monoclonal antibodies used in this work (kindly supplied by Dr. F. Horaud of Institute Pasteur, Paris) were designated Io which reconizes only wild type polio 1 and lo which recognizes vaccine-related strains of poliovirus type 1. All the strains under study were tested against the two classes of antibodies by neutralization methods as described by Crainic et al. (1982, Develop. biol. Standard, 50:229-234) and classified as Sabin-like or non Sabin-like (Table I).

Work supported by PADCT/FINEP.
Received September 11, 1986.
Accepted December 29, 1986.

TABLE I
Type I Poliovirus isolates

Isolate	Year of isolation	Place of isolation	Reaction by monoclonal antibodies
14165	1980	Rio de Janeiro	SL
14485	1980	Rio de Janeiro	SL
14548	1980	Rio de Janeiro	SL
3827	1981	São Paulo	NSL
18058	1981	Pernambuco	NSL
18062	1981	Pernambuco	NSL
20294	1981	Pernambuco	NSL
20300	1982	Pernambuco	NSL
21517	1982	Pernambuco	NSL
25232	1984	Pernambuco	NSL
25236	1984	Sergipe	NSL
25243	1984	Alagoas	NSL
25245	1984	Rio Grande do Norte	SL
25815	1985	Pernambuco	NSL
25817	1985	Rio Grande do Norte	NSL
25820	1985	Pernambuco	NSL
26072	1985	Bahia	NSL

SL – Sabin-like; NSL – Non Sabin-like.

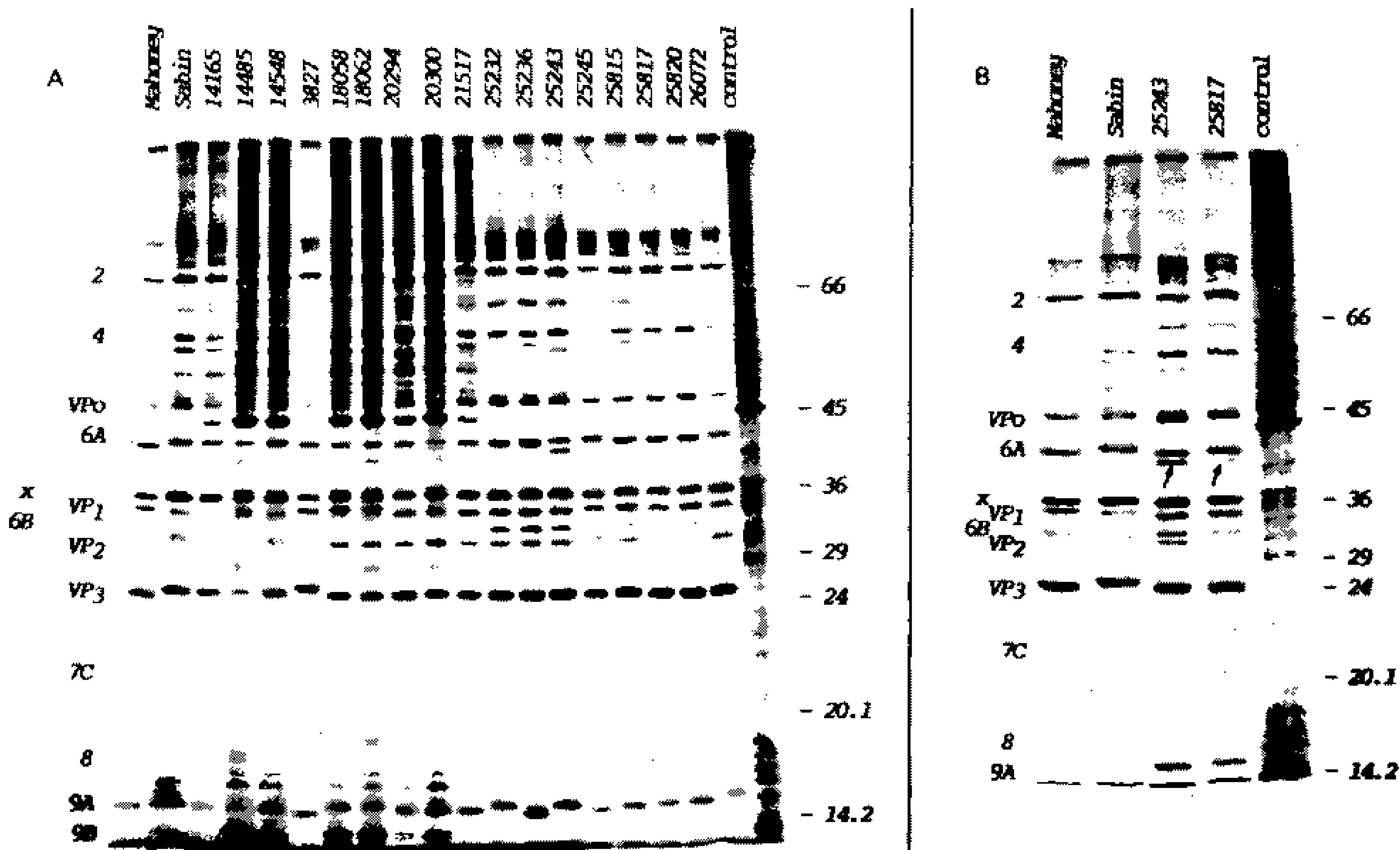

Fig. 1 A – Autoradiogram of an SDS-PAGE of [35s] methionine-labeled proteins of the seventeen isolates of type 1 poliovirus. B – Autoradiogram of an SDS-PAGE of [35s] methionine-labeled **proteins of Mahoney strain,** Sabin strain, 25243, 25817 and uninfected cells (control). Arrows indicate the unusual polypeptide found. Markers sizes are shown in kilodaltons (KD). Nomenclature of polioviral proteins follows that of Nottay et al. (1981).

As it can be seen in Fig. 1, the electrophoretic migration of VP1 was slightly slower in strains 3827, 18058 and 18062. VP2 showed an electrophoretic pattern relatively constant among the wild strains. However, the lower mobility of VP2 and its vicinity to the polypeptide 6B was the main characteristic which distinguished among wild strains and the Sabin-related strains (14165, 14485, 14548).

Strain 25245 (a Sabin-like strain as classified by monoclonal antibodies) displayed an intermediate electrophoretic patterns with respect to VP_2. This findings has been considerad as a possible reversion to virulence from an originally attenuated vaccine-strain.

With regard to VP_3, strain 3827 shows a slower migration pattern when compared to the other strains.

A high correlation was found between the results of monoclonal analysis and the electrophoretic pattern in the determination of the origin (vaccine or non vaccine) of the isolates.

Among the non-structural polypeptides, differences were also noted mainly in polypeptide 2 but not in its cleavage product 4. Some differences were also noted in polypeptide 9A.

The most striking feature among all viruses studied was shown by strains 25243 and 25817 that displayed an extra polypeptide with MW around 40.000, localized between 6A and x (note the arrows) and not found in the uninfected cells nor in Mahoney or Sabin reference strains (Fig. 1B). At the moment the meaning of this finding is not understood. It is possible that with the use of more specific and sensitive techniques (fingerprinting analysis are under way), this matter will be elucidated.

In spite of being a simple method, the SDS-PAGE was sensitive enough to demonstrate close relationship among virus strains and clearly distinguished the origin (vaccine or non vaccine) of field isolates of poliovirus, as has been shown previously by Minor (1980, J. Virol, 34: 73-84) and Nottay et al. (1981, Virology, 108:405-423).

The present results show heterogeneity among poliovirus strains isolated on Brazil. This can be partially explained by (i) the wide dissemination of vaccinal viruses in the community, where in the last five years in a single day each year more than 20 million children have been vaccinated during the two national vaccination days (ii) the selective pressure of antibodies and (iii) the high rate of mutation of the poliovirus during its replication in the intestinal tract.

Acknowledgements: We would like to thank Dr. M. V. Tedeschi for suggestions and critical reading of the manuscripts and also José da Costa Farias Filho and Alexandre Madi Fialho for technical assistance.