معمل بحوث تلوث المياه ـ الدقي ـ الجيزة
رئيس المعمل د٠/ فاطمة الجوهري

انتشار الفيروسات في مياه النيل الطبيعية خلال الفترة من ١٩٧٥ ـ ١٩٨١

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تم خلال فترة الدراسة الممتدة من ١٩٧٥ الى ١٩٨١ جمع ١٦٤ عينة على امتداد مجرى نهر النيل من أسوان ـ أسيوط ـ القاهرة الكبرى وفرعي دمياط ورشيد ٠

أجري فحص العينات للكشف عن وجود الفيروسات بها وذلك باستخدام أنواع مختلفة من الانسجة الحية ، وقد أمكن عزل وتصنيف خمسة مجموعات مختلفة من الفيروسات هي فيروس شلل الأطفال ، الايكو ، الككساكي ، الانفلونزا ، الهيربس وذلك من ٣٥ عينة (٣ر٢١٪) ٠

وقد وجد أن مجموعة الفيروسات المعوية تمثل الغالبية العظمى بين المجموعات المعزولة حيث وصلت نسبتها الى ٧ر٦٥٪ ٠

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PREVAILANCE OF VIRUSES IN NATURAL NILE WATER (1975-1981)

(With 2 Tables & 2 Figs.)

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SUMMARY

A total of 164 samples were collected along the River Nile during the period from 1975 to 1981. The samples were examined for the presence of cytopathogenic active agents in different tissue culture cell lines. Of these samples 35 (21.3%) were found the harbour 5 different viruses. These were identified by the neutralization test in tissue culture cells. The identified agents were Polio 3 (one isolate), Echo (13 isolates), Coxsackie B (9 isolates), Influenza (8 isolates) and Herpes simplex (4 isolates).

The enteric viruses group, constituted the most prevalent part of the isolates (65.7%). Other viruses as Influenza (22.8%) and Herpes simplex (11.5%) were occasionally detected.

INTRODUCTION

The problem of viruses in water is directly related to the technical deficits of reliable and low costing methods for the recovery of viruses that occur in low multiplicities in these sources (HILL, et al. 1971 & 1972; GELDEREICH and KENNEDY, 1978 and SPROUL, 1983). However, WALLIS and MELNICK (1967) presented the membrane adsorption technique as a promising method for virus recovery from various types of waters.

Several investigators reportd that the membrane adsorption and elution technique may hold greatest potential for satisfying the basic requirement of a method for recovering low multiplicities of viruses from large volume of water (RAO and LABZOFFSKY, 1969 and MOOR, et al. 1970).

Foods including milk, animal feeds, in addition to polluted waters have been implicated epidemiologically with several outbreaks of viral diseases (AYCOCK, 1927; LIPARI, 1951; BERG, 1964; BACKER, 1966; CLIVER, 1967; AKIN, 1981 and SPROUL, 1983). Even more, there are many viruses which can spread by a water supply but yet not recognized by the available epidemiological tools.

After High Dam construction, the regime of River Nile hydrology was changed. Unfortunately, there is no available data on the distribution of viruses in Nile water before or after water reservation. The present work was carried out to show the distribution of enteric viruses in River Nile water.

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MATERIAL and METHODS

1- Collection of samples and concentration procedure:

Sub-surface samples of 2 litres volume were collected in sterile botles and refrigerated in an ice box during the collection trip. Within the same day of collection, samples were concentrated by using WALLIS and MELNICK procedure (1967). Water sample was adjusted to pH 4.5-5 by adding 1:1 H Cl, and filtered through 0.45 u cellulose nitrate membrane filters, (Sartorius Membrane filter GMBH, Gottingen, West Germany, Cat. No. 11406) by negative pressure. The adsorped viral agents were eluted off by passing 10ml 3x nutrient broth (pH 9), through each membrane in two portions. The eluate of each sample was aseptically adjusted to pH 7.0-7.5 by adding 1 N HCl and mixed well. Penicillin (100 IU) and streptomycin (50 mg) per 1ml eluate were added with thorough mixing. Concentrated samples were packed in crushed ice (1-2 days) according to the location and transported to the Water Poll. Cont. Laboratory of the National Research Centre, where they were frozen at -20°C till examined.

2- Cell cultures:

A continuous monkey kidney cell line, designed as MS, grown in MEM growth medium, was supplied from the Virology Section, Serum and Vaccine Institute, El-Agoza, Cairo, was used for virus isolation. In few occasions, primary chicken embryo fibroblast cells grown in Hank's balanced salt solution (HBBS) fortified with lactalbumine hydrolysate (0.5%), 5% (v/v) calf serum and $NaHCO_3$. The latter tissue was prepared in Water Pollution Control Laboratory, NRC as directed by SOKKER, et al. (1965) and EL-HAWAARY (1977).

3- Virus assay:

All assays were performed in tubes of the usable monolayer tissue (3 tubes per sample). Each tube received 0.2ml of the concentrated sample and incubated at 37°C for 30 min. as a period of contact between the suspected viral agents and tissue cells. After the contact time, the inoculum was discarded, then tubes received the maintenance medium as 1ml/tube from MEM or Hank's lactalbumin, without calf serum and incubated at 37°C for 7-10 days. During this period tubes were examined daily for the appearance of cytopathogenic effects (CPE) compared with the non-inoculated control tubes of the same cell culture. Tubes showing CPE, were frozen and thawed twice and inoculated again into a new cell culture tube, incubated and observed for further 10 days for CPE appearance. CPE active cultures were frozen at -20°C and kept for further identification by the neutralization test (BUSBY, et al. 1964) in the corresponding tissue cells, using the known standardized antisera, that were available in the laboratory.

RESULTS

1- Greater Cairo:

Concerning the source of domestic, industrial and unknown factors affecting water quality, detailed studies were focused on Greater Cairo area. This area covers 50 miles along River Nile from Helwan to El-Kanater, down stream. From this segment 92 water samples were collected during the years 1975 to 1981. Forty one samples were collected from Helwan site, 34 from Cairo and 17 samples from El-Kanater sites (Fig. 1).

The examination of these samples for the presence of viral agents is based on the activity

of such agents against tissue culture cells, i.e. cytopathogenic activity effects (CPE). The obtained results are indicated in tables 1&2 and Fig. 2. A total of 18 isolates were successfully recovered along this area, representing 19.5% of the total examined samples (92). These isolates were identified by the neutralization test as 7 isolates of Influenza virus, 5 isolates of ECHO virus, 3 isolates of Coxsackie B virus, 2 isolates of Herpes simplex virus and 1 isolate of Poliovirus type 3. Still other cytopathogenic or cytotoxic agents (15 cases - 16.3%) of the total examined samples were not identified by the available antisera. These cases were detected among samples collected from Helwan (9 samples) and Cairo (6 samples). It was noticed that these agents were very active against the tissue cells, i.e. showed complete sloughing of the cell sheet even in the second passage. The enteric viruses group represented by Echo, Coxsackie B and Polioviruses constituted 50% and 27.3% from the identified isolates and CP active agents respectively. Other virus types as Influenza virus (30.0%) and Herpes simplex (11.1%) were occasionally detected among the identified isolates.

2- Demietta branch:

During May 1975 to March 1979, 28 samples were collected from Demietta branch. Sixteen of these samples were collected from the begining of this Branch, at El-Kanater, and 12 samples at El-Mansora City during November 1978 and March 1979 (Fig. 1).

Eleven samples showed CP activity effects (39.3%). Seven samples contained Echo virus (4), Coxsackie (2) and Herpes simplex (1) viruses. The other 4 samples contained untyped active agents (Table 2).

3- Rosetta Branch:

During 1975 to 1977, 17 samples were collected from the begining of Rosetta Branch at El-Kanater and 8 samples were collected during November 1978 and March 1979 at Kafr El-Zait City. Echo virus was isolated from two samples. Influenza and Herpes simplex viruses were isolated separately from two samples. Untyped viral agents were detected in 4 samples (Table 2).

4- Upper Egypt:

During November 1978, March 1979 and October 1981, 12 samples from Asswan and 7 samples from Assiut were collected. The obtained results (Table 2) indicated that 44.4% of the samples collected from Asswan were harboring viral agents. Coxsackie B and Echo viruses were detected separately in 3 and 2 cases of the tested samples. Coxackie B virus was isolated only from one sample of thsoe collected at Assiut.

DISCUSSION

Results of the prelimenary experiments (using the alginate filters, after prefilteration of raw Nile water samples via 0.45 u membranes), indicated that problems associated with clogging of the alginate filters is still a limiting factor. Though, membrane adsorption technique is the best method for concentrating and recovering viruses from water. Indeed, virus recovery was observed to be considerably low (21.3%), when compared with the bacterial parameters of faecal pollution (EL-ABAGY, et al. 1979).

ECHO, Coxsackie B and Influenza viruses constituted 37.5%, 25% and 12.5% respectively, of the identified isolates recovered at Helwan site. On the other hand, the same virus types

constituted 25%, 0% and 75% among Cairo isolates. Furthermore, Polio 3 and Herpes simplex viruses were undetected at Cairo site, mean while reached 12.5% from Helwan isolates. These findings could support the assumption that River Nile hydrology is a major factor affecting virus recoveries from Nile water. Erosion of deposits (silt, clays and other sediments) from river banks and/or from the edges of the in water islands may take place. It is entirely possible that viral agents could be adsorped to such sediments as well as organic materials. This was followed by sedimentation at another places. These sediments could carry the adherant or absorbed viral particles to the bottom. Henceforth, reduction of certain viruses in the flowing water between Helwan, Cairo and El-Kanater sites, could not be due to normal death of these agents, but it could be rather due to normal deposition, at low flow rates in certain wider areas, between any two successive sites.

The isolation rate of viruses at El-Kanater site, especially before Delta barrage and at the begining of Demietta and Rosetta branches, was somewhat lower than the expected rate. However, about the middle of Demietta branch or at El-Mansora city, virus recovery rate was increased up to 41.6%. Thus, reflecting the surrounding environmental factors concerning human activities and population density.

An interesting finding in this study was the higher isolation rate of viral agents from Asswan site (41.6%) than at Assuit site (14.3%). These unexpected results may be due to the wastewater discharged from El-Sail drain into the River Nile; at Asswan district. It must be taken into consideration that the effluent discharges carry out the municipal wastes of Asswan city and Kema factory.

These results indicate that enteric viruses group constituted the most prevailant part of the identified isolates (65.7%). ECHO, Coxsackie B and Polio virus type 3, were identified by the neutralization test in tissue culture cells. In Roumania, NASTOR and COSTIN (1976) isolated Coxsackie A and B from 15% of their river water samples.

In addition to the enteric viruses group, other viruses as Influenza and Herpes simplex were occasionally detected from River Nile water. Furthermore, it was found that 13.4% of the examined samples showed active cytopathogenic activity or cytotoxic effects and could not be identified by the available methods. This can indicate shortage of these techniques or denotes to the presence of unknown active factors. All available evidence points up to the possibility that mutagenic or any toxic compounds may serve as a potential toxic agent against the living tissues, but it felt that some links are still missing between this assumption and what is occuring exactly. These untyped active agents were recorded by many investigators (TYLOR and BRUCE, 1962; PRIMAVESI, 1966; EL-HAWAARY, 1977 and SOBSEY, 1978). It may be worthy to mention that the presence of these agents were correlated with human activity and industrialization along the River Nile and lack of any antipollution policy. While these agents were undetected at Asswan, Assiut and at Delta barrage sites (El-Kanater), they were detected at the other sites in different percentages ranging between 22% at Helwan and 14.3% in Demietta branch.

In general, it can be concluded that water quality, considering viruses, was solely influenced not only by the presence or absence of humanbeings, but rather by river hydrology and human activities in the community through which the stream flowed.

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Table (2)
Incidence of viruses along River Nile (1975-1981)

Location*	No. of CPE+ve samples	Viral type	Incidence
Upper Egypt:			
Asswan	5	Coxsackie B	3 (60%)
		ECHO	2 (40%)
Assiut	1	Coxsackie B	1 (100%)
Greater Cairo:			
Helwan	17	Polio 3	1 (5.9%)
		Coxsackie B	2 (11.8%)
		ECHO	3 (17.6%)
		Influenza	1 (5.9%)
		Herpes simplex	1 (5.9%)
		Untyped	9 (52.9%)
Cairo	14	ECHO	2 (14.3%)
		Influenza	6 (42.8%)
		Untyped	6 (42.9%)
El-Kanater	2	Coxsackie B	1 (50.0%)
		Herpes simplex	1 (50.0%)
Lower Egypt:			
Demitta branch	11	Coxsackie B	2 (18.2%)
		ECHO	4 (36.4%)
		Herpes simplex	1 (9.0%)
		Untyped	4 (36.4%)
Rosetta branch	7	ECHO	2 (28.6%)
		Influenza	1 (14.3%)
		Herpes simplex	1 (14.3%)
		Untyped	3 (42.8%)

* = Results are arranged according to geographical distribution of the sampling sites.

Table (1)
Distribution and date of sampling along River Nile (1975-1981)

Date	Upper Egypt		Greater Cairo			Lower Egypt	
	Asswan	Assiut	Helwan	Cairo	El-Kanater	Demietta Branch	Rosetta Branch
May 1975	ND	ND	3	2	1	2	3
Aug.	ND	ND	2	3	1	2	1
Oct.	ND	ND	3	2	1	1	2
Nov.	ND	ND	3	2	1	1	1
Dec.	ND	ND	3	2	1	1	1
Jan. 1976	ND	ND	3	2	1	1	1
Feb.	ND	ND	3	2	1	1	1
Mar.	ND	ND	3	2	1	1	1
Apr.	ND	ND	3	2	1	1	1
May	ND	ND	3	2	1	1	1
Jun.	ND	ND	3	2	1	1	1
Oct.	ND	ND	3	2	1	1	1
Jan. 1977	ND	ND	3	2	1	1	1
Mar.	ND	ND	3	2	1	1	1
Nov. 1978	5	3	ND	2	1	6	4
Mar. 1979	4	3	ND	2	1	6	4
Oct. 1981	3	1	ND	1	1	ND	ND
Total	12	7	41	34	17	28	25
C.P.E. +:	5	1	8	8	2	7	4
Typed	(41.6%)	(14.3%)	(19.5%)	(23.5%)	(11.7%)	(25.0%)	(16.0%)
Untyped	0	0	9	6	0	4	3
			(21.9%)	(17.6%)		(14.3%)	(12.0%)

ND = Not Done

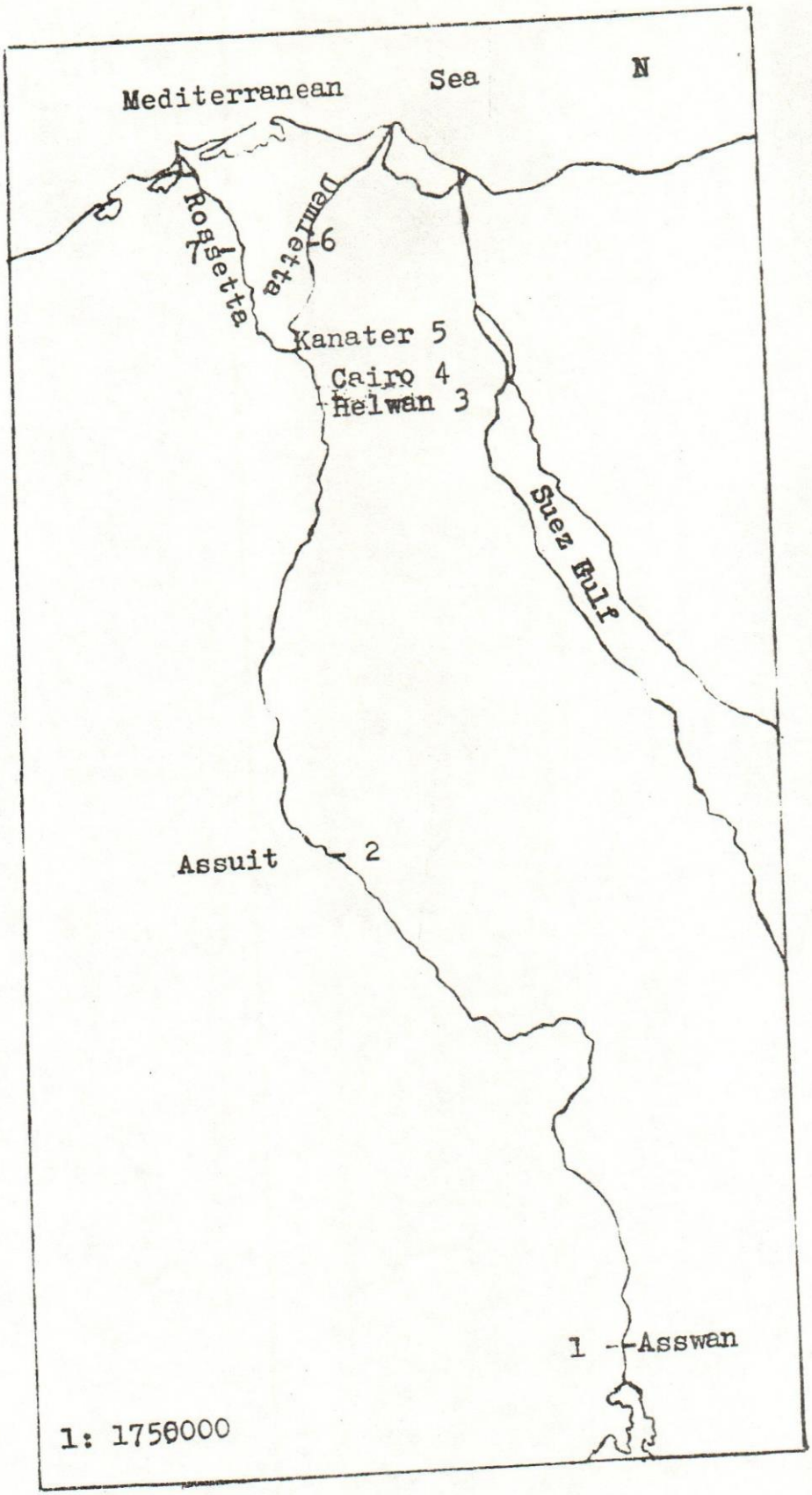

Fig. 1- Sampling locations

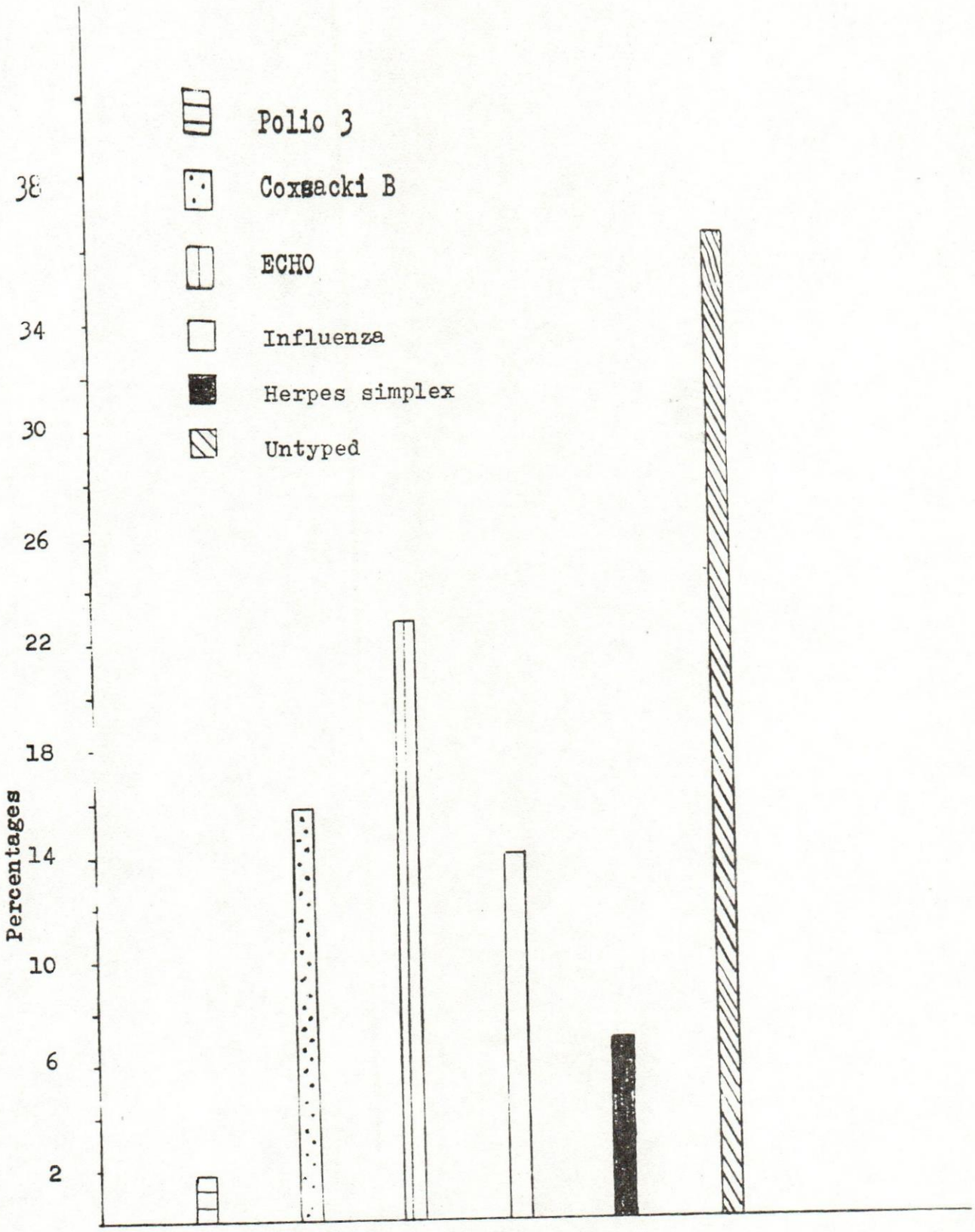

Fig.2- The prevailance of different recovered viruses in Nile water.