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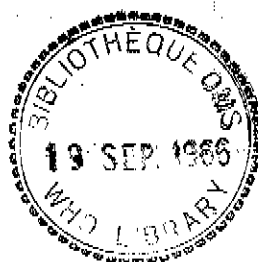
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ENGLISH ONLY

REPORT
ON THE WHO SERUM REFERENCE BANK IN PRAGUE
(covering period 1 July 1964 - 31 March 1966)

by

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INTRODUCTION

The activities of the Prague Serum Reference Bank in the period indicated mainly consisted in:

- I. The reception and processing of serum collections from abroad and from Czechoslovakia
- II. Laboratory examination of sera in Prague and abroad
- III. Aid in planning and execution of serological studies abroad and in Czechoslovakia
- IV. Laboratory research projects

I. NEW COLLECTIONS OF SERA FROM CZECHOSLOVAKIA AND FROM ABROAD

1. Sera collected in Czechoslovakia

The Serum Bank participated in the execution of several serological studies in Czechoslovakia, from which the following new collections were received:

1.1 Poliovirus antibody surveys (K. Žáček). (a) 320 specimens were collected from children up to 15 years of age in one region in Moravia 1965; (b) 726 sera were collected from all age-groups in the same region in 1966.

1.2 Influenza viruses antibody survey (M. Drašnar). In 1965, 200 specimens were obtained among the population of Prague City and approximately 200 specimens in five other localities in the Czech regions. Altogether 1100 serum specimens were collected and examined for antibodies to six influenza virus strains.

1.3 E. pertussis and parapertussis surveys (B. Vysoká-Burianová). A total of 1200 serum specimens were collected in 1965 among the population of five localities subjected to long-term follow-up in the Czech regions.

1.4 Multipurpose immunological survey (K. Žáček). A total of approximately 1200 serum specimens were obtained by strict random sampling among the normal population of all age-groups in a district of about 100 000 inhabitants in autumn 1964. Further specimens will be taken from the same donors in autumn 1966 for the purposes of longitudinal studies.

1.5 Sera for cholesterol level studies (J. Fodor). The Serum Bank received 1135 blood specimens in several consecutive consignments; the serum were separated and distributed into a total of 4874 aliquots.

2. Serum collections from abroad

The following serum collections arrived at the Prague Bank and were processed into aliquots:

Source (country)	Assembled or sent by	Number of specimens received in				Number of aliquots	
		2nd half 1964	1965	First quarter 1966	Total	Frozen	Lyophi- lized
Mongolia	WHO Mongolia Project	67	4 553	-	4 620	11 993	-
Ceylon	WHO Consultant (Dr Pecenka)	429	-	-	429	-	-
Nigeria	WHO Centre for Treponematoses (Copenhagen)	-	1 871	-	1 871	-	-
Togo	WHO Treponematoses Advisory Team	346	3 474	750	4 570	8 481	7 257
Pakistan	WHO Consultant (Dr Žáček)	-	496	-	496	1 494	726
TOTAL		842	10 394	750	11 986	21 968	7 983

Apart from this, the Serum Bank received blotting-paper rondelles with dried blood, viz, 62 rondelles from Nigeria and approximately 500 specimens collected by the WHO team in Western Samoa.

3. Withdrawal of specimens from the collections stored at the Prague Serum Reference Bank

From the collections assembled in Czechoslovakia were withdrawn:

- (i) approximately 200 serum specimens as control specimens for serological tests with Vibrio cholerae to be carried out at the SEATO Cholera Research Laboratory in Dacca, Eastern Pakistan
- (ii) approximately 300 specimens for examination for rubella virus antibodies as a part of the WHO sponsored pilot study organized by J. L. Melnick; the tests will be carried out by Dr J. Strauss of the Institute of Epidemiology and Microbiology, Prague.

The following withdrawals were made from the foreign collections:

- (i) 100 specimens of the Togo collection were sent to Dr Gear, WHO Serum Reference Bank in Johannesburg, for examination against different bacterial, viral and parasite antigens
- (ii) 100 specimens of the Philippine collection were sent for the same purpose to Dr Paul, WHO Serum Bank at Yale
- (iii) 457 serum specimens from Pakistan were sent for examination for arbovirus antibodies to Dr Charles L. Wisseman of University of Maryland, United States of America
- (iv) 200 serum specimens were sent for examination for cholesterol level determination to Dr James M. White of CDC, Atlanta, United States of America.

II. LABORATORY EXAMINATIONS PERFORMED IN PRAGUE

Apart from the homeland serological surveys for antibodies to polioviruses, influenza viruses, B. pertussis and parapertussis, laboratories of the Institute of Epidemiology and Microbiology in Prague and other laboratories in Czechoslovakia examined the following "foreign" serum collections:

Collection from	Year of tests	Number of sera tested	Tested for antibodies to
Ceylon	1964	429	all poliovirus types
Thailand	1965	100	polioviruses, morbilli, <u>pertussis</u> and <u>parapertussis</u> , ASLO, toxo- and histoplasmosis, leptospira, brucellosis arboviruses, mumps
		500	ASLO
Pakistan	1965	301	polioviruses, morbilli, <u>pertussis</u> and <u>parapertussis</u> , ASLO, toxoplasmosis, adenoviruses, RS virus, Mycobacteria pneumoniae
Togo	1965	189	polioviruses, morbilli, <u>pertussis</u> and <u>parapertussis</u>
Eastern Nigeria	1965	182	<u>pertussis</u> and <u>parapertussis</u> , morbilli, diphtheriae, ASLO
	1966	500	ASLO
Mongolia	1966	55	all poliovirus types

The results of these examinations together with brief descriptions of the methods employed in antibody determinations and the keys used for sub-sampling of larger collections are presented in the tables and figures contained in Annex A of this report. The results of ASLO level determinations have already been submitted for publication in the WHO Bulletin while the other results will be submitted for publication in the nearest future.

III. ASSISTANCE OF THE SERUM REFERENCE BANK WITH THE PLANNING AND EXECUTION OF SEROLOGICAL STUDIES ABROAD

The Acting Director, Statistician and other members of the staff of the Serum Reference Bank provided active help to some countries or institutions in the planning or carrying out of serological studies. This especially relates to the following cases:

- (i) minor collections of serum specimens were undertaken in three localities in Pakistan in 1965 for examination of the presence or absence of antibodies to certain arboviruses and some other viral and bacterial agents
- (ii) minor serum collections were compiled in Turkey and in Iraq in 1966 for examination of antibodies to cholera and possibly some other infections
- (iii) in Pakistan, Turkey and Iraq plans for conducting multipurpose immunological surveys were discussed
- (iv) in December 1965 Dr K. Žáček and Mr J. Jelínek took part at WHO headquarters in Geneva in discussions and preparation of plans relating to further exploitation of the serum collections assembled in different countries by the WHO Treponematoses Advisory Team
- (v) a certain extent of technical aid was provided to the WHO team that was collecting sera in Mongolia during 1964 and 1965.

IV. RESEARCH PROJECTS

1. Testing of blotting-paper rondelles with dried blood

Some results of an orientational experiment in which sera and eluates from paper rondelles taken from 20 volunteering donors were simultaneously tested for antibodies to polioviruses, have already been presented in the Annual Report of the Prague Serum Reference Bank for 1963. The following tests were performed in the subsequent period:

(i) sixty-two serum specimens and paper rondelle eluates taken from identical donors by the Treponematoses Advisory Team in Nigeria in 1965 were simultaneously tested for antibodies to polioviruses

(ii) comparative experiments were undertaken to show whether the lower titres in paper rondelle eluates than in serum specimens obtained in the previous orientational tests were due to a determination of antibodies as a result of the drying up of the blood on the rondelles or simply to the difference in the actual amount of plasma contained in the given dilution of sera and eluates, which in the previous tests were diluted experimentally in the ratios employed by Vaisman et al.¹ Some of the results of these experiments are summarized in the tables and figures of Annex B.

2. Stability of cholesterol levels at different temperatures

The Serum Reference Bank co-operated with Dr D. Grafnetter and Dr J. Fodor of the Institute for Cardiovascular Diseases in Prague in a WHO-sponsored study relating to the effect of serum storage at different temperatures on cholesterol levels. This study is comparable to similar projects on the stability of serum at different temperatures carried out at all three WHO Serum Reference Banks in 1963 and 1964. The results of this study were submitted for publication in English to the "Cor et Vasa" journal, Prague.

3. Diphtheric antitoxin determination by metabolic-inhibition test in tissue culture

This test, developed by Dr Kříž of the Institute of Epidemiology and Microbiology in Prague in close co-operation with workers of the Serum Reference Bank, was employed in a serological survey in Czechoslovakia and in examining 180 serum specimens collected by the WHO Treponematoses Advisory Team in Eastern Nigeria.

¹ Vaisman A., Hamelin A. & Guthe T. (1963) Bull. Org. mond. Santé, 29, 1-6.

The results obtained so far suggest that it will be possible to utilize this economical test for large-scale surveys of diphtheric antitoxin levels among the population and for other purposes. The method of the test and the preliminary results of its application to serological studies will be submitted for publication in the near future.

4. Documentation

The system of recording and processing of data used at the Prague Serum Reference Bank has been improved and is warranted by the system utilizing a five-track teleprinter tape. The transfer of data and information from individual record cards is carried out by means of numerical recording and teleprinting, which provides a primary protocol of data strictly corresponding to the original set of record cards. The supplementing of this primary protocol with results of subsequently performed serological tests is carried out by automatic rewriting of the primary tape, which is automatically arrested at the appropriate serum number to allow the addition of further data. In this way a new enlarged protocol of data is obtained in which the previous data are included without an error. Obviously, such an additional tape can be provided every time the Serum Bank withdraws serum aliquots and receives serological results. The important point is that data entered on a teleprinter tape can be fed directly into the computer for automatic statistical elaboration. A draft of a programme for such statistical processing of serological data has been worked out in detail at the Prague Serum Reference Bank. The first step is classification, tabulation and graphic presentation of the results obtained. The possible utilization of the IBM digital computer to be installed at the WHO headquarters in Geneva is envisaged. Classified and tabulated data will further be analysed from the aspects of:

time regression of antibody titres

shape of distribution curves of antibody titres

mathematical formulation of the immunity process among the population under study.

To meet these tasks, the Solartron analogue computer installed at the Institute of Epidemiology and Microbiology in Prague (provided by WHO) has been specially adjusted. This computer has been put into operation recently.

V. EQUIPMENT AND FACILITIES

Workers of the Serum Bank have developed and now currently employ a simple apparatus for semi-automatic sealing of glass ampoules (with a capacity of 1200 ampoules per hour) and an apparatus for measuring and dispensing 0.5-1.0 ml aliquots from individual specimens of sera.

After having obtained glass vials of required dimensions from Denmark and special rubber stoppers developed and produced in Czechoslovakia, a new lyophilization apparatus, model 30 P. I.T. was put in operation.

VI. THE STAFF

Acting Director: Dr K. Žáček, Head, Enterovirus Department, Institute of
Epidemiology and Microbiology in Prague, (IEM), part-time

Professional

Assistants:

Dr V. Teplý, Virologist, IEM, part-time

Mr J. Jelínek, Statistician, IEM, part-time

Technical staff:

Mrs Y. Rendlová, IEM, full-time

Mrs M. Šimáčková, IEM, full-time

Mrs Y. Bláhová, IEM, full-time

Miss O. Stará, IEM, full-time

Documentarists:

Mrs E. Adamcová, IEM, full-time

Mrs. A. Urbanová, IEM, part-time

VII. PUBLICATIONS

1. Rotta, J., Jelínek, J. & Žáček, K. Antistreptolysin O surveys as an indicator of the prevalence of streptococcal infections among the population. WHO Bulletin, in press.
2. Grafnetter, D., Fodor, J., Teplý, V. & Žáček, K. On the effect of serum storage on cholesterol levels as measured by a simple direct method, Cor et Vasa (Prague), in press.
3. Vysoká-Burjánová, B. et al. Surveillance programme of whooping cough in Czechoslovakia, J. Hyg. Epid. Microb. Immunol. (Prague), in press
4. Surveillance Report, immunological surveys of A. Pertussis, B. Poliomyelitis, C. Measles in Thailand, Pakistan, Nigeria (Eastern) and Togo, GES/SR/66.3
5. Rotta, J., Jelínek, J., Žáček, K., de Vries, J. L., Chanthranetra, P. & Antal, G. M. Surveillance report, antistreptolysin O surveys as an indicator of the prevalence of streptococcal infections among the population of Thailand, Pakistan and Eastern Nigeria, GES/SR/66.4.

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ANNEX A

PRAGUE SERUM REFERENCE BANK

Serological Tests on Thailand, Pakistan,
Togo and Nigeria Serum Collections

ANNEX A

to Report for 1965

METHODS USED FOR ANTIBODY DETERMINATIONS

1. Polioviruses - Serum-neutralizing antibodies were determined by the colorimetric (pH) test in plastic panels as outlined in the 1958 Report of the WHO Expert Committee on Poliomyelitis. In calculating age-specific curve levels of antibody of 1:4 or above have been designated as positive.
2. Mumps - Antibodies were determined by the complement-fixation microtest on plexiglass plates using two units of antigen in the reaction; titres equal to or above 1:4 have been designated as positive.
3. Adenoviruses, RS virus and Mycoplasma pneumoniae - by complement-fixation tube test with two units of antigen and two units of complement. Titres equal to or above 1:4 have been designated as positive.
4. Measles - Antibodies were determined by the haemagglutination-inhibition test using Takatsy's micromethod. After preliminary absorption of sera to concentrated monkey erythrocytes four units of antigen (prepared from the "Boston" strain according to Rosen, 1961) were added per dilution of serum (from 1:2 or 1:4 to 1:256) and the mixture was held one hour at room temperature. Then 0.05 ml of Cercopithecus asthiops erythrocytes was added per well and the reaction was incubated one hour in a moist chamber at 37°C. In calculating age-specific curve levels of antibody of 1:2 or above have been designated as positive.
5. B. pertussis and parapertussis - By the agglutination test in plastic panels with a suspension of 20 000 million bacteria in the reaction. The sera were diluted in twofold steps from 1:4 to 1:512 and titres equal to or above 1:8 have been designated as positive.
6. Brucellosis - By agglutination and complement-fixation tests with Brucella Abortus Bang using the techniques outlined in the 1961 and 1964 Reports of the WHO Expert Committee on Brucellosis.
7. Toxoplasmosis - By the complement-fixation test using three units of antigen.
8. Histoplasmosis - By the quantitative precipitation reaction in agar using the following antigens: histoplasmin, coccidioidin, paracoccidioidin, and blastomycin.

9. Thailand sera - Out of the total collection of about 4000 serum specimens assembled by the WHO Treponematoses Advisory Team in Thailand in 1962, 100 specimens were selected according to random numbers so that the individual age-groups would include the following predetermined numbers of specimens:

Age-group (in years)	Number of specimens
2-3	10
3-4	10
4-5	10
5-6	10
6-10	20
11-20	20
20+	20

These 100 specimens were examined for antibodies to the following antigens:

All types of polioviruses

Measles

Mumps

Streptolysin O

B. pertussis and B. parapertussis

Brucellosis

Toxoplasmosis

Histoplasmosis

Arboviruses (some types)

The test for arbovirus antibodies was performed in the WHO Reference Laboratory for Arboviruses in Bratislava; the other tests were carried out in the Institute of Epidemiology and Microbiology in Prague, CSSR.

The tables and charts with the determinations of antibodies to polioviruses, mumps, B. pertussis and parapertussis are attached to this report; the results of tests for ASLO have already been submitted to WHO headquarters.

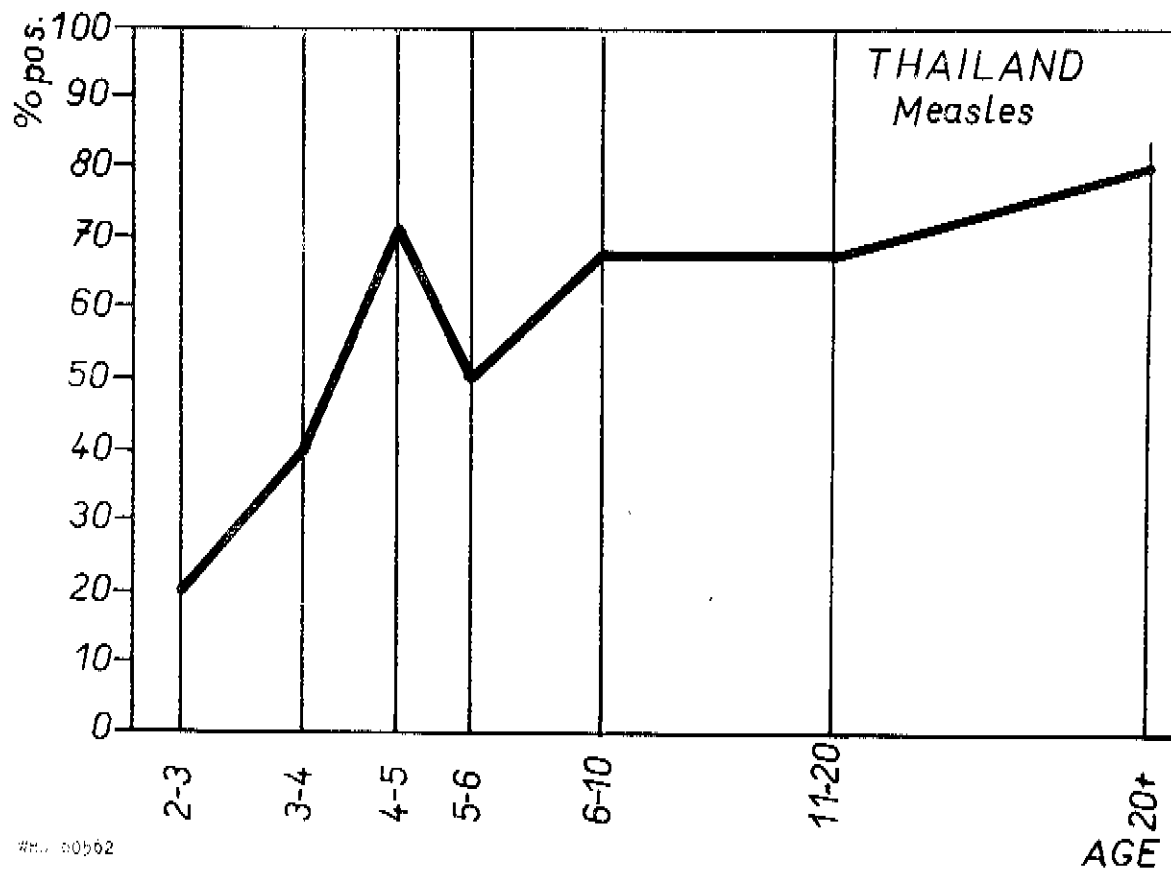
All tests for brucellosis, toxoplasmosis and histoplasmosis were negative.

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HI ANTIBODIES TO MEASLES

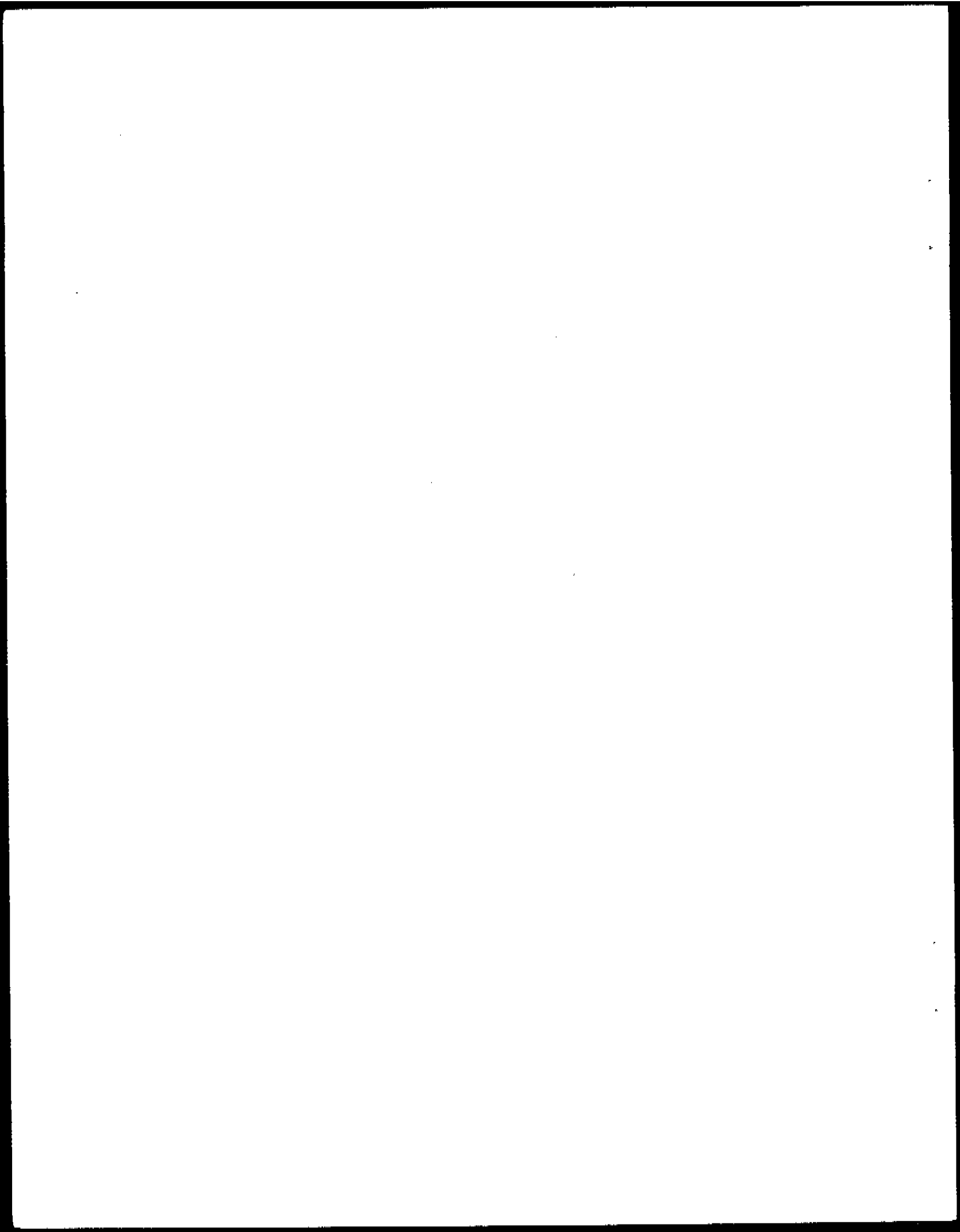
Age-group	No. of sera tested	Titres								Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
2 - 3	10	8	0	0	1	0	1	0	0	20
3 - 4	10	6	0	0	2	1	1	0	0	40
4 - 5	10	3	0	1	2	1	1	1	1	70
5 - 6	10	5	0	1	4	0	0	0	0	50
6 - 10	19	6	0	3	4	4	2	0	0	68
11 - 20	19	6	1	2	3	6	1	0	0	68
20+	20	4	2	5	0	4	4	1	0	80
Total	98	38	3	12	16	16	10	2	1	

THAILAND
C-F ANTIBODIES TO MUMPS

Age-group	No. of sera tested	Titres				Per cent. of positives
		Neg.	1/4	1/8	1/16	
2 - 3	10	9	0	0	1	10
3 - 4	10	8	1	0	1	20
4 - 5	10	7	1	0	2	30
5 - 6	10	8	1	0	1	20
6 - 10	20	13	2	0	5	35
11 - 20	20	14	2	1	3	30
20+	20	11	2	3	4	45
Total	100	70	9	4	17	



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POLIOVIRUS ANTIBODIES BY TEST

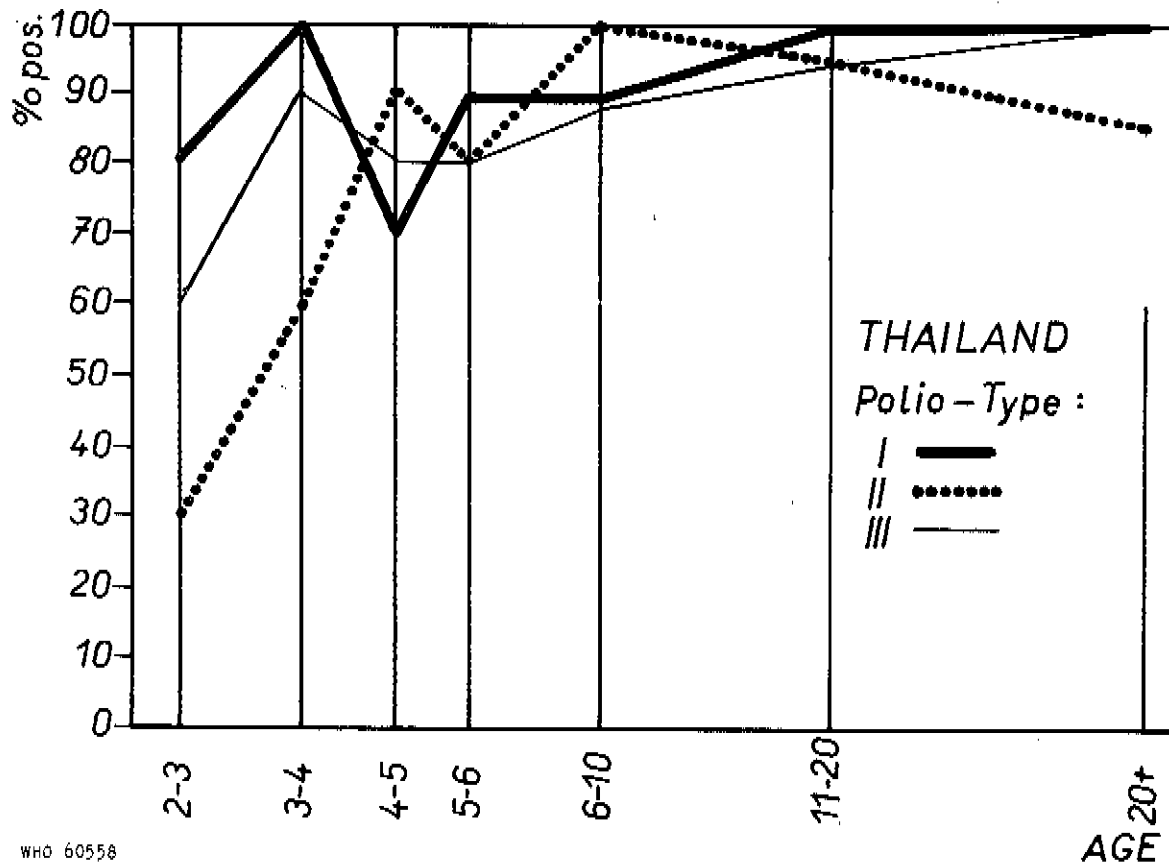
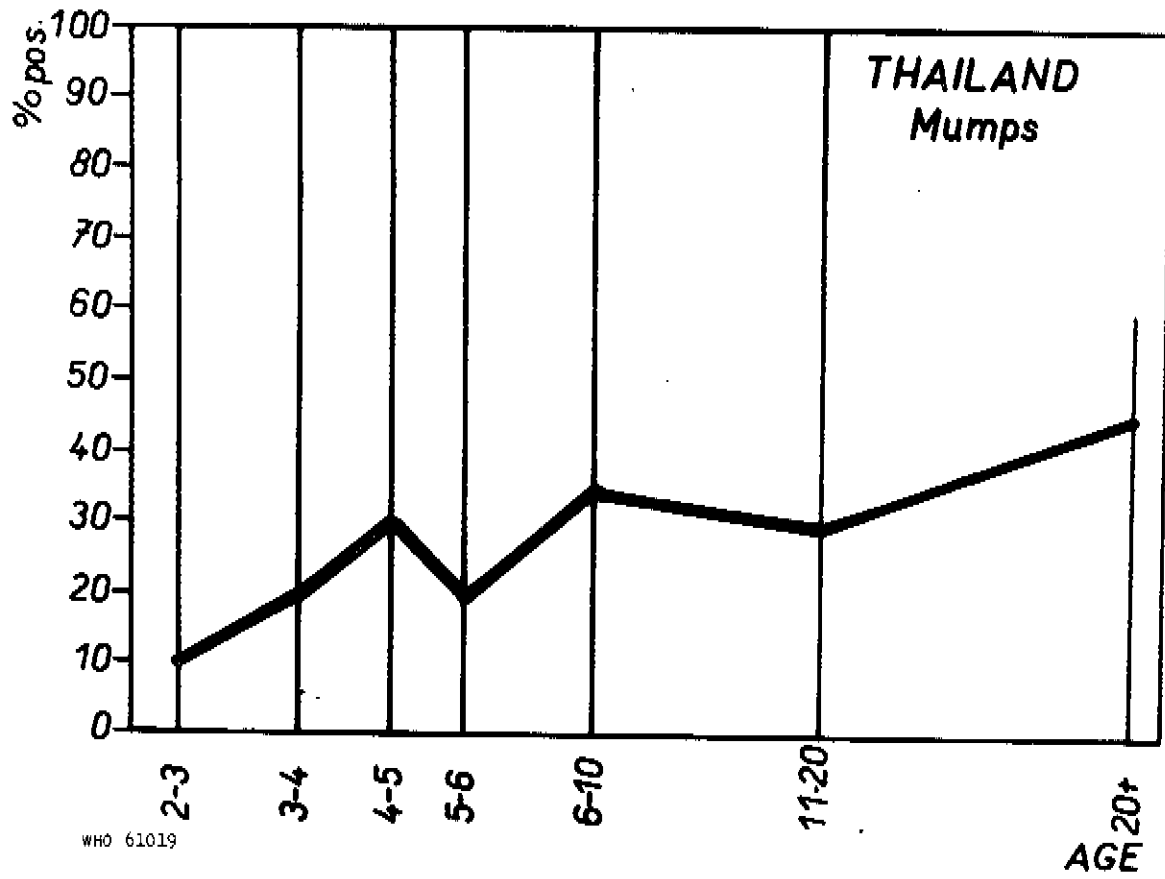
Age-Group	No. of sera tested	Type																		
		I				II				III										
		Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives	
2-3	10	2	0	0	2	6	80	7	1	1	1	0	0	30	4	0	1	1	4	60
3-4	10	0	1	2	2	5	100	4	0	1	1	4	60	1	1	3	4	1	1	90
4-5	10	3	0	0	1	6	70	1	0	2	0	7	90	2	0	0	6	2	2	80
5-6	10	3	0	2	2	5	90	2	1	0	4	3	80	2	0	3	2	3	3	80
6-10	20	2	0	2	4	12	90	0	1	3	7	9	100	2	1	2	9	6	6	90
11-20	20	0	0	3	9	8	100	1	2	2	7	8	95	1	1	5	7	6	6	95
20+	20	0	0	4	8	8	100	3	2	6	7	2	85	0	0	5	13	2	2	100
Total	100	8	1	13	28	50		18	7	15	27	33		12	3	19	42	24	24	

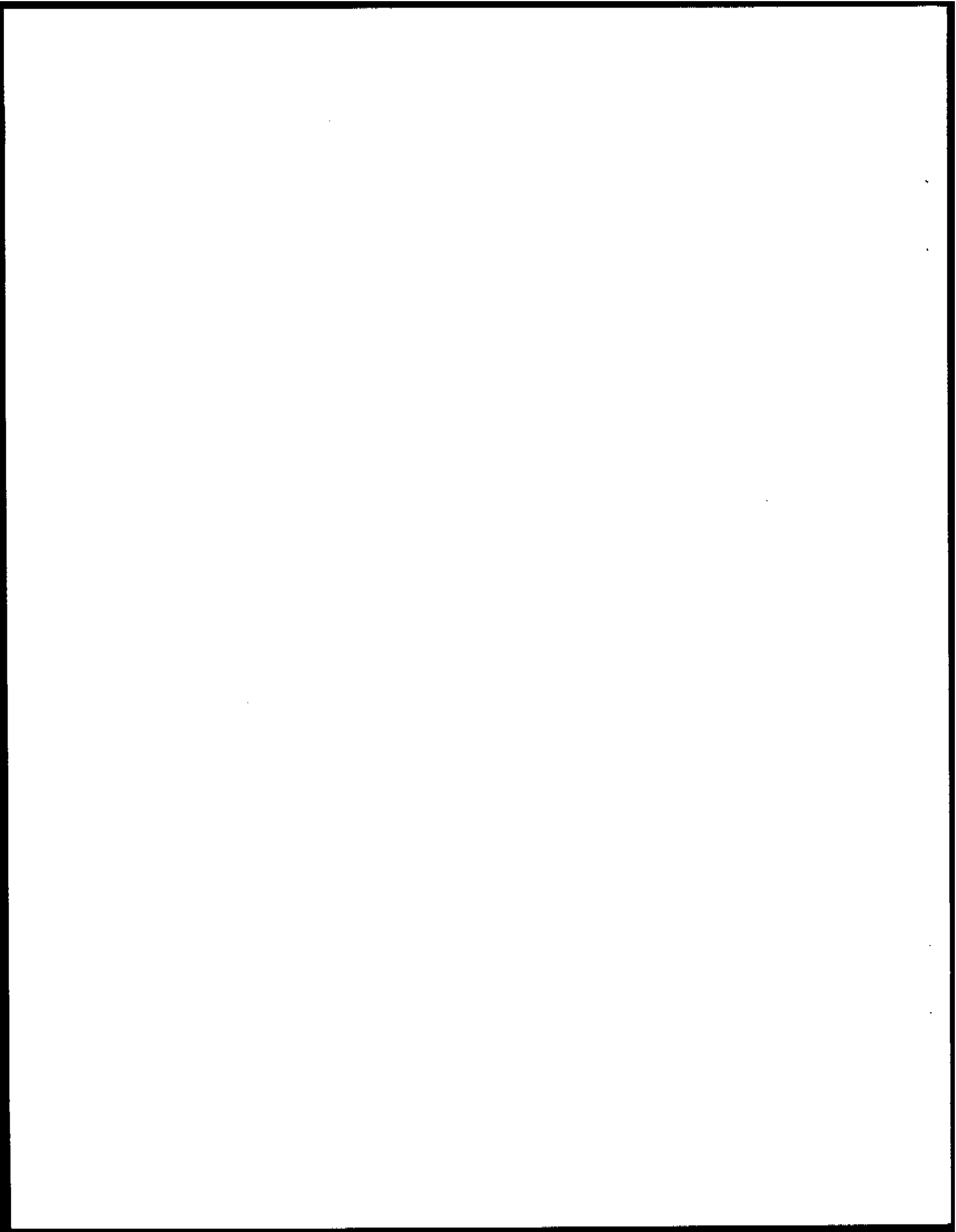
THAILAND
HAEMAGGLUTINATION ANTIBODIES TO B. PERTUSSIS

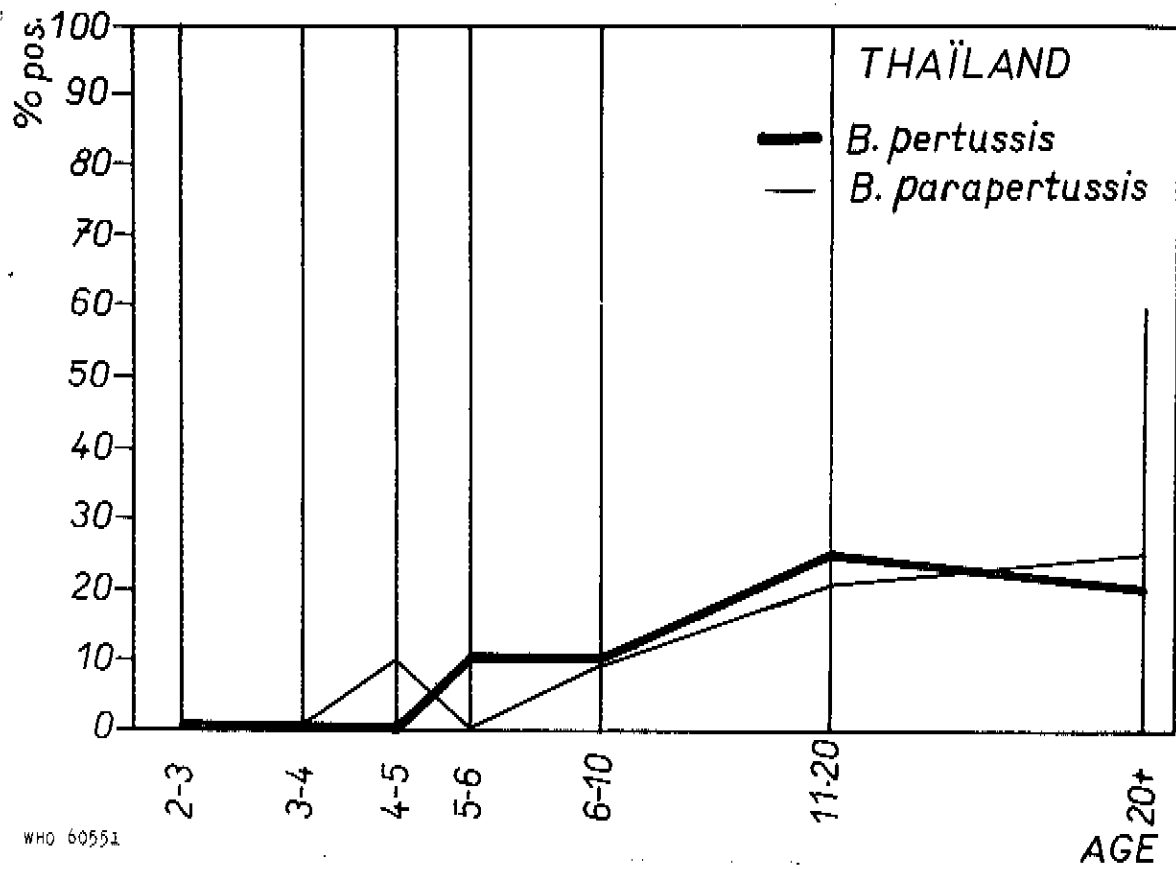
Age-groups	No. of sera tested	Titres									Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	
2 - 3	10	10	0	0	0	0	0	0	0	0	0
3 - 4	10	10	0	0	0	0	0	0	0	0	0
4 - 5	10	10	0	0	0	0	0	0	0	0	0
5 - 6	10	9	0	1	0	0	0	0	0	0	10
6 -10	20	18	0	0	0	1	1	0	0	0	10
11-20	20	15	0	1	0	2	1	1	0	0	25
20+	20	16	0	1	0	1	1	1	0	0	20
Total	100	88	0	3	0	4	3	2	0	0	

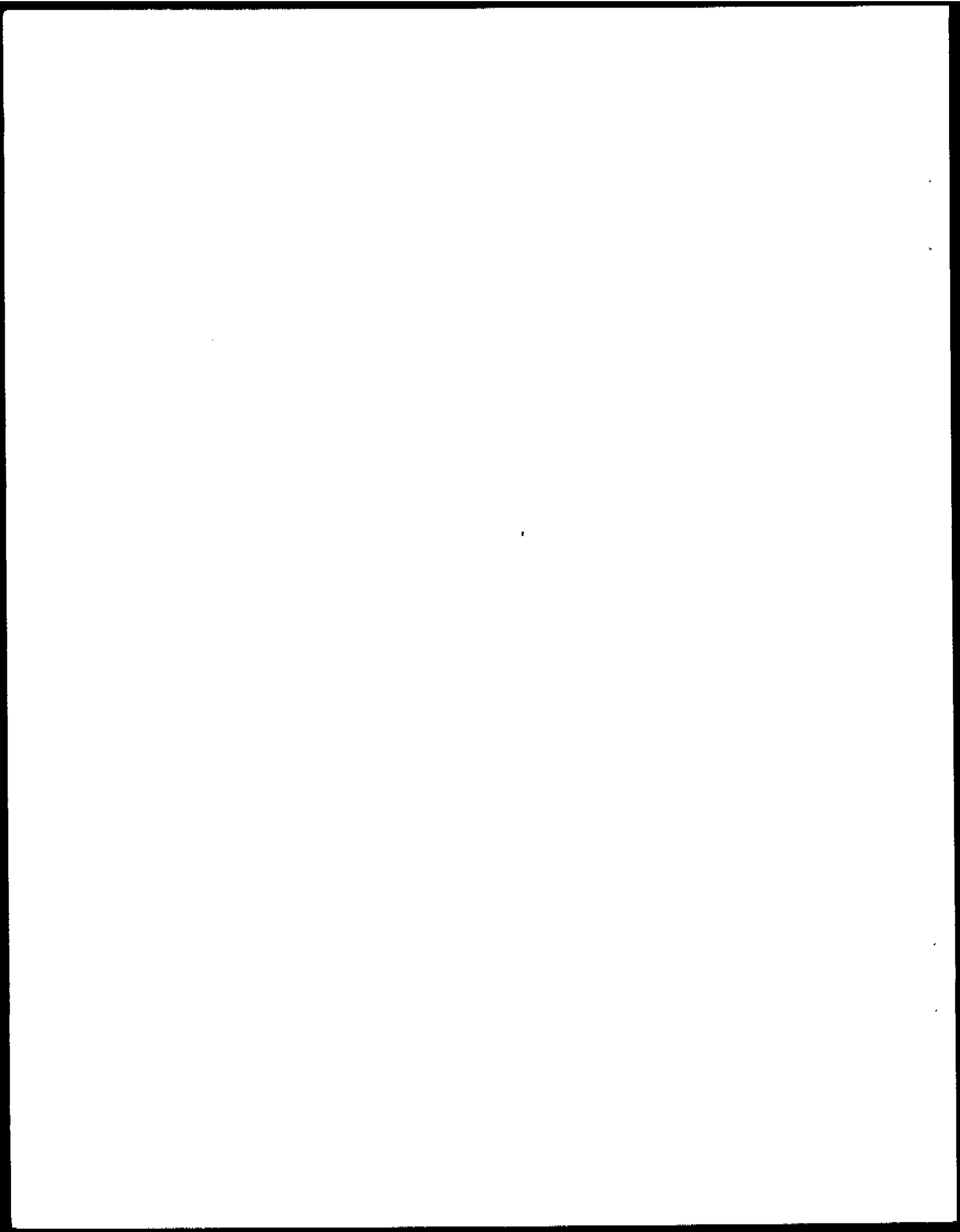
HAEMAGGLUTINATION ANTIBODIES TO B. PARAPERTUSSIS

Age-groups	No. of sera tested	Titres									Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	
2 - 3	10	10	0	0	0	0	0	0	0	0	0
3 - 4	10	10	0	0	0	0	0	0	0	0	0
4 - 5	10	9	0	0	1	0	0	0	0	0	10
5 - 6	10	10	0	0	0	0	0	0	0	0	0
6 -10	20	18	0	0	0	1	0	0	1	0	10
11-20	20	16	0	1	0	2	1	0	0	0	20
20+	20	15	0	0	0	1	1	3	0	0	25
Total	100	88	0	1	1	4	2	3	1	0	









THAILAND
ANTIBODIES TO ARBOVIRUSES BY HAEMAGGLUTINATION-INHIBITION TEST

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Annex A

page 7

Serum number	WEE	EEE	Sindbis	Chikungunya	Dengue				Yellow fever	Tick-borne encephalitis	West Nile	Jap. B.
					1	2	3	4				
1003												
1300												
1406					1:40							
1757												
2384												
2902												
3069												
3104					1:40							1:160
3434												1:40
3616												
208								1:20				
990												
1359		1:20										
1678												
2217												
2982												
3065					1:40	1:20						
3263					1:160	1:40	1:80	1:40			1:40	

THAILAND
ANTIBODIES TO ARBOVIRUSES BY HAEMAGGLUTINATION-INHIBITION TEST (continued)

Serum number	WEE	EEE	Sindbis	Chikungunya	Dengue				Yellow fever	Tick-borne encephalitis	West Nile	Jap. B.
					1	2	3	4				
3356		1:20			1:1280	1:320	1:640	1:320	1:160	1:40	1:320	1:320
3424					1:80	1:20	1:40	1:20	1:20	-	1:20	-
663												
857		1:160			1:40	1:80	1:80	1:40	1:40	-	1:40	1:40
1016												
1039												
1111						1:20						
2967												
3184					1:160	1:40	1:80	1:80	1:80	1:40	1:40	-
3292	1:20	1:320		1:40	1:320	1:320	1:320	1:80	1:80	1:40	1:160	1:160
3336	1:20	1:320		1:40	1:640	1:640	1:640	1:160	1:160	1:80	1:320	1:320
3585					1:160	1:80	1:160	1:80	1:160	1:20	1:160	1:80
356												
902		1:160		1:20	1:80	1:80	1:320	1:40	1:40	1:40	1:40	1:160
1044		1:20										
1813												
2747												
3029					1:80	1:160	1:80	1:80	1:160	1:20	1:160	1:160
3212					1:80	-	1:20	-	1:20	-	-	-
3367					1:20	1:20	1:20	-	1:20	-	-	-
3579					1:80	1:40	1:80	1:40	1:40	-	1:40	1:40

THAILAND
ANTIBODIES TO ARBOVIRUSES BY HAEMAGGLUTINATION-INHIBITION TEST (continued)

Serum number	WEE	EEE	Sindbis	Chikungunya	Dengue				Yellow fever	Tick-borne encephalitis	West Nile	Jap. B.
					1	2	3	4				
3313		1:80			1:80	1:40	1:40	1:20	1:40	-	1:20	1:80
223		1:320			1:40	1:40	1:80	1:20	1:40	-	1:20	1:40
267					1:20	-	1:40	-	1:40	-	1:20	-
872	1:20	1:320			1:80	1:80	1:320	1:40	1:40	1:40	1:80	1:160
1010	-	-		-	-	-	-	-	-	-	-	-
1331		1:40			-	-	-	-	-	-	-	1:40
1333												
1335												
1471												
2388		1:40				1:80						
2966												
2985		1:160				1:80	1:80		1:20	1:20	1:20	1:80
3176	1:40	1:640		1:40	1:320	1:320	1:640	1:320	1:320	1:160	1:640	1:1280
3177		1:160			1:320	1:80	1:320	1:40	1:40	1:80	-	1:160
3261		1:160			1:320	1:160	1:320	1:160	1:160	1:20	1:320	1:640
3295					1:80	1:20	1:40	1:20	1:40	-	1:40	1:20
3362					1:320	1:160	1:320	1:320	1:160	1:40	1:320	1:160
3432		1:20	1:40	1:1280	1:160	1:160	1:160	1:160	1:320	1:40	1:640	1:640
3437	1:20	1:320		1:320	1:320	1:320	1:320	1:40	1:40	1:40	1:80	1:160
3775			1:20	1:1280	1:1280	1:320	1:640	1:640	1:640	1:80	1:1280	1:640
222		1:160		1:40	1:80	1:160	1:320	1:80	1:80	1:40	1:80	1:160

THAILAND
ANTIBODIES TO ARBOVIRUSES BY HAEMAGGLUTINATION-INHIBITION TEST (continued)

Serum number	WEE	EEE	Sindbis	Chikungunya	Dengue				Yellow fever	Tick-borne encephalitis	West Nile	Jap. B.
					1	2	3	4				
744				1:160	1:320	1:160	1:160	1:160	1:160	1:40	1:320	1:160
871		1:40			1:320	1:160	1:320	1:160	1:160	1:40	1:160	1:320
878	1:40	1:640		1:320	1:640	1:320	1:640	1:320	1:320	1:160	1:640	1:640
935					1:640	1:320	1:640	1:320	1:320	1:40	1:320	1:320
1073					1:160	1:160	1:320	1:160	1:160	1:40	1:160	1:160
1301					1:320	1:160	1:160	1:160	1:160	1:40	1:320	1:640
1342				1:20	1:160	1:320	1:320	1:80	1:160	1:20	1:160	1:160
1357												
1664					1:320	1:160	1:320	1:320	1:1280	1:80	1:320	1:160
1949		1:320	1:20	1:20	1:320	1:160	1:320	1:160	1:160	1:80	1:2560	1:160
2225					-	-	1:80	1:40	1:80	-	1:80	1:20
3041				1:320	1:320	1:160	1:320	1:160	1:160	1:40	1:160	1:320
3108					1:320	1:160	1:160	1:160	1:160	1:160	1:160	1:160
3152					1:160	1:80	1:160	1:80	1:160	-	1:160	1:160
3288					1:80	1:40	1:160	1:80	1:160	1:20	1:160	-
3379		1:320			1:320	1:320	1:320	1:80	1:160	1:40	1:80	1:160
3386	1:20	1:320	1:20	1:20	1:320	1:320	1:320	1:320	1:320	1:80	1:640	1:320
3396				1:80	1:160	1:160	1:160	1:160	1:320	1:80	1:320	1:320
3486				1:640	1:5120	1:640	1:1280	1:1280	1:640	1:80	1:640	1:1280

10. Pakistan sera - In 1965 three small collections of sera were obtained among the populations of Karachi, Dacca and Lahore and shipped to the Prague Serum Reference Bank.

Aliquots for arbovirus antibody determination (the main purpose of the collection) were sent to Dr Wisseman of Maryland University, United States of America; results have not arrived so far.

The remaining aliquots were tested in the Institute of Epidemiology and Microbiology in Prague for antibodies to the following antigens:

All types of polioviruses

Measles

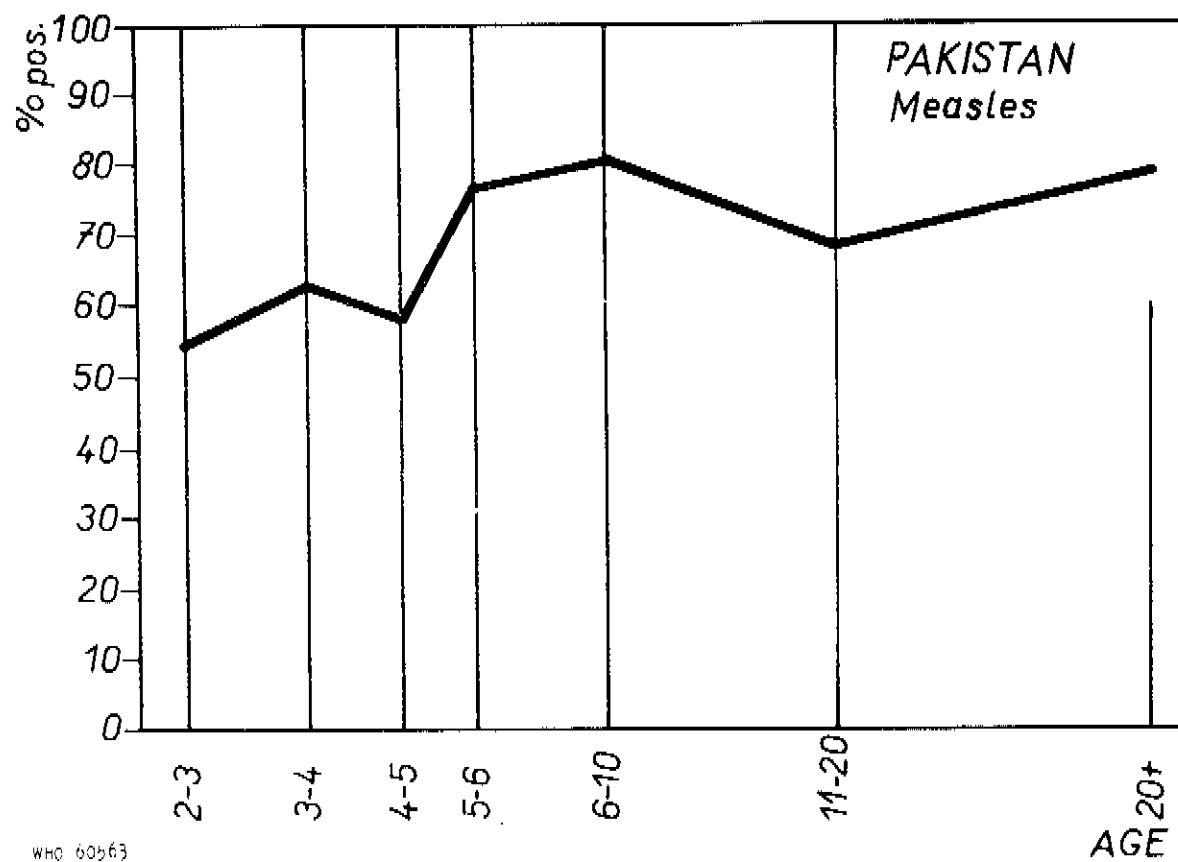
Adenoviruses, RS virus and Mycoplasma pneumoniae

B. pertussis and parapertussis.

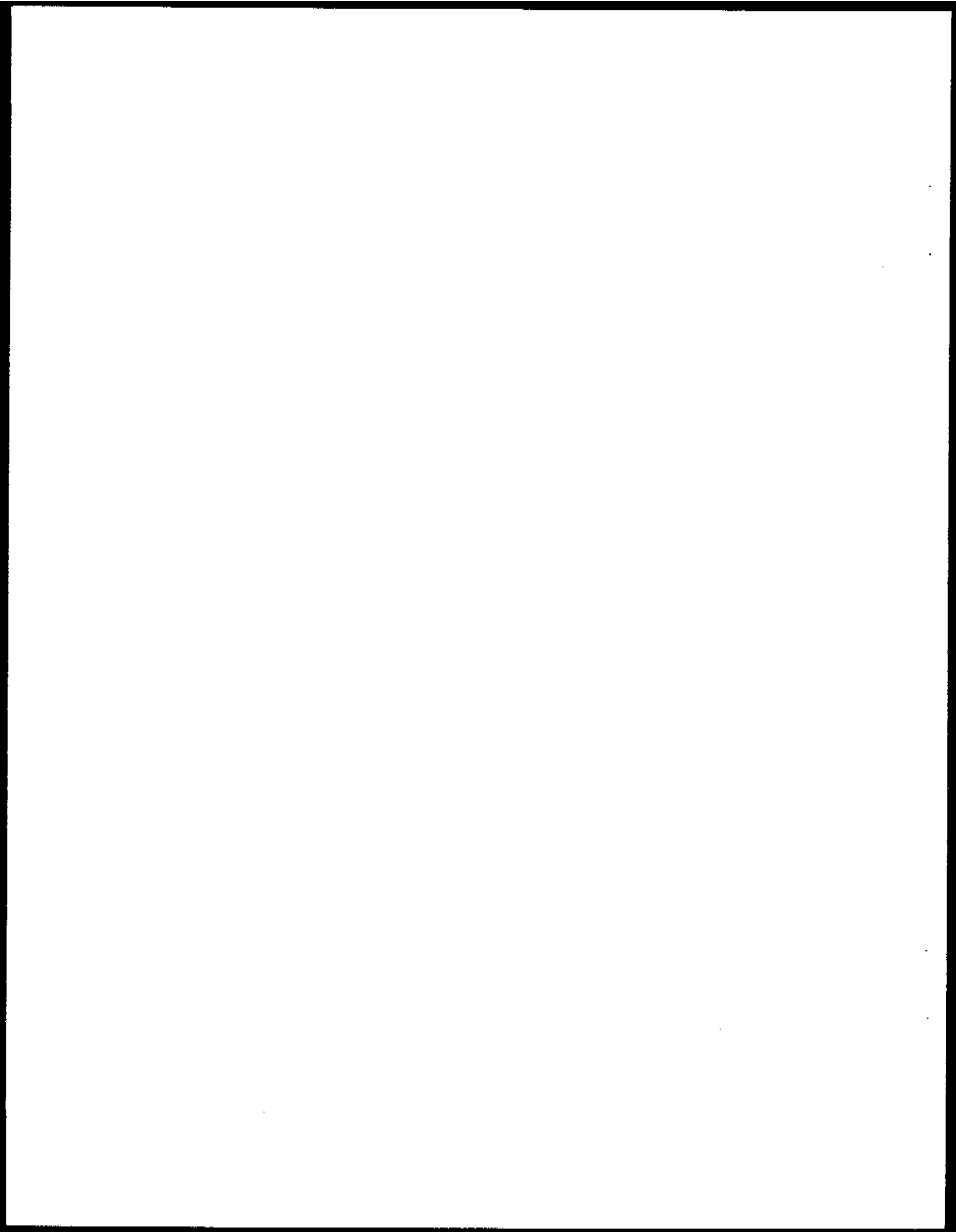
As the results were similar in specimens from all the three localities, they are presented summarized in the attached tables and charts.

PAKISTAN
HI ANTIBODIES TO MEASLES

Age-groups	No. of sera tested	Titres								Per cent. of positives
		Neg.	4	8	16	32	64	128	256	
2 - 3	13	6	0	0	0	2	2	3	0	54
3 - 4	21	8	2	0	4	3	3	1	0	63
4 - 5	12	5	0	1	1	3	1	1	0	58
5 - 6	17	4	2	1	4	3	0	2	1	77
6 - 10	60	12	1	12	12	7	7	6	3	80
11 - 20	87	17	6	15	28	15	3	2	1	69
20+	174	36	27	29	42	26	10	4	0	79
Total	384	88	38	58	91	59	26	19	5	



WHO 60563



PAKISTAN
CF ANTIBODIES TO ADENOVIRUSES

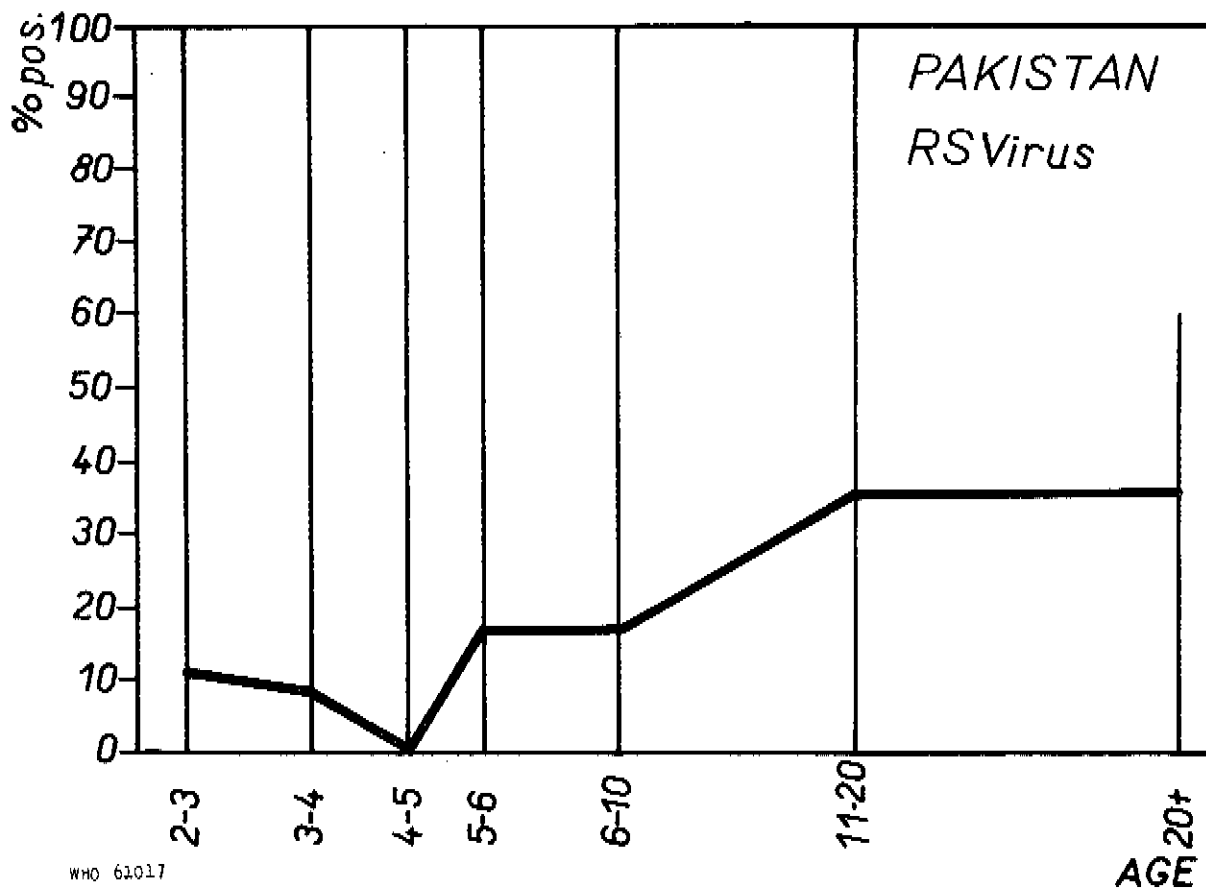
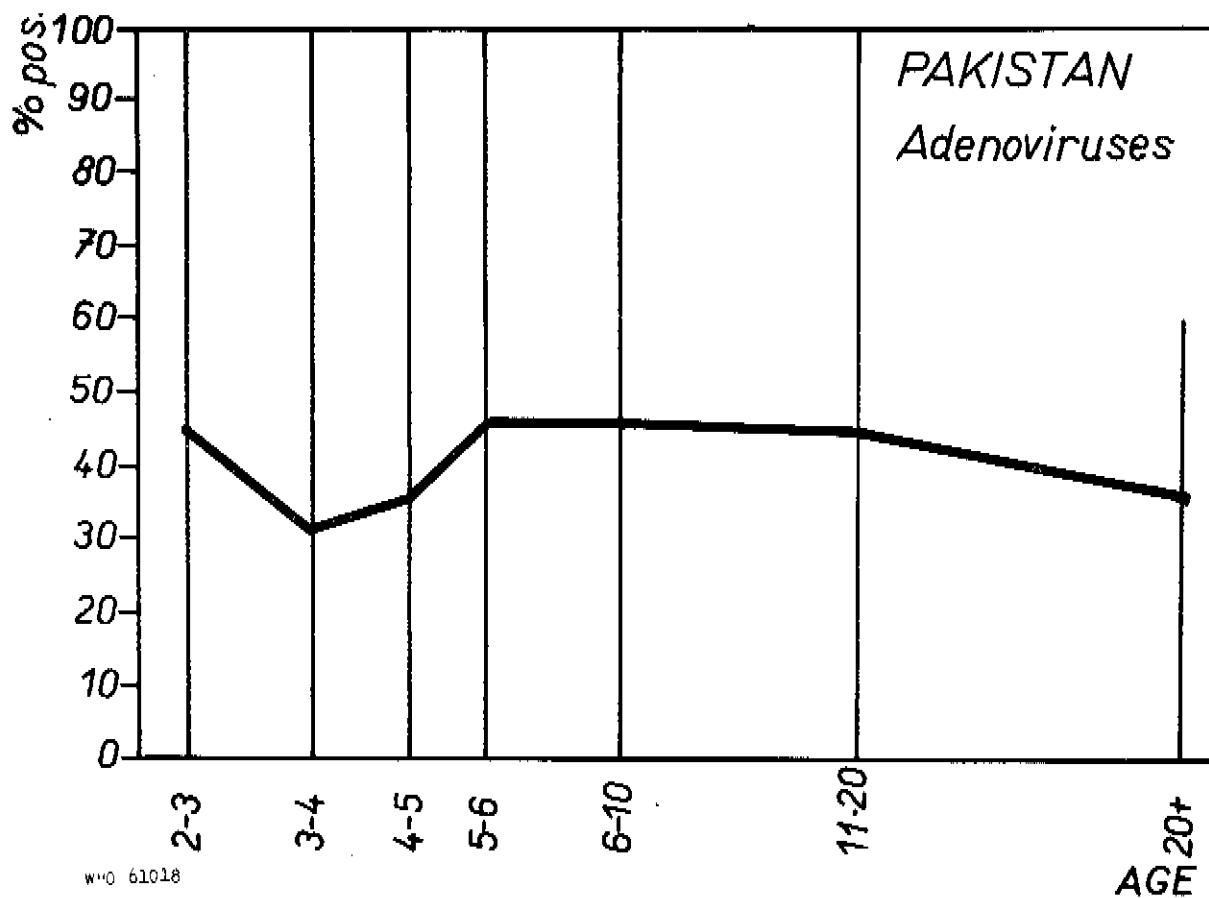
Age-group	No. of sera tested	Titres							Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	
2 - 3	9	5	2	2	0	0	0	0	44
3 - 4	13	9	1	1	1	1	0	0	31
4 - 5	9	6	1	1	1	0	0	0	33
5 - 6	13	7	4	1	0	1	0	0	46
6 - 10	46	25	12	3	1	4	0	1	46
11 - 20	76	42	7	7	12	8	0	0	45
20+	132	85	15	13	13	6	0	0	36
Total	298	179	42	28	28	20	0	1	

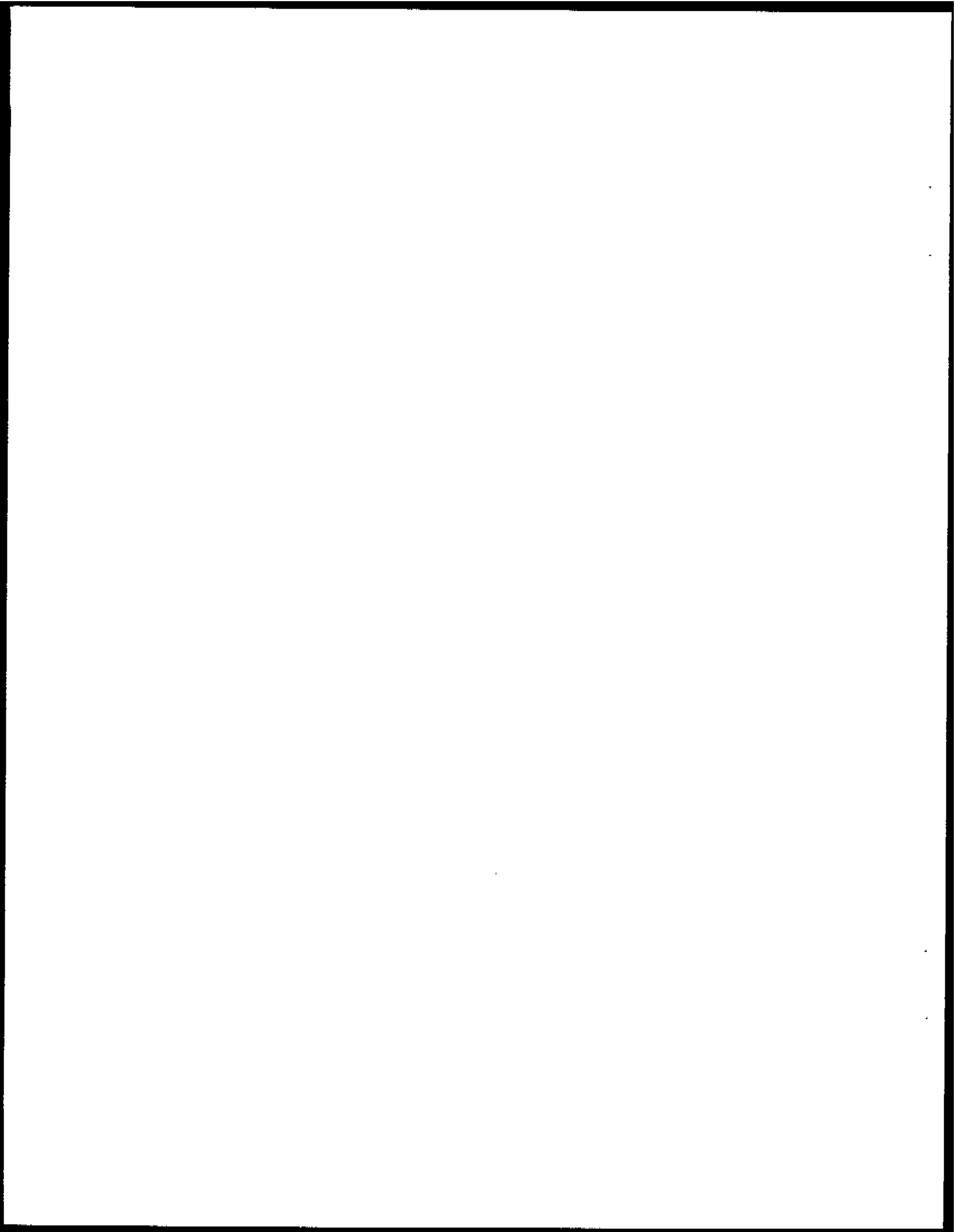
CF ANTIBODIES TO RS VIRUS

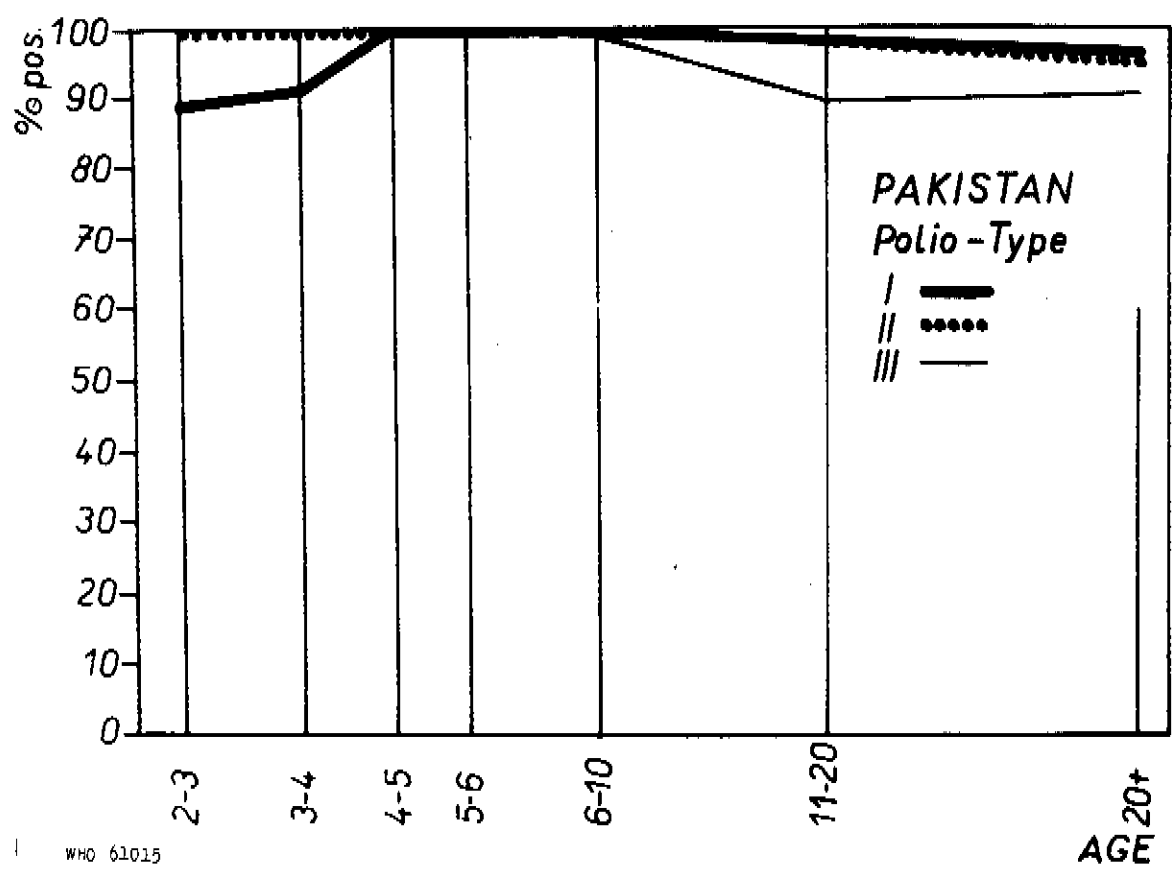
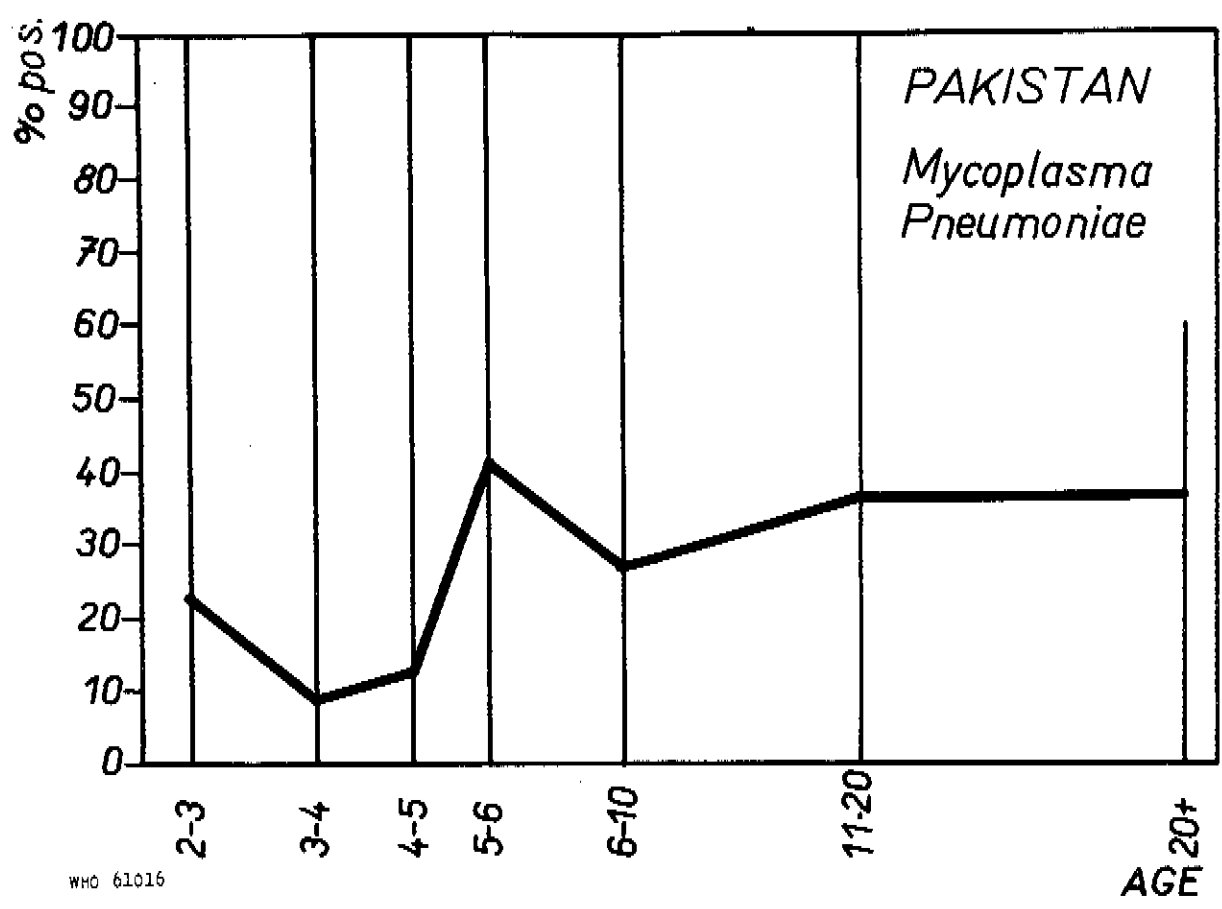
Age-group	No. of sera tested	Titres							Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	
2 - 3	9	8	0	0	0	1	0	0	11
3 - 4	13	12	0	0	0	1	0	0	8
4 - 5	9	9	0	0	0	0	0	0	0
5 - 6	13	11	0	1	1	0	0	0	15
6 - 10	46	39	2	2	1	1	1	0	15
11 - 20	76	52	4	3	9	7	1	0	32
20+	132	90	16	12	9	5	0	0	32
Total	298	221	22	18	20	15	2	0	

PAKISTAN
CF ANTIBODIES TO MYCOPLASMA PNEUMONIAE

Age-group	No. of sera tested	Titres							Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	
2 - 3	9	7	0	2	0	0	0	0	22
3 - 4	13	12	1	0	0	0	0	0	8
4 - 5	9	8	1	0	0	0	0	0	11
5 - 6	13	8	3	0	2	0	0	0	38
6 - 10	46	34	5	5	1	1	0	0	26
11 - 20	76	50	7	6	7	5	1	0	34
20+	132	87	17	18	7	2	1	0	34
Total	298	206	34	31	17	8	2	0	







PAKISTANI
POLIOVIRUS ANTIBODIES BY PH TEST

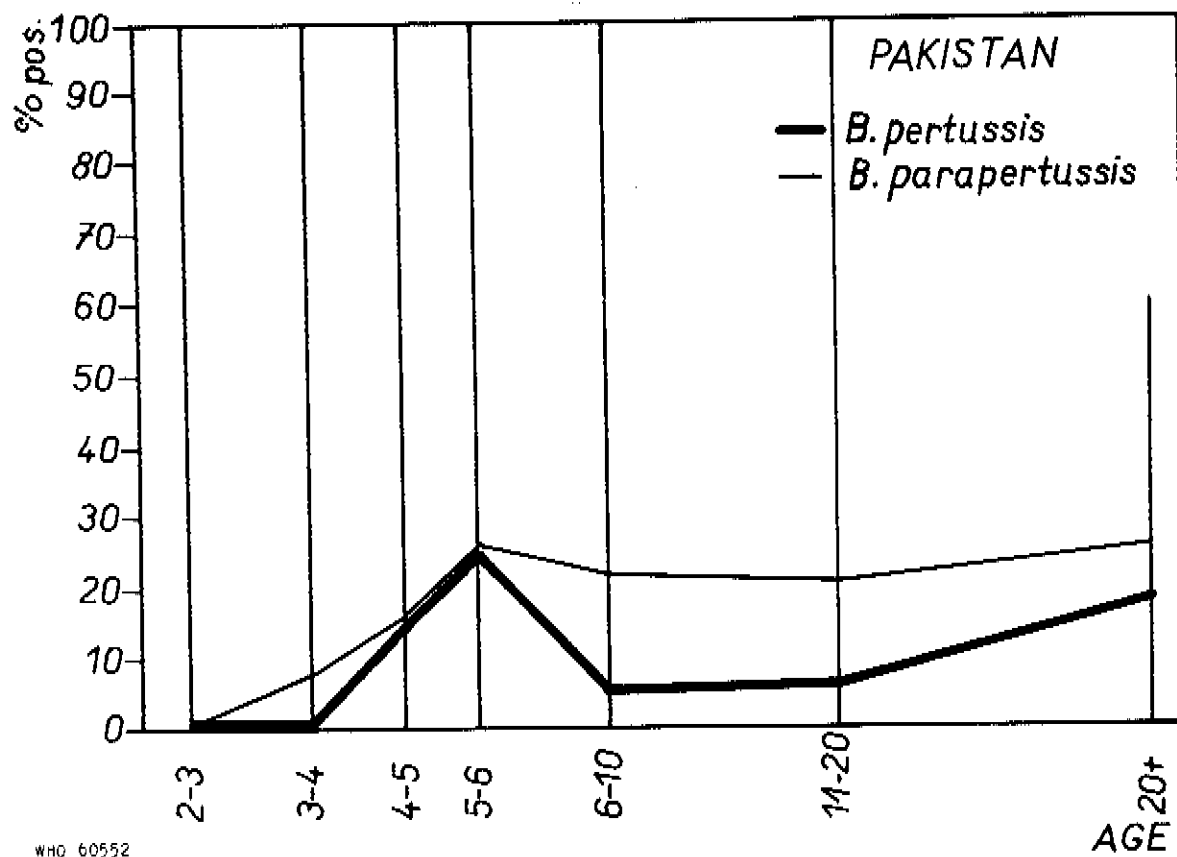
Age-group	No. of sera tested	Type																	
		I				II				III									
		Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives
2-3	9	1	1	2	2	3	89	0	2	2	1	4	100	1	4	1	2	1	89
3-4	13	1	1	3	5	3	92	0	0	3	7	3	100	1	3	3	3	3	92
4-5	7	0	0	0	4	3	100	0	0	3	3	1	100	0	1	1	4	2	100
5-6	9	0	0	2	2	5	100	0	1	3	3	2	100	0	0	5	3	1	100
6-10	38	0	1	9	22	6	100	0	3	9	21	5	100	0	7	14	15	2	100
11-20	73	1	5	30	25	12	99	1	6	35	22	9	99	7	18	35	6	7	90
20+	152	4	15	78	49	6	98	8	17	73	49	5	96	14	36	76	26	0	91
Total	301	7	23	124	109	38		9	29	128	106	29		23	63	135	59	16	

PAKISTAN
AGGLUTINATION ANTIBODIES B. PERTUSSIS

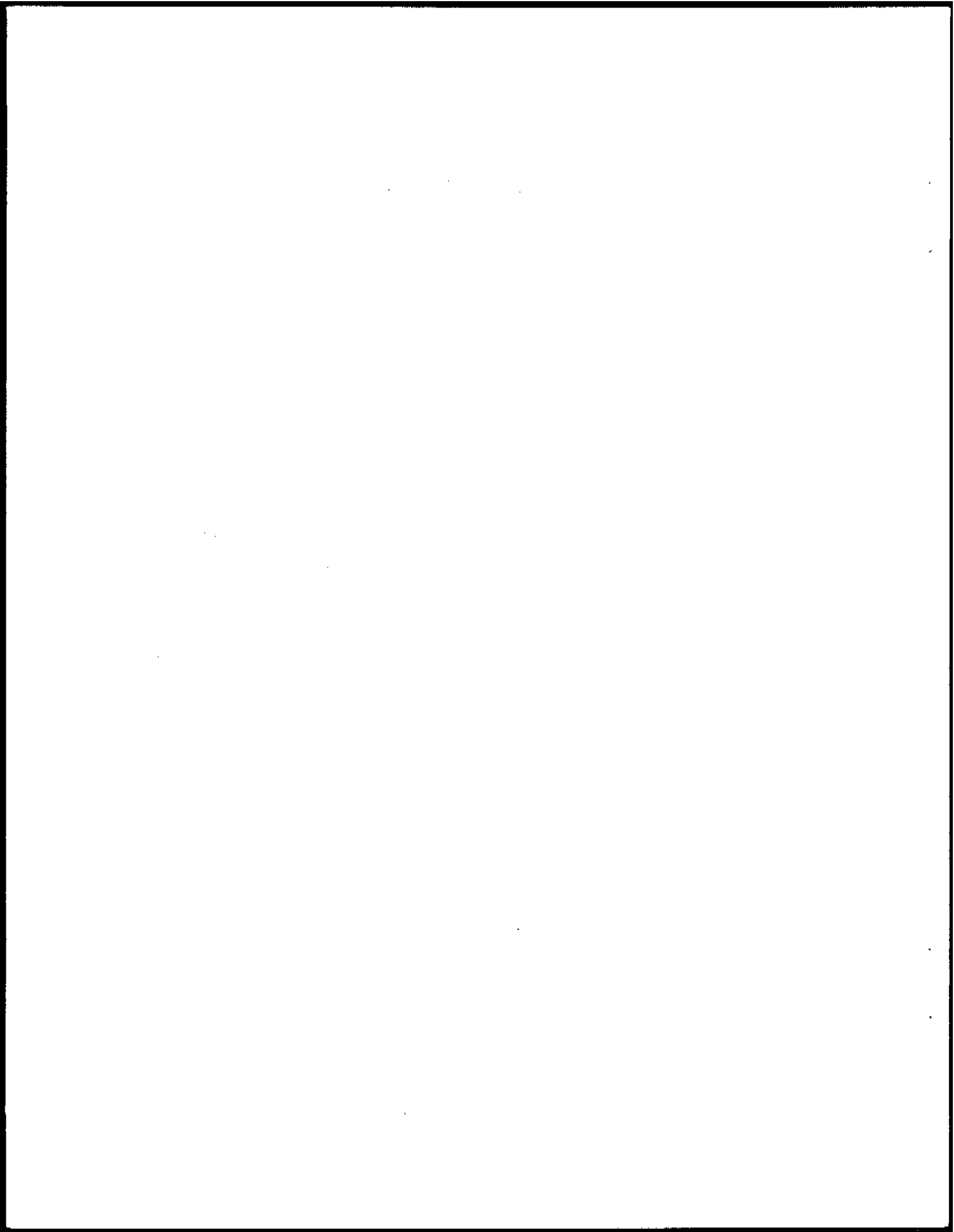
Age-group	No. of sera tested	Titres									Per cent. of positives
		Neg.	4	8	16	32	64	128	256	512	
2 - 3	8	8	0	0	0	0	0	0	0	0	0
3 - 4	13	13	0	0	0	0	0	0	0	0	0
4 - 5	7	6	0	0	0	1	0	0	0	0	14
5 - 6	8	6	0	0	1	0	0	0	0	1	25
6 - 10	39	37	0	0	1	1	0	0	0	0	5
11 - 20	71	66	0	0	1	2	1	1	0	0	7
20+	152	124	0	0	4	8	10	3	1	2	18
Total	298	260	0	0	7	12	11	4	1	3	

PAKISTAN
AGGLUTINATION ANTIBODIES TO B. PARAPERTUSSIS

Age-group	No. of sera tested	Titres									Per cent. of positives
		Neg.	4	8	16	32	64	128	256	512	
2 - 3	8	8	0	0	0	0	0	0	0	0	0
3 - 4	13	12	0	0	0	0	1	0	0	0	8
4 - 5	7	6	0	0	1	0	0	0	0	0	14
5 - 6	8	6	0	0	2	0	0	0	0	0	25
6 - 10	39	30	0	0	4	3	1	0	0	1	23
11 - 20	71	56	0	0	3	10	2	0	0	0	21
20+	152	113	0	0	4	17	8	7	2	1	26
Total	298	231	0	0	14	30	12	7	2	2	



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11. Togo sera - Out of the collection of sera obtained so far by the WHO Serum Reference Bank in Prague from Togo (assembled by WHO Treponematoses Advisory Team in 1964 and 1965), 186 specimens were selected. All these specimens had been collected at sampling points one to five situated at the south of Togo. This sub-sample was selected according to random numbers so that the individual age-groups would include approximately the following predetermined numbers of specimens:

Age-group (in years)	Number of sera
2 - 3	20
3 - 4	20
4 - 5	20
5 - 6	20
6 - 10	40
11 - 20	40
20+	40

These 186 specimens were examined for antibodies to the following antigens:

- All types of polioviruses
- Measles
- Streptolysin O
- B. pertussis and parapertussis

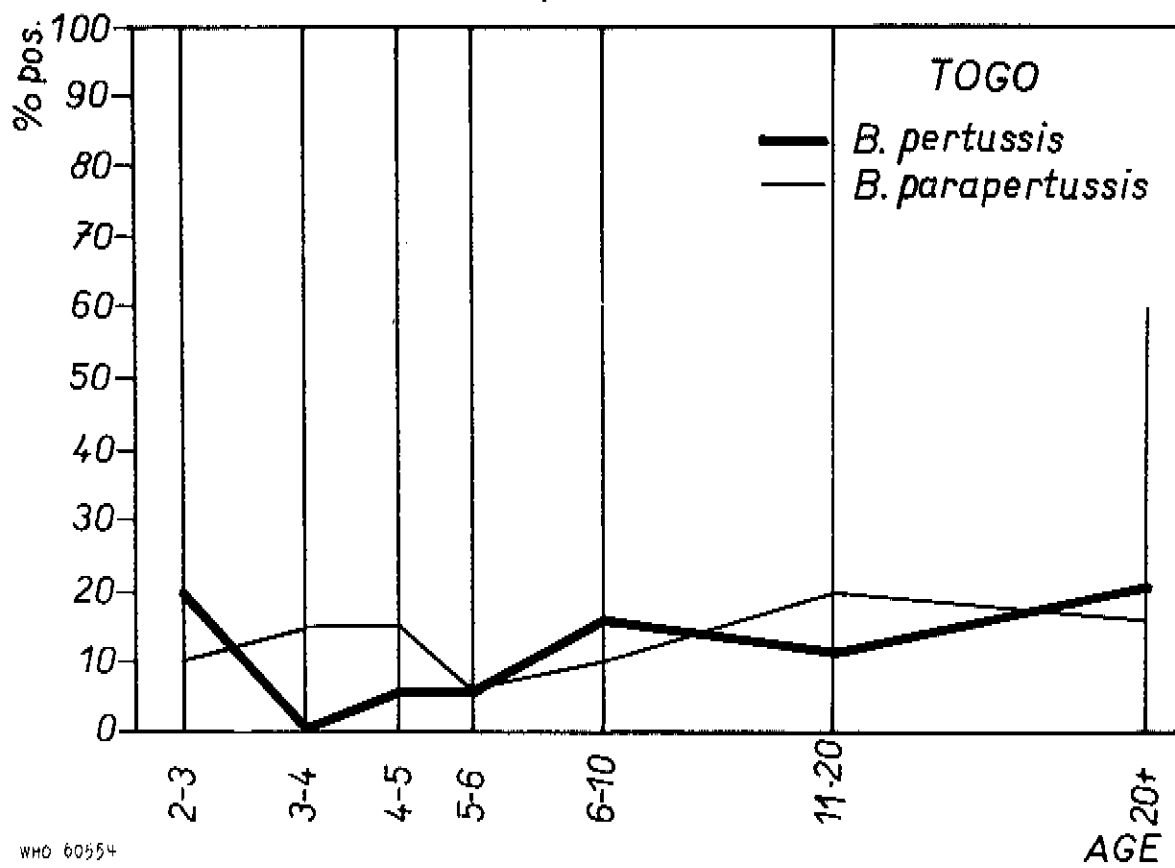
The tables and charts with these determinations accompany this report, the results of tests for ASLO levels have already been submitted to WHO headquarters.

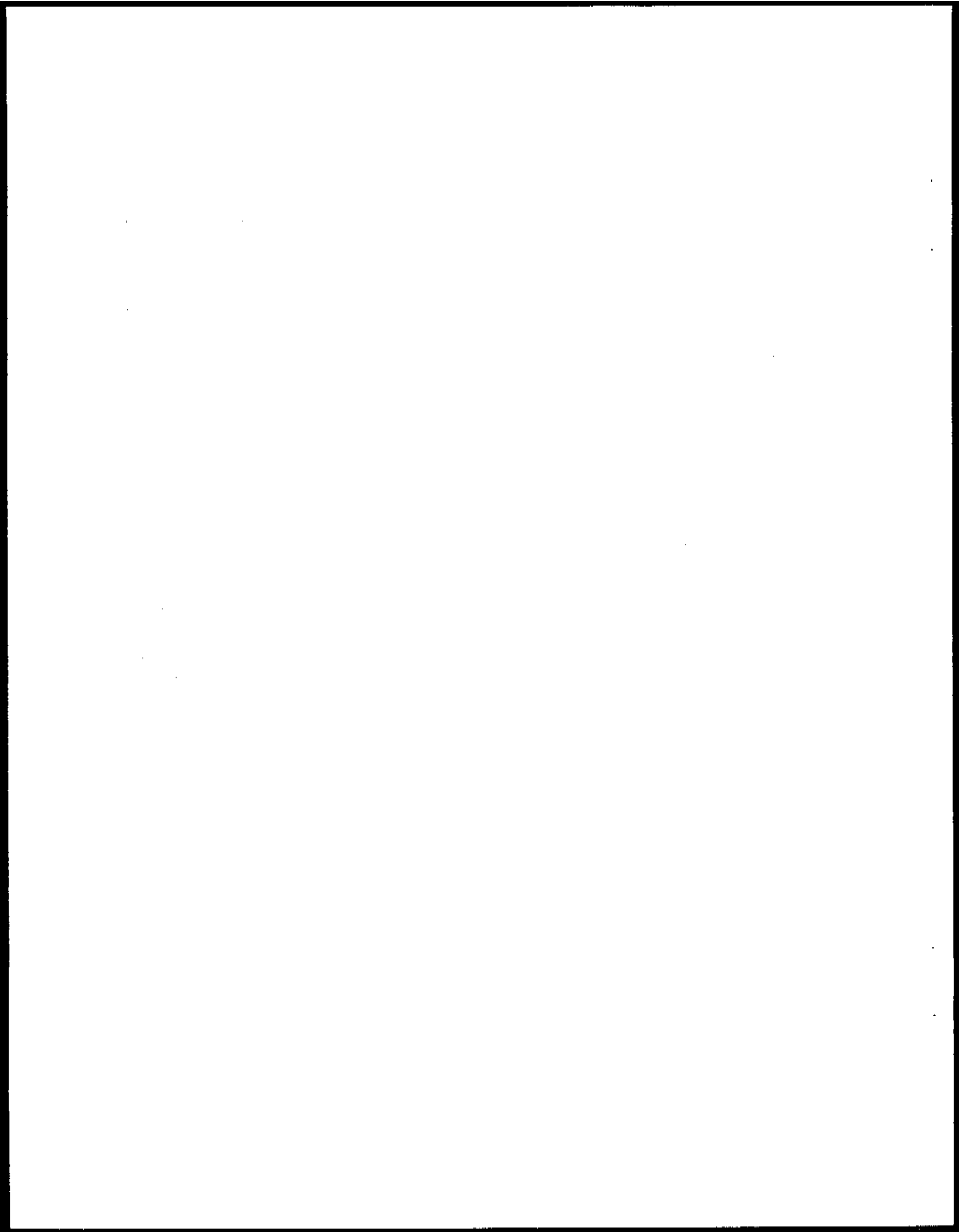
TOGO
AGGLUTINATION ANTIBODIES TO B. PERTUSSIS

Age-group	No. of sera tested	Titres									Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	
2 - 3	10	8	0	0	0	2	0	0	0	0	20
3 - 4	19	19	0	0	0	0	0	0	0	0	0
4 - 5	19	18	0	0	1	0	0	0	0	0	5
5 - 6	20	19	0	0	0	0	1	0	0	0	5
6 - 10	40	33	0	0	3	2	0	1	1	0	17
11 - 20	40	35	0	0	1	2	2	0	0	0	12
20+	40	31	0	0	6	2	1	0	0	0	22
Total	188	163	0	0	11	8	4	1	1	0	

AGGLUTINATION ANTIBODIES TO B. PARAPERTUSSIS

Age-group	No. of sera tested	Titres									Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	
2 - 3	10	9	0	0	0	1	0	0	0	0	10
3 - 4	19	16	0	0	3	0	0	0	0	0	16
4 - 5	19	16	0	0	3	0	0	0	0	0	16
5 - 6	20	19	0	0	0	1	0	0	0	0	5
6 - 10	40	36	0	0	2	1	0	1	0	0	10
11 - 20	40	32	0	0	4	2	0	2	0	0	20
20+	40	34	0	0	4	2	0	0	0	0	15
Total	188	162	0	0	16	7	0	3	0	0	



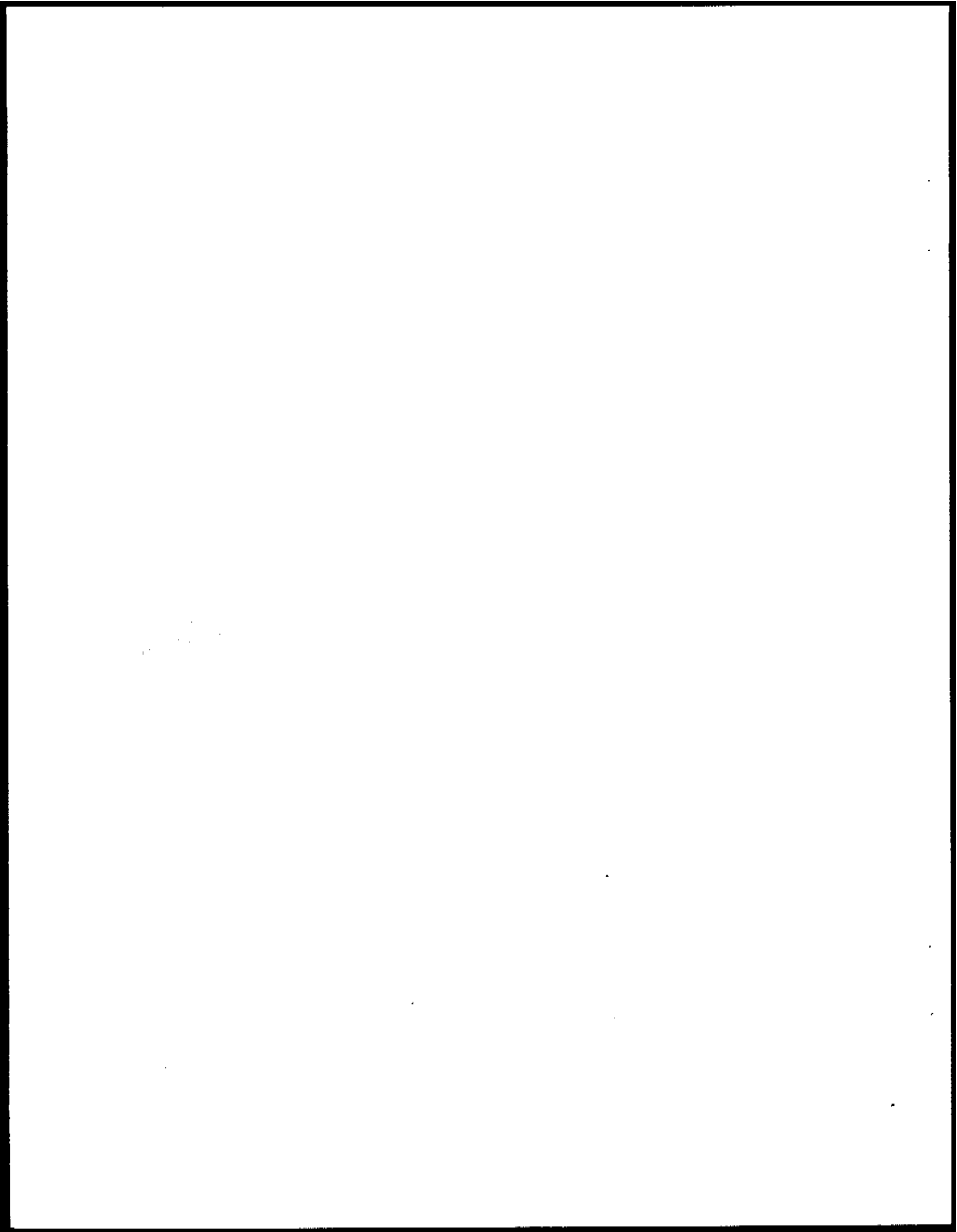


TOGO
HI ANTIBODIES TO MEASLES

Age-group	No. of sera tested	Titres								Per cent. of positives
		Neg.	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
2 - 3	12	8	0	1	1	2	0	0	0	33
3 - 4	19	10	1	3	1	3	0	0	1	48
4 - 5	21	7	1	2	5	4	2	0	0	67
5 - 6	20	10	0	2	2	3	2	0	1	50
6 - 10	40	15	4	3	6	8	1	1	2	63
11 - 20	40	15	5	9	3	3	3	1	1	63
20+	39	12	7	9	5	6	0	0	0	70
Total	191	77	18	29	23	29	8	2	5	

1000
 PCELEPHUS ANTENODIES BY AGE TEST

Age- group	No. of sera tested	Type																	
		I					II					III							
		Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives
2-3	9	2	1	3	1	2	76	2	1	4	1	1	78	4	3	2	0	0	56
3-4	19	1	2	5	6	5	95	0	2	6	7	4	100	5	5	8	0	1	74
4-5	20	1	2	9	7	1	95	1	3	6	8	2	95	1	4	11	1	3	95
5-6	20	1	2	9	6	2	95	3	3	4	8	2	85	1	5	10	3	1	95
6-10	40	2	7	16	12	3	95	1	15	16	5	3	98	2	16	16	6	0	95
11-20	39	0	10	16	11	2	100	3	6	22	5	3	92	2	12	21	2	2	95
20+	40	2	9	19	7	3	95	0	7	19	10	4	100	2	22	15	3	0	95
Total	187	9	33	77	50	18		10	37	77	44	19		17	67	81	15	7	



12. Eastern Nigeria sera - The Prague Serum Reference Bank obtained a total of 2591 serum specimens collected by the WHO Treponematoses Advisory Team in 50 sampling points in Eastern Nigeria during 1964. The age distribution of these specimens is as follows:

Age-group	No. of specimens	Per cent.
2	33	1.2
2 - 3	45	1.7
3 - 4	114	4.4
4 - 5	128	4.9
5 - 9	970	37.0
10 - 14	556	21.0
15 - 19	62	2.4
20 - 24	63	2.4
25 - 29	108	4.2
30 - 39	206	8.0
40 - 49	147	5.7
50 - 59	80	3.1
60+	79	3.1
Total	2 591	100

The collection has been divided into the following three sub-samples:

Sub-sample	Area	Sampling point number	No. of specimens tested
I	North-east (thinly populated)	1, 2, 3, 4, 5, 6, 20, 21, 46	158
II	North-west (densely populated)	13, 14, 15, 16, 17, 18, 19, 22, 23, 24, 25, 26, 32	164
III	Southern coastal area	7, 8, 9, 10, 11, 12, 34, 37, 40, 41, 42, 48, 49	239
		Total	561

As the number of sera in the age-groups up to five years and above 15 years was small, all the specimens available were examined; in the age-groups five to nine and 10-14 years only part of the specimens were included in the sub-samples. A total of 561 specimens were examined for antibodies to the following antigens:

All types of polioviruses

Measles

B. pertussis and parapertussis

and in addition to this 180 specimens were tested for diphtheria antitoxin levels.

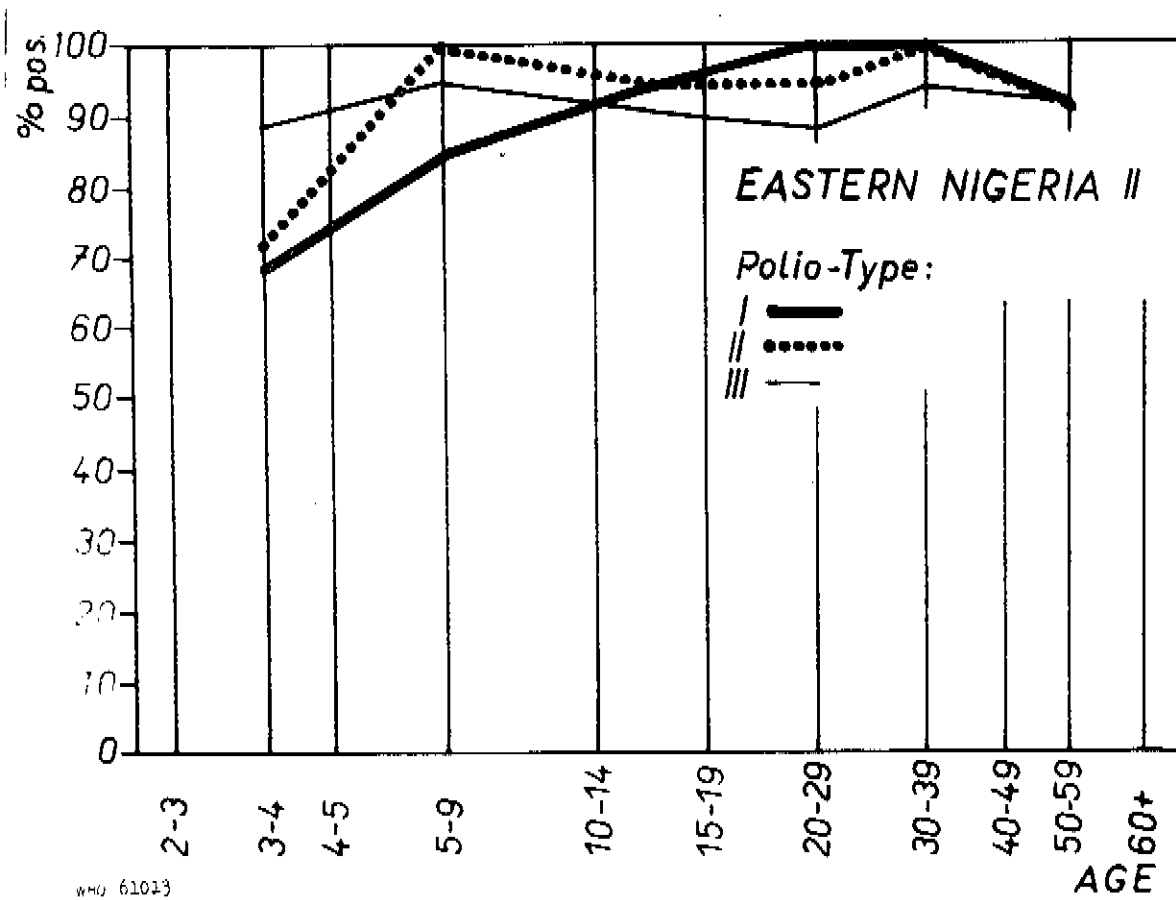
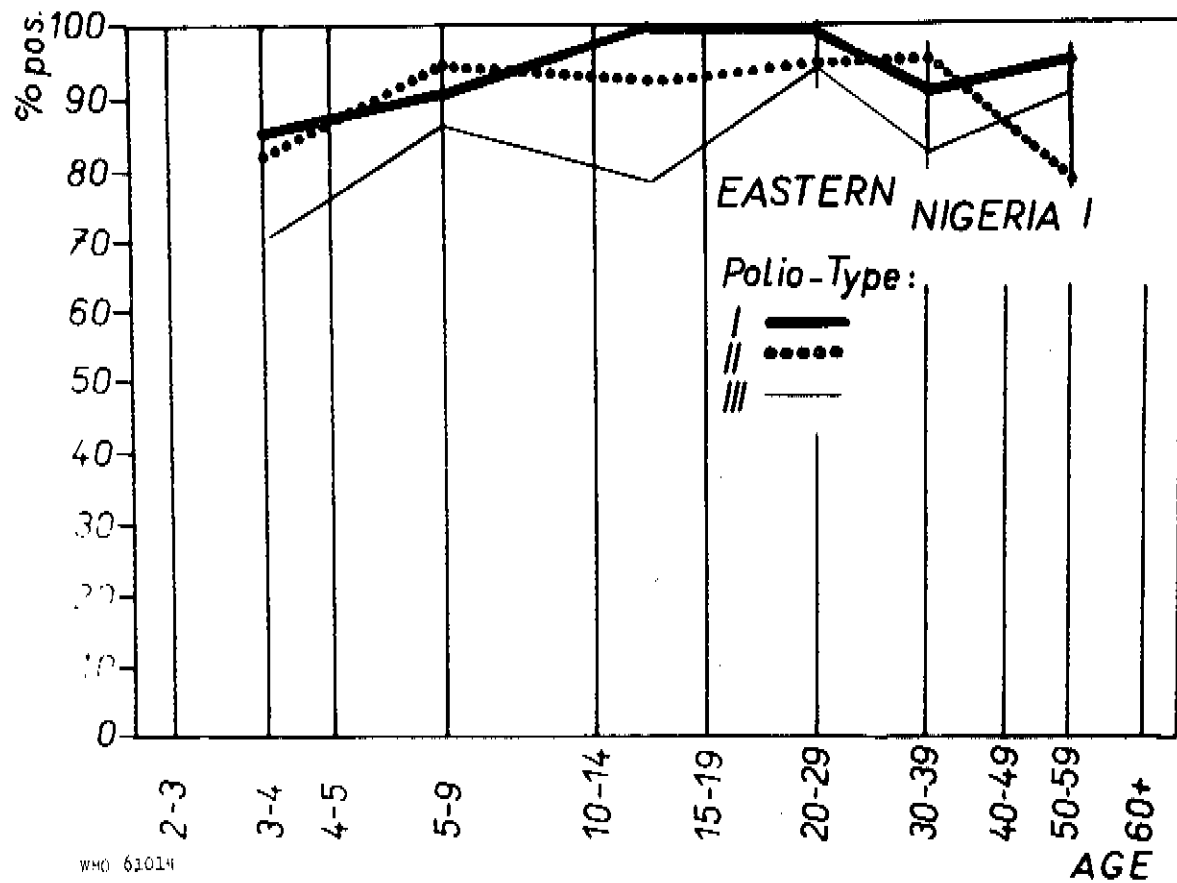
The results of testing the individual sub-samples and a table and chart summarizing the determinations for the whole area of Eastern Nigeria is attached to this report.

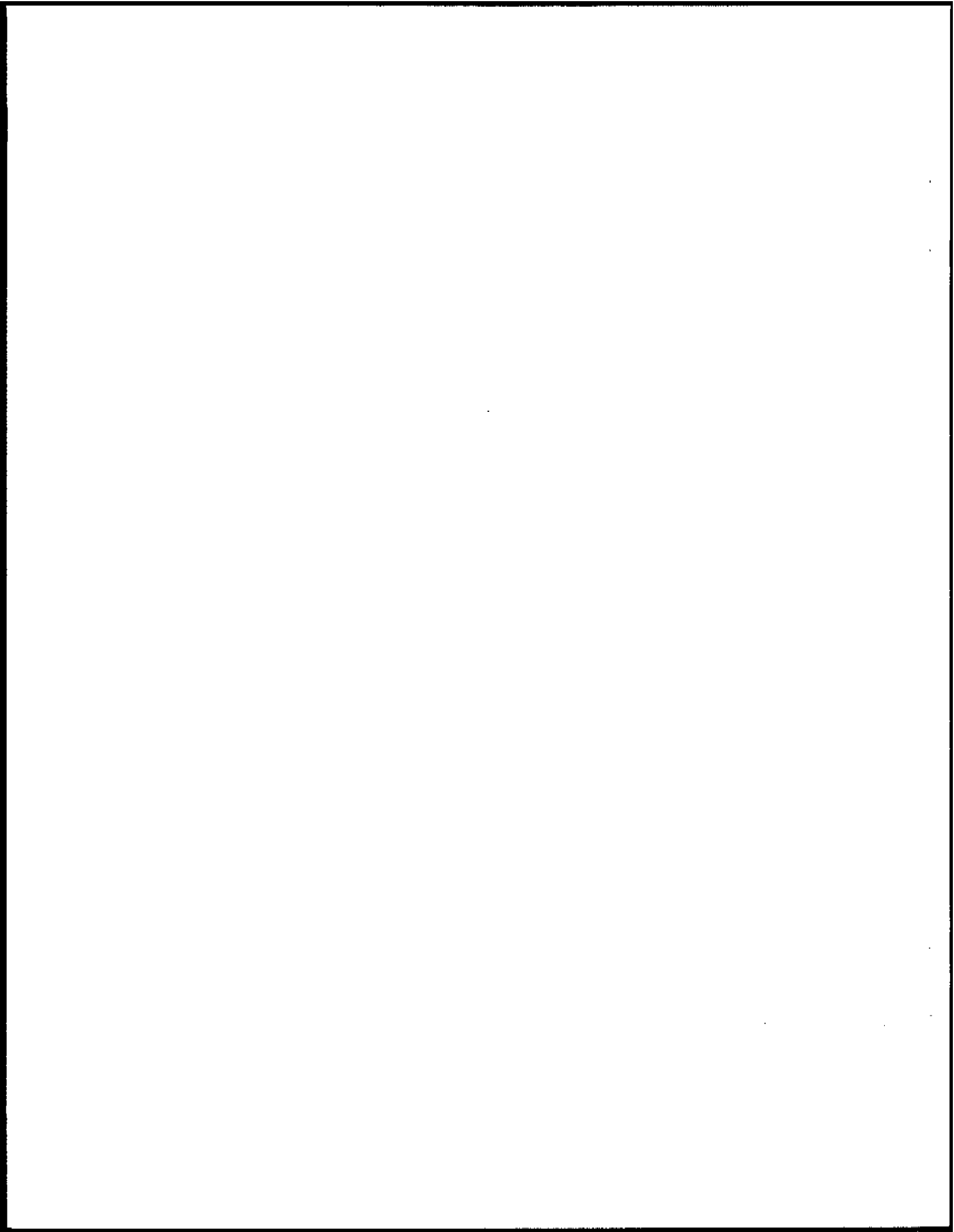
EASTERN NIGERIA (NORTHERN PART I)
POLIOVIRUS ANTIBODIES BY I_H TEST

Age-group	No. of sera tested		Type															
			I					II					III					
			Neg.	16	5-	25G+	Per cent. of positives	Neg.	4	15	6+	25G+	Per cent. of positives	Neg.	4	16	6+	25G+
2-5	1	2	14	1	3	87	5	4	8	7	6	33	9	5	7	7	2	70
6-9	2	2	10	7	3	92	1	7	10	4	2	96	3	10	7	4	0	87
10-19	0	9	14	1	1	100	2	6	8	12	3	93	6	5	13	4	1	79
20-29	0	5	10	0	0	100	1	3	10	5	3	95	1	10	5	3	0	95
30-39	2	6	14	2	0	92	1	12	7	4	0	96	4	10	9	0	1	83
40+	1	8	10	5	0	96	5	6	8	5	0	79	2	16	5	1	0	92
Total	9	32	72	30	7		15	38	51	37	9		25	56	46	19	4	

EASTERN NIGERIA (NORTHERN PART II)
 POLIOVIRUS ANTIBODIES BY pH TEST

Age-group	No. of sera tested	Type																	
		I						II						III					
		Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives
2-5	26	8	1	11	4	2	69	7	5	6	7	1	73	3	7	10	4	2	89
6-9	29	4	1	8	10	6	86	0	5	8	12	4	100	1	7	17	3	1	96
10-19	37	2	8	16	13	1	95	2	7	12	14	2	95	3	15	14	4	1	92
20-29	18	0	4	11	3	0	100	1	8	7	2	0	95	2	9	6	0	1	89
30-39	17	0	7	4	4	2	100	0	6	6	4	1	100	1	6	7	2	1	94
40+	27	2	9	13	7	0	93	2	12	8	5	0	93	2	13	9	3	0	93
Total	154	16	30	63	54	11		12	43	47	44	8		12	57	63	16	6	



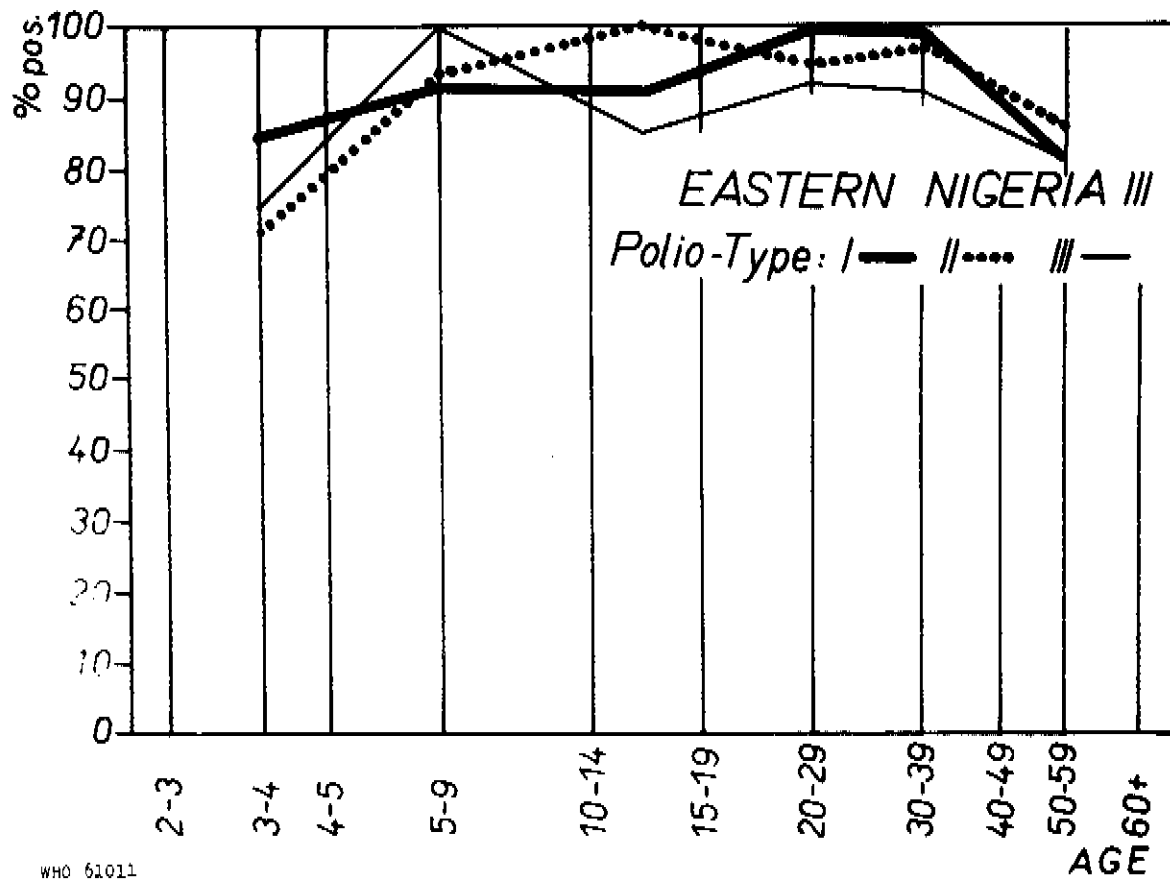
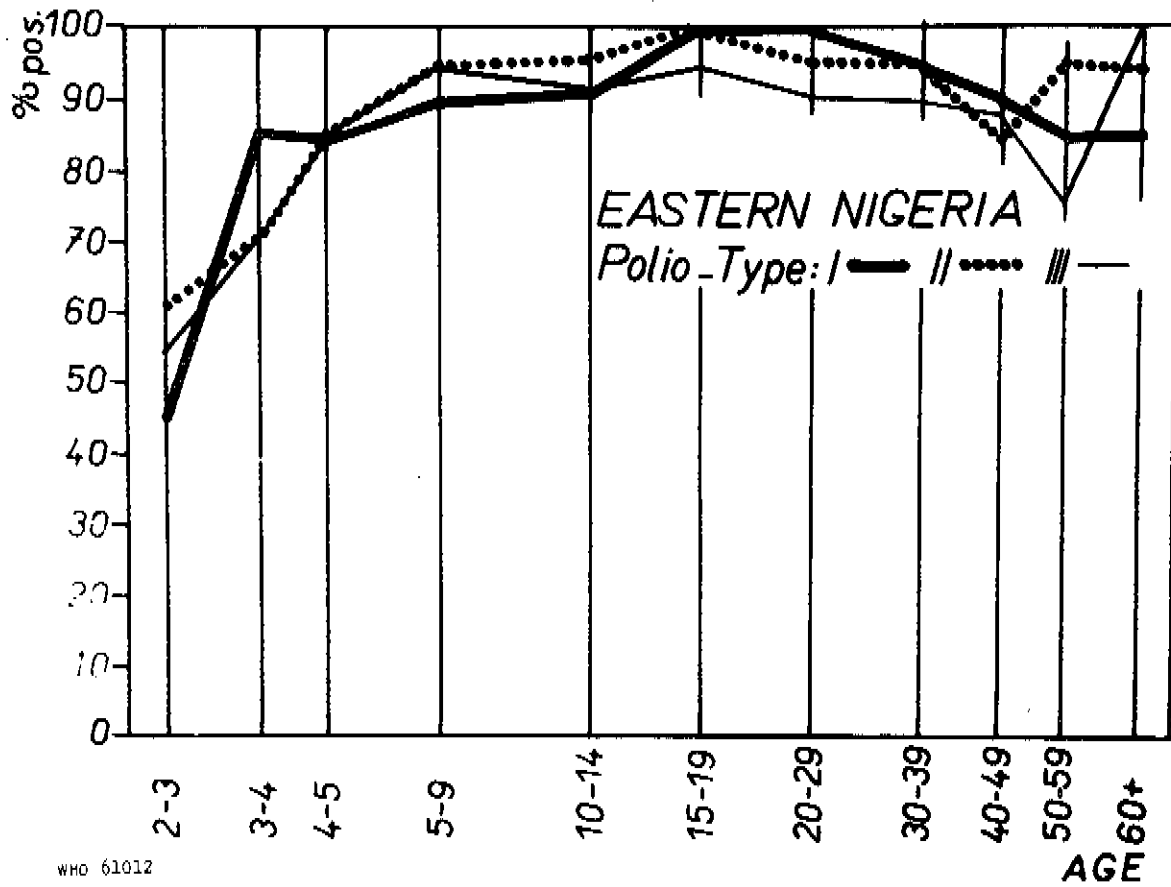


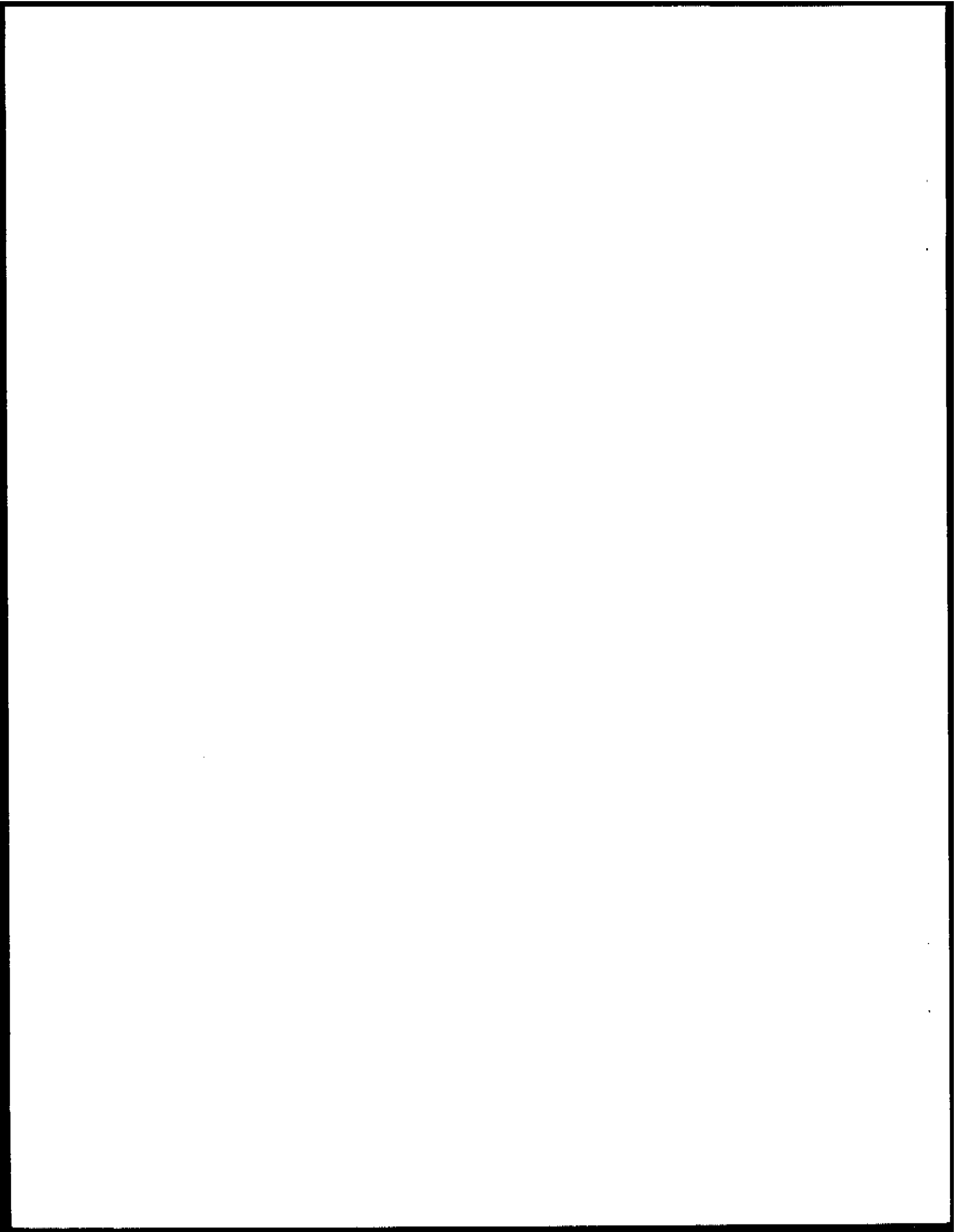
EASTERN NIGERIA (SUMMARY)
POLIOVIRUS ANTIBODIES BY pH TEST

Age- group	No. of sera tested	Type																	
		I					II					III							
		Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives
2-3	13	7	1	2	3	0	46	5	2	2	2	62	6	4	2	0	0	1	54
3-4	31	4	3	15	5	4	87	9	5	9	5	71	9	5	12	5	5	0	71
4-5	37	5	3	13	11	5	86	5	5	10	8	86	5	10	11	3	3	3	86
5-9	81	8	8	26	26	13	90	3	16	22	11	96	4	26	32	15	4	4	95
10-14	92	6	21	41	21	3	93	3	13	34	3	97	8	27	43	12	2	2	91
15-19	21	0	4	13	4	0	100	1	5	8	5	95	7	4	1	1	0	0	66
20-29	64	0	16	34	11	3	100	3	15	26	3	95	5	27	21	10	1	1	92
30-39	79	3	22	36	14	4	96	2	30	29	2	97	8	25	36	6	4	4	90
40-49	56	5	19	23	8	1	91	6	18	25	7	84	7	22	19	8	0	0	88
50-59	34	5	9	16	4	0	85	9	15	8	2	37	8	14	9	3	0	0	76
60+	26	4	8	8	6	0	95	1	10	9	6	96	0	15	5	3	0	0	100
Total	534	47	114	227	117	23		47	122	178	141		67	179	194	71	15	15	

EASTERN NIGERIA (SOUTHERN PART)
POLIOVIRUS ANTIBODIES BY pH TEST

Age-group	No. of sera tested	Type															Per cent. of positives		
		I					II					III							
		Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16	64	256+	Per cent. of positives	Neg.	4	16		64	256+
2-5	25	4	4	5	8	4	84	7	1	3	6	8	72	8	7	8	2	0	76
6-9	28	2	5	8	9	4	93	2	4	4	13	5	93	0	9	8	8	3	100
10-14	47	4	8	24	10	1	92	0	5	22	18	2	100	6	16	20	5	0	87
20-29	27	0	7	13	4	3	100	1	4	9	10	3	96	2	8	10	7	0	93
30-39	38	1	9	18	8	2	98	1	12	16	8	1	98	3	9	20	4	2	92
40+	65	11	19	24	10	1	83	9	25	26	5	0	86	11	22	22	10	0	85
Total	220	22	52	92	49	15		20	51	80	60	19		30	71	88	36	5	





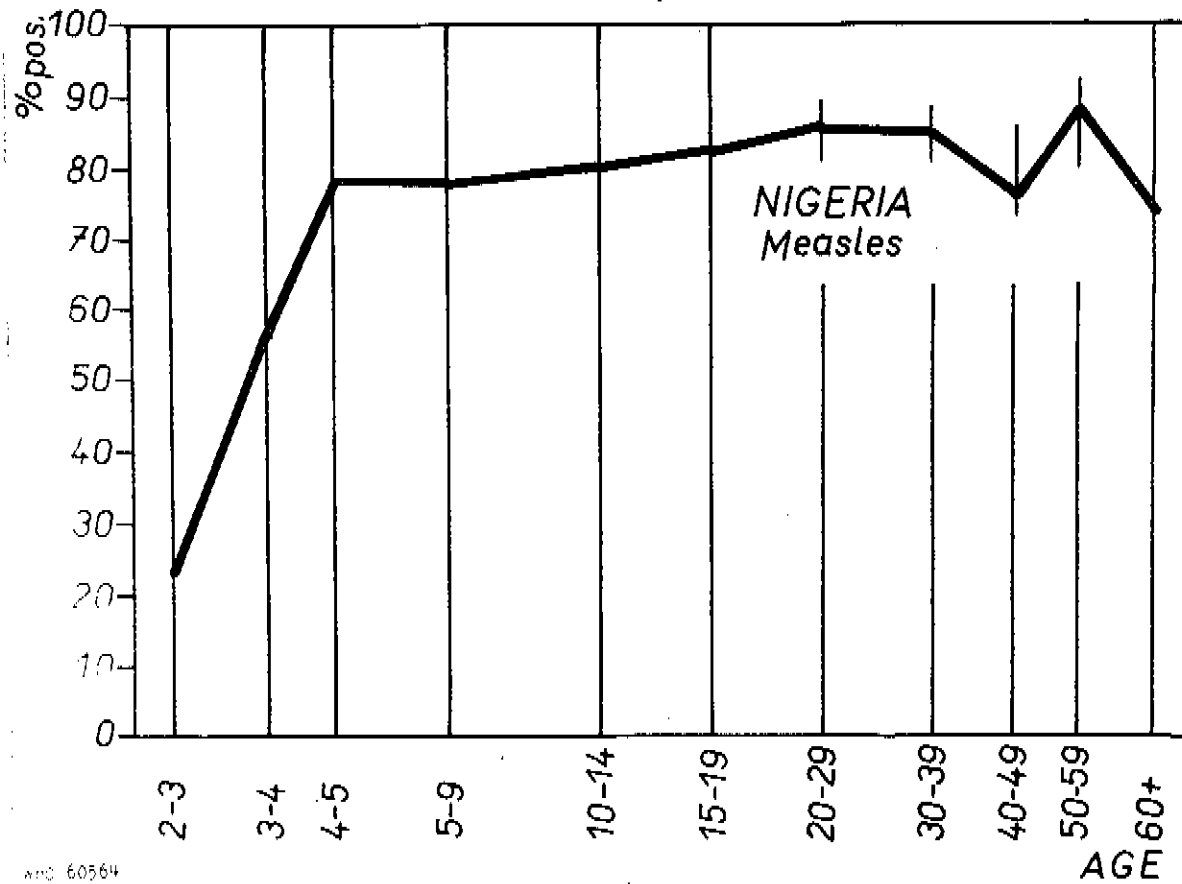
EASTERN NIGERIA
HI ANTIBODIES TO MEASLES

Age-group	No. of sera tested	Titres										Per cent. of positives
		Neg.	2	4	8	16	32	64	128	256	512	
2 - 3	13	10	0	0	0	1	1	1	0	0	0	23
3 - 4	33	15	1	0	3	2	4	1	2	5	0	55
4 - 5	40	9	2	3	7	10	4	1	3	1	0	78
5 - 9	88	19	9	4	12	10	12	11	8	3	0	78
10 - 14	89	18	9	11	12	18	9	7	2	2	1	80
15 - 19	23	4	1	4	5	4	3	0	1	1	0	82
20 - 29	66	9	5	9	16	7	11	5	3	1	0	86
30 - 39	82	12	8	16	15	13	8	7	1	2	0	85
40 - 49	56	15	6	9	7	9	6	3	1	0	0	73
50 - 59	34	4	3	6	8	2	3	6	0	2	0	88
60+	29	8	4	1	4	5	3	3	0	1	0	72
Total	553	123	48	63	89	81	64	45	21	18	1	

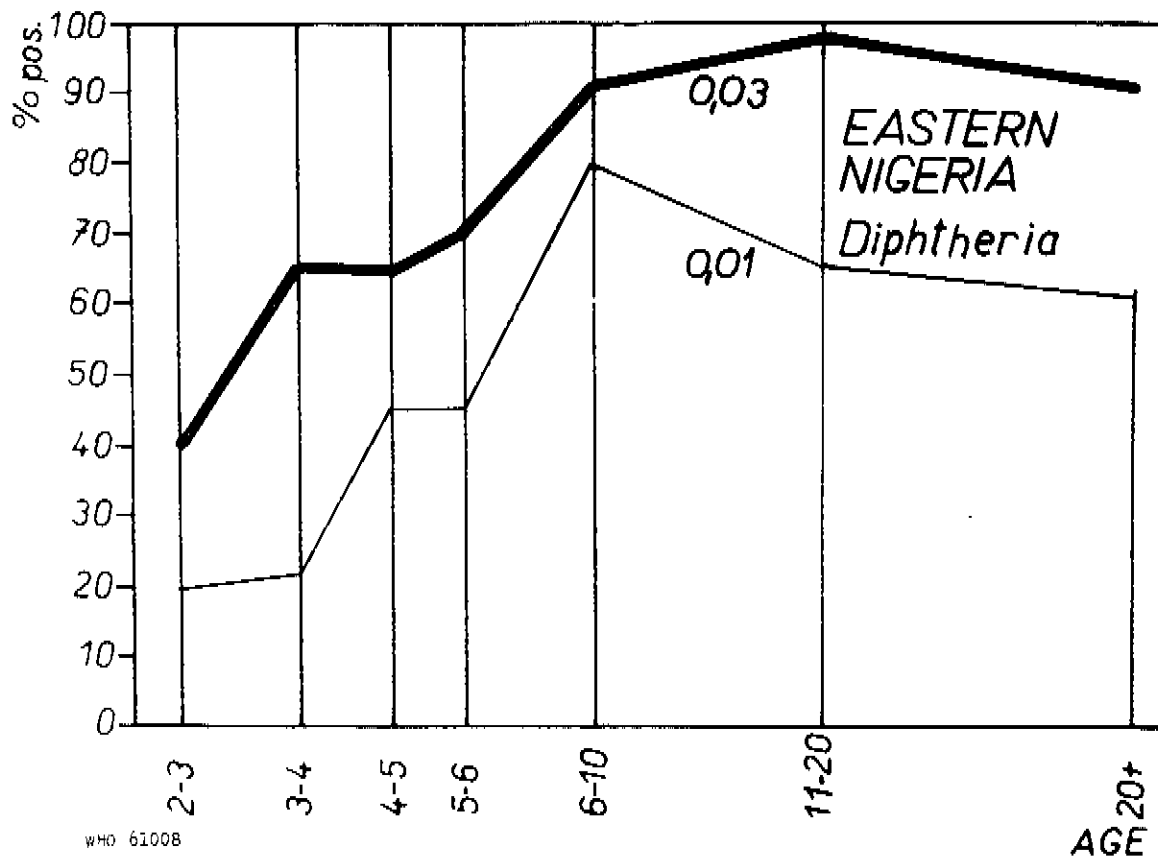
EASTERN NIGERIA
DIPHTHERIA ANTITOXIN LEVELS

Age-group	No. of sera tested	Titres (I U/ml)					Per cent. of positives (0.03 and over)
		<0.03	0.03-0.09	0.1-0.49	0.5-0.9	>1	
2 - 3	5	3	1	1	0	0	40
3 - 4	17	6	7	4	0	0	65
4 - 5	20	7	4	4	1	4	65
5 - 6	20	6	5	9	0	0	70
6 - 10	39	3	4	14	12	6	92
11 - 20	40	1	12	14	9	4	98
20+	39	3	12	15	7	2	92
Total	180	29	45	61	29	16	

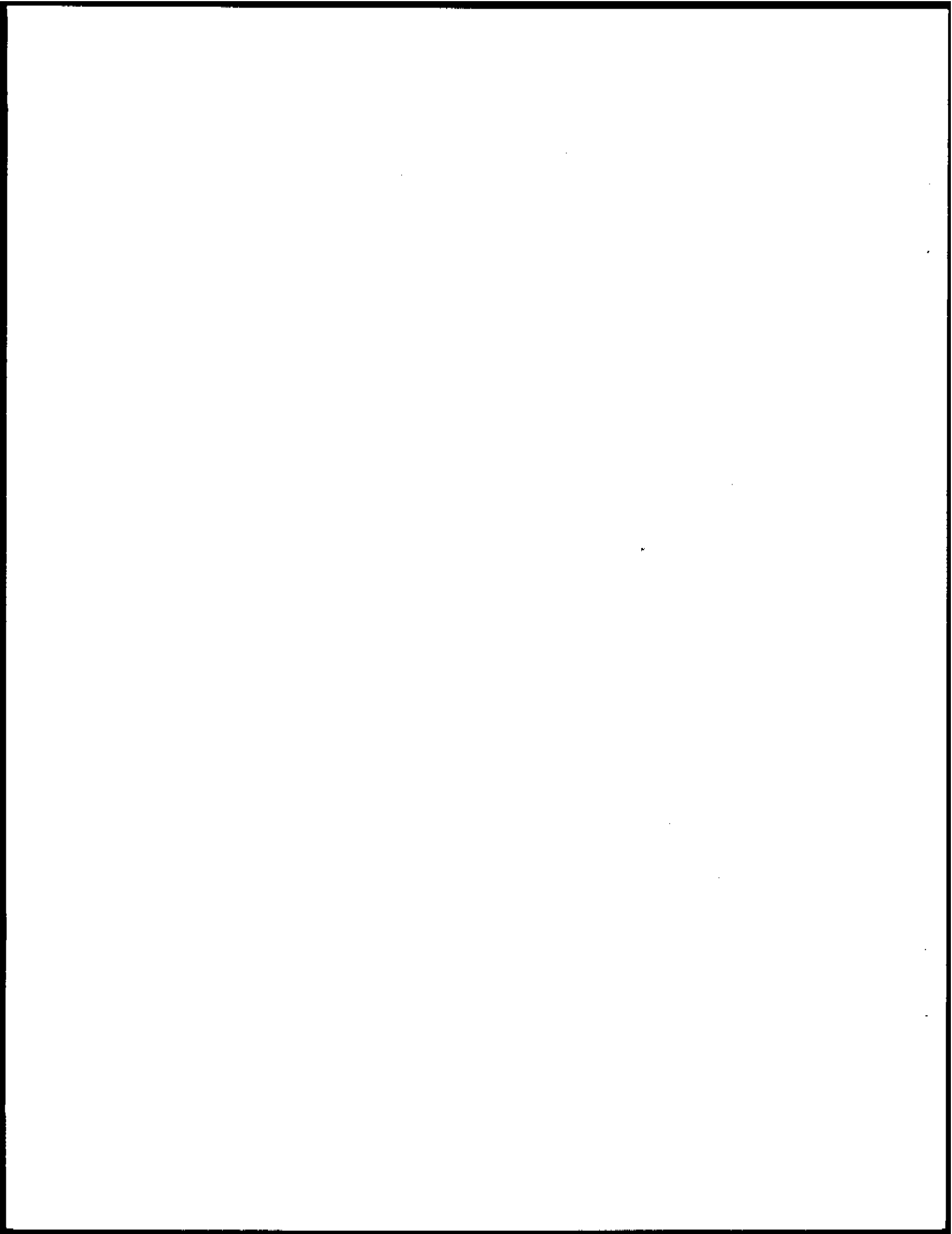
Summary



WHO 60564



WHO 61008



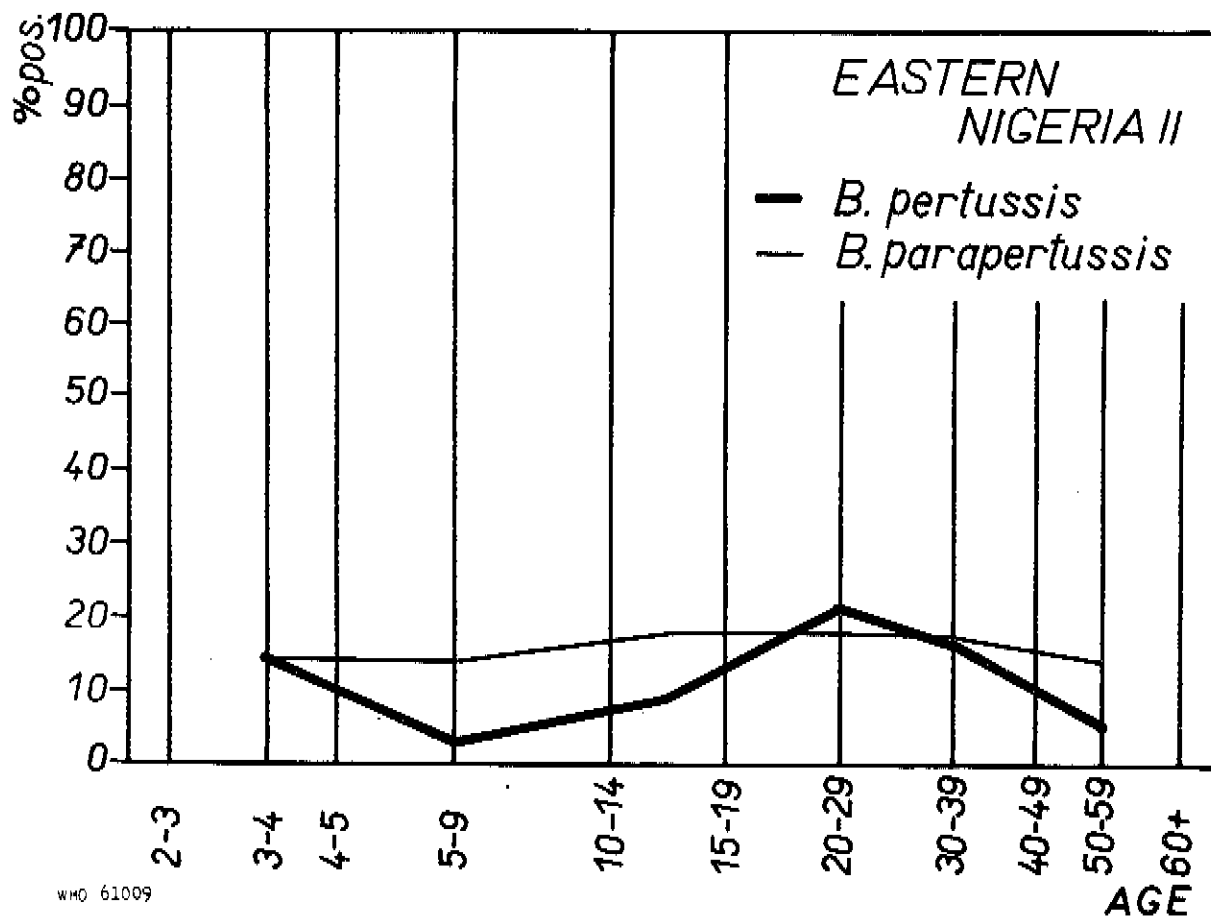
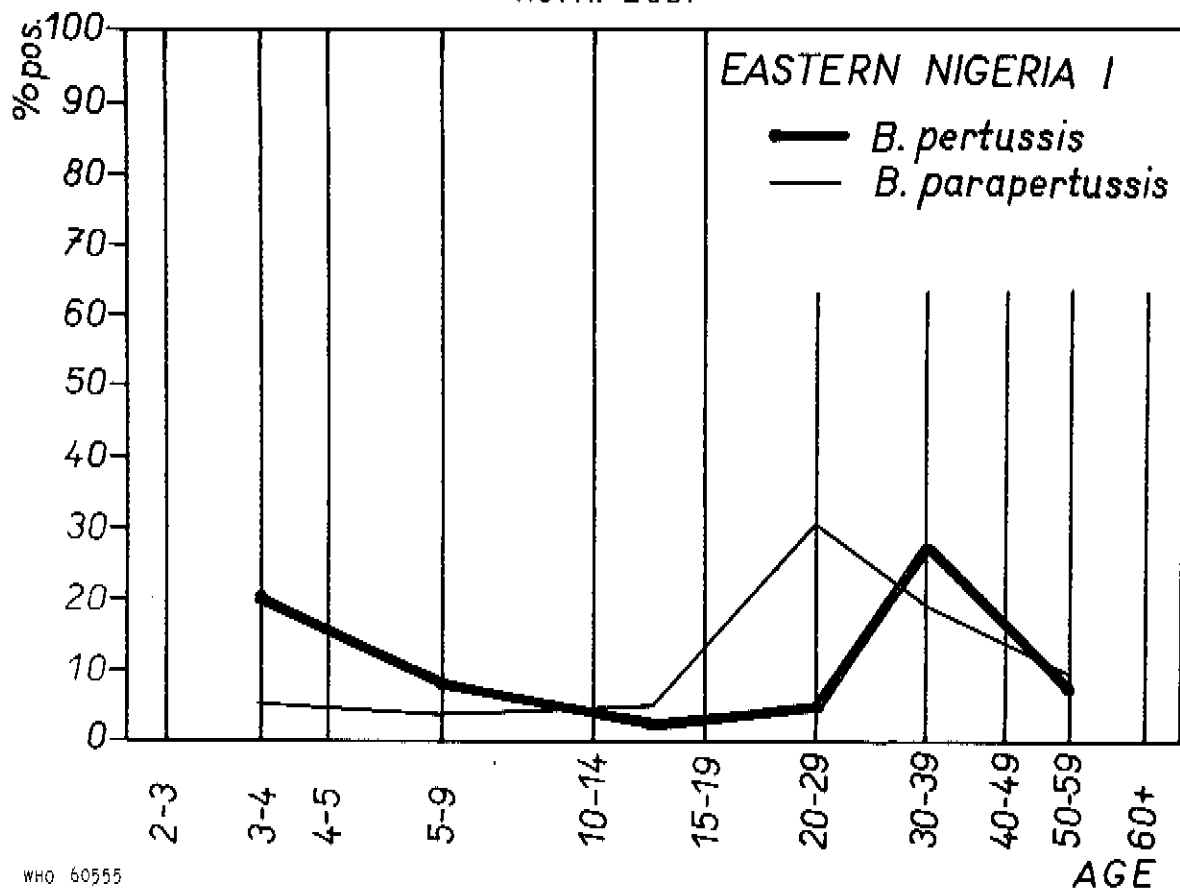
EASTERN NIGERIA (NORTHERN PART I)

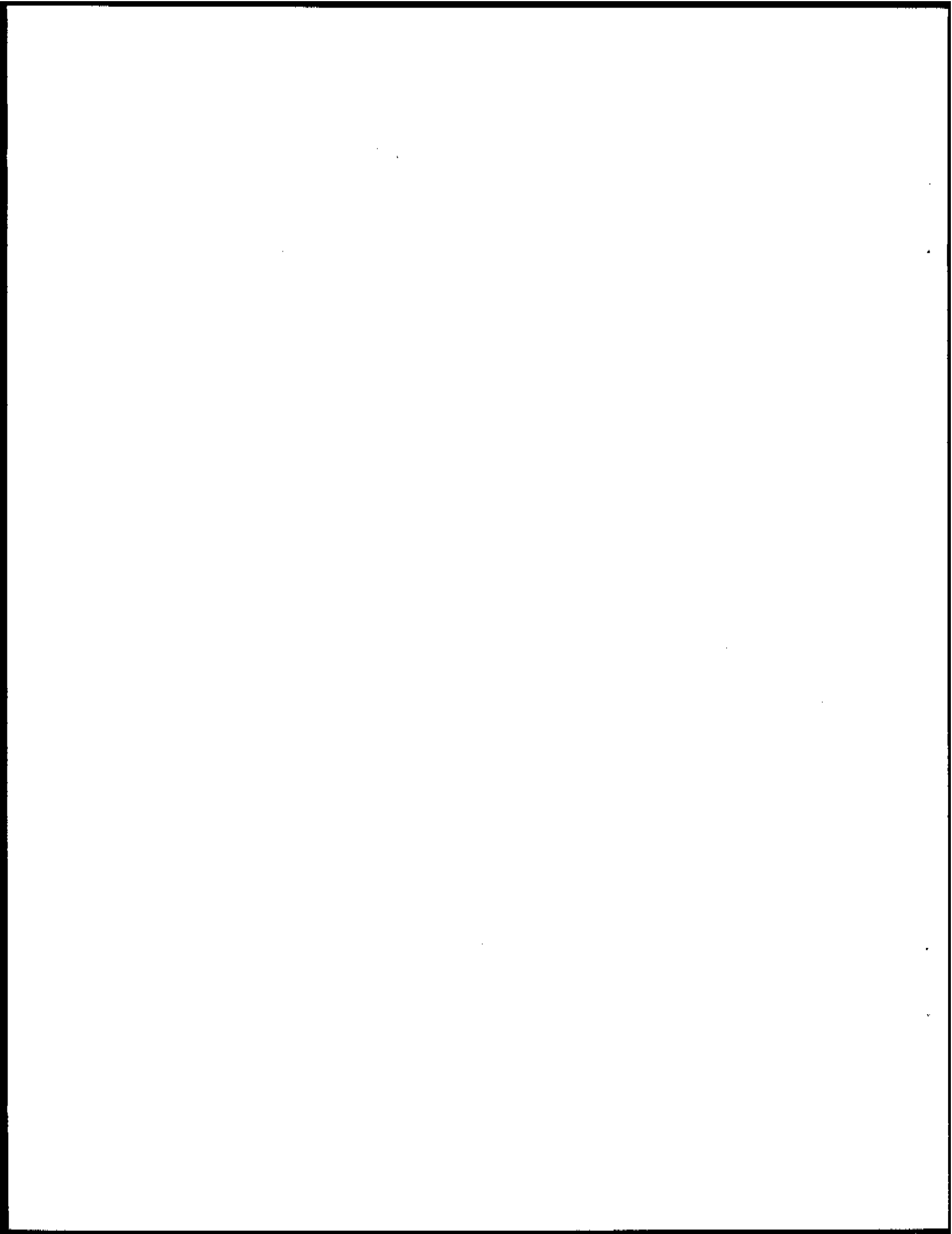
Age- Group	No. of sera tested	B. pertussis antibodies							B. parapertussis antibodies							Per cent. of positives			
		Reg.	1:8	16	32	64	128	256	512	Per cent. of positives	Reg.	1:8	16	32	64		128	256	512
2 - 5	30	24	0	2	1	1	2	0	0	20	28	0	0	0	2	0	0	0	7
6 - 9	25	23	0	1	1	0	0	0	0	8	24	0	0	0	0	0	0	0	4
10 - 19	32	31	0	0	1	0	0	0	0	3	30	0	0	0	0	0	0	0	6
20 - 29	19	18	0	0	0	1	0	0	0	5	13	0	0	4	1	0	0	0	31
30 - 39	28	20	1	3	0	1	3	0	0	28	22	1	2	3	0	0	0	0	21
40+	24	22	0	0	0	2	0	0	0	8	21	0	1	2	0	0	0	0	12
Total	158	130	1	6	3	5	5	0	0		138	1	10	6	3	0	0	0	

EASTERN NIGERIA (NORTHERN PART II)

Age- Group	No. of sera tested	B. pertussis antibodies							B. parapertussis antibodies										
		Neg.	1:8	16	32	64	128	256	512	Per cent. of positives	Neg.	1:8	16	32	64	128	256	512	Per cent. of positives
2-5	28	24	2	1	0	1	0	0	0	14	24	0	1	2	1	0	0	0	14
6-9	30	29	1	0	0	0	0	0	0	3	26	2	2	0	0	0	0	0	13
10-19	41	37	1	1	0	1	1	0	0	10	34	3	0	2	1	1	0	0	17
20-29	18	14	0	1	2	1	0	0	0	22	15	1	1	1	0	0	0	0	17
30-39	18	15	2	1	0	0	0	0	0	17	15	3	0	0	0	0	0	0	17
40+	29	27	0	1	0	0	0	1	0	7	25	2	1	1	0	0	0	0	14
Total	164	145	6	5	2	3	1	1	0		139	11	5	6	2	1	0	0	

North-East



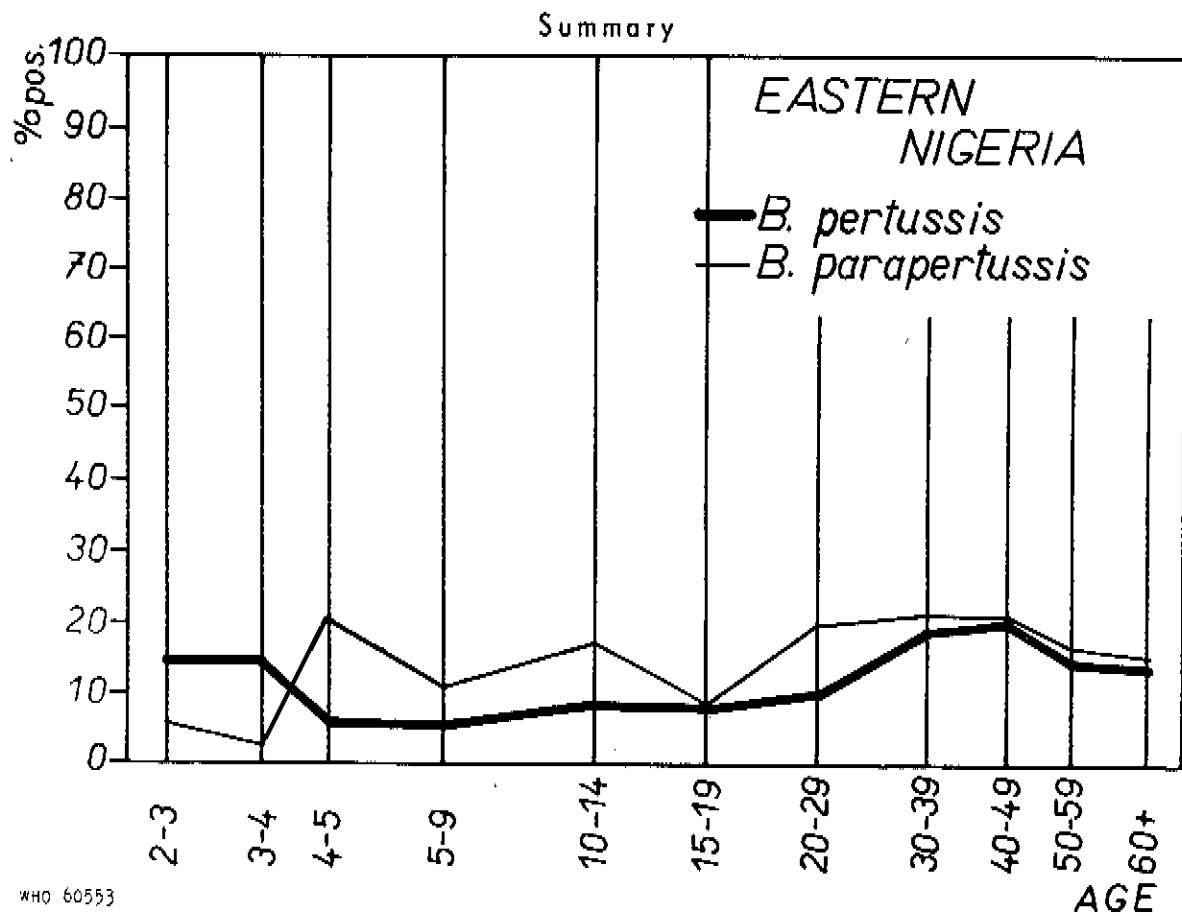
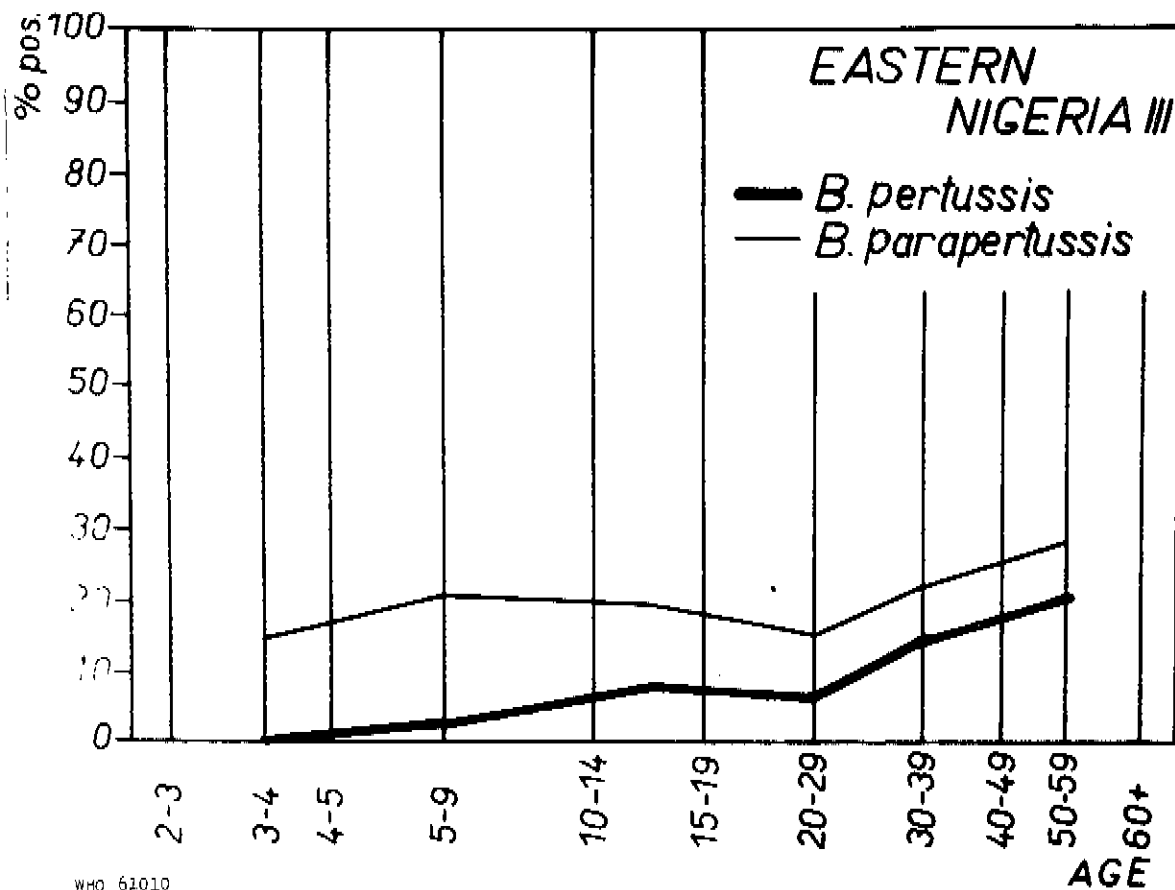


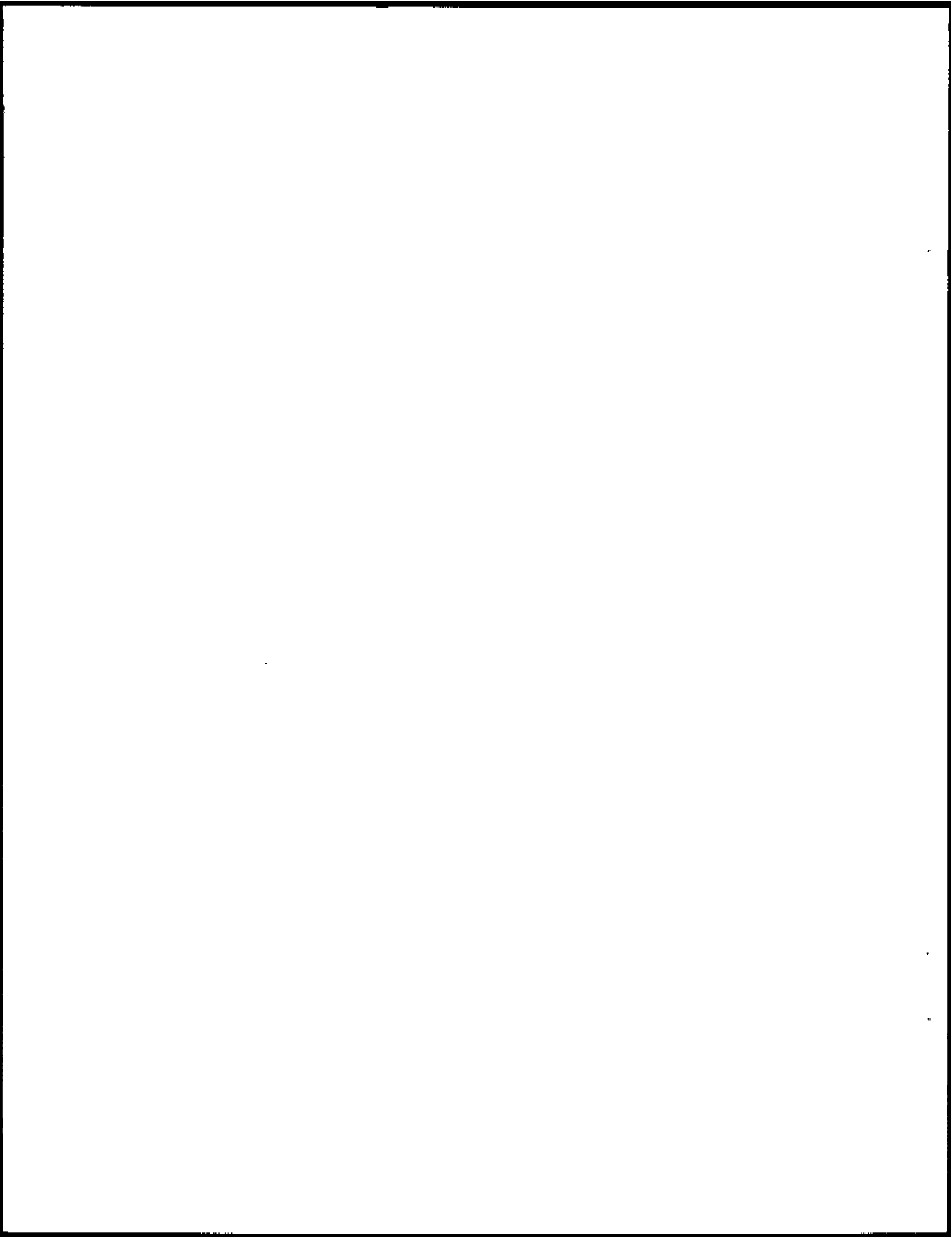
EASTERN NIGERIA (SOUTHERN PART III)

Age-group	No. of sera tested	B. pertussis antibodies						B. parapertussis antibodies						Per cent. of positives					
		Neg.	1:8	16	32	64	128	256	512	Per cent. of positives	Neg.	1:8	16		32	64	128	256	512
2-5	28	28	0	0	0	0	0	0	0	0	24	0	1	1	1	1	0	0	14
6-9	32	30	0	0	0	1	0	0	0	3	24	0	4	2	1	0	0	0	22
10-19	49	45	1	1	0	2	0	0	0	8	39	3	2	3	1	0	0	1	20
20-29	28	26	0	1	1	0	0	0	0	7	23	0	4	1	0	0	0	0	18
30-39	38	32	0	0	0	3	2	1	0	16	29	2	2	3	0	0	2	0	24
40+	65	52	0	2	2	2	5	2	2	20	49	4	8	4	0	0	0	0	25
Total	239	213	1	4	6	10	3	2	0		188	9	21	14	2	1	3	1	

EASTERN NIGERIA (SUMMARY)

Age-group	No. of sera tested	B. pertussis antibodies							B. parapertussis antibodies										
		Neg.	1:8	16	32	64	128	256	512	Per cent. of positives	Neg.	1:8	16	32	64	128	256	512	Per cent. of positives
2-3	13	11	1	0	0	0	1	0	15	12	0	0	0	1	0	0	0	0	8
3-4	33	28	1	2	0	1	1	0	15	32	0	0	0	1	0	0	0	0	3
4-5	41	38	0	0	2	1	0	0	7	33	0	0	3	1	1	1	0	0	19
5-9	86	81	1	1	1	1	0	1	6	76	2	6	1	1	0	0	0	0	12
10-14	98	99	2	2	1	3	1	0	9	80	6	5	4	1	1	0	1	0	18
15-19	23	21	0	0	1	1	0	0	9	21	0	0	1	1	0	0	0	0	9
20-29	65	57	0	3	3	2	0	0	12	52	1	8	3	1	0	0	0	0	20
30-39	82	65	3	5	3	2	4	0	20	64	6	4	6	0	0	2	0	0	22
40-49	57	45	0	2	4	4	0	2	21	45	4	4	4	0	0	0	0	0	21
50-59	34	29	0	1	2	2	0	0	15	28	1	4	1	0	0	0	0	0	18
60+	29	25	0	0	1	1	1	1	14	25	1	2	1	0	0	0	0	0	14
Total	561	489	8	16	18	18	8	4	0	468	21	35	24	7	2	3	1	0	





PRAGUE SERUM REFERENCE BANK

Determination of Antibodies to Polioviruses
and B. pertussis and parapertussis
in Eluates from Dried Blood on Paper Rondelles

ANNEX B

to Report for 1965

1. Determination of antibodies to polioviruses and B. pertussis and parapertussis in eluates from dried blood on paper rondelles

1.1 Poliovirus antibodies in 62 specimens from Nigeria (Dr V. Teplý)

Antibodies to all three poliovirus types were tested simultaneously in 62 serum specimens and eluates from corresponding dried blood specimens on paper rondelles collected by the WHO Treponematoses Advisory Team in Nigeria in May 1964. The rondelles were shipped to Prague via WHO headquarters in Geneva by air mail in sealed polyethylene envelopes at normal temperature and were kept in the laboratory about 14 days at 4°C before being examined.

Eluates were prepared by two rondelles from each donor being cut to pieces, extracted together in 3.2 ml of sterile buffered saline for two hours in a shaker, stored overnight at 4°C, next day inactivated 30 min at 56°C, and prior to inoculation into tissue culture centrifuged 10 min at 3000 rev/min. The volume of 3.2 ml saline used for the extraction of two rondelles was in accordance with the experiences of Vaisman & associates),¹ who assumed that at this ratio the resulting dilution of plasma in the eluate would approximately equal 1:20.

The results of the simultaneous testing of the 62 sera and eluates from paper rondelles collected in Nigeria are summarized in Figs. 1-3; they show that on the average two to three times higher amounts of antibody were determined in the sera than in the rondelles extracted by the volume of diluent indicated.

1.2 Quantitative tests of rondelles with dried blood (Dr B. Vysoká-Burianová)

Since in all previous tests a two to three times smaller amount of antibodies was found in eluates diluted in accordance with Vaisman at the ratio one rondelle to 0.8 ml of saline (assumed dilution of plasma 1:10) than in corresponding serum dilutions, several experiments were carried out to determine whether the deficiency in antibody titre in eluates was due to a certain antibody deterioration in the dry blood on the rondelles or whether the responsible factor was merely quantitative relations in that the amount of plasma on the rondelles did not correspond to Vaisman's calculations.

¹ Vaisman, A. et al. (1963) Bull. Org. mond. Santé, 29, 1-6.

For the purpose, venous blood specimens and simultaneously two to four rondelles from finger puncture per donor were collected from 72 child volunteers (vaccinated against pertussis eight months previously). The sera and in parallel the eluates in concentrations to correspond to the hypothetical ratios of 1:10, 1:5 and 1:2.5 (i.e. obtained by extracting one, two or four rondelles, respectively, in 0.8 ml of saline) were tested for the level of agglutination antibodies to B. pertussis and parapertussis.

The results of these experiments are presented in Figs. 4-6, which show that the amount of antibodies detected in sera initially diluted approximately 1:5 corresponded to the "dilution" of rondelle extract 1:2.5, i.e. four rondelles extracted in 0.8 ml of saline.

In a further experiment, 10 rondelles were taken from one donor. The rondelles were weighed accurately on an analytical balance before and after blood absorption, and were eluated at the ratio of 1:5 with respect to the individual blood volumes ascertained in the rondelles. Similarly, corresponding dilutions of whole heparinized blood were prepared (the blood was weighed) and in a simultaneous test the eluated and the whole blood were titrated for antibody content. Table 1 shows the results.

TABLE 1. TITRES OF ANTIBODIES TO B. PERTUSSIS IN

	Rondelles (1:5 dilution)			Whole blood (1:5 dilution of equal volumes of blood as in rondelles)		
1	10+++	20++		10+++	20++	40+
2	10+++	20++		10+++	20++	
3		-		10+++	20++	
4	10+++	20++		10+++	20++	
5		-		10+++	20++	
6	10+++	20++	40+	40+++	80+	
7	10+++			10+++	20++	
8	10+++	20++		10+++	20++	40±
9	10+++	20++		10+++	20++	
10	10+++	20++	40+	10+++	20++	40+

The table indicates that an approximately equal amount of antibody was detected in whole blood as in blood dried-up on blotting-paper rondelles. Hence, most probably antibodies do not deteriorate qualitatively as a result of the drying of blood on rondelles, but the ratio of one rondelle to 0.8 ml of saline does not correspond to serum dilution 1:10. To this dilution of serum corresponds rather an eluate obtained by extraction of four rondelles in approximately 0.8 ml of saline.

FIG. 1

POLIOVIRUS ANTIBODIES TYPE 1 IN 62 SERA FROM NIGERIA

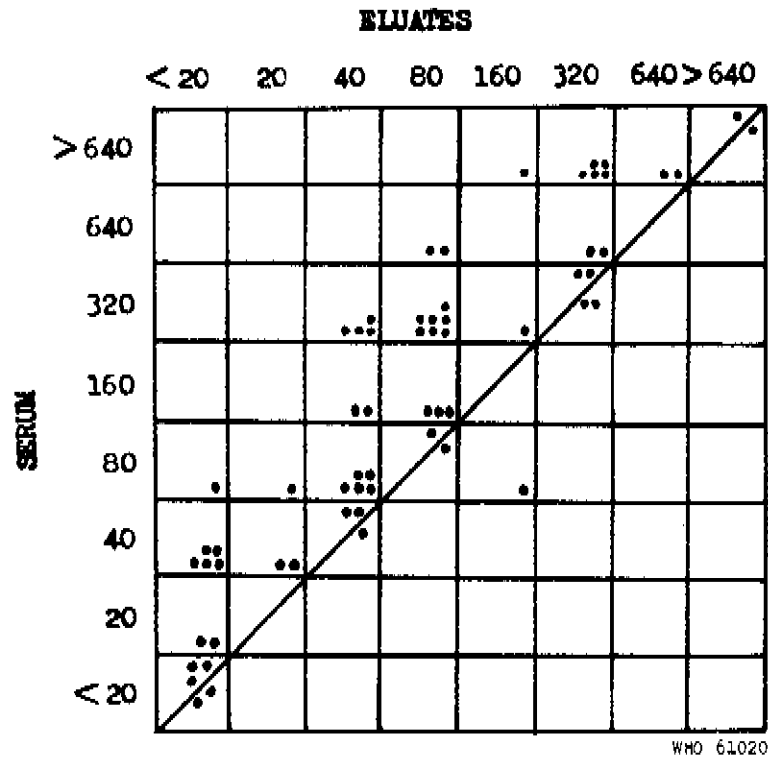
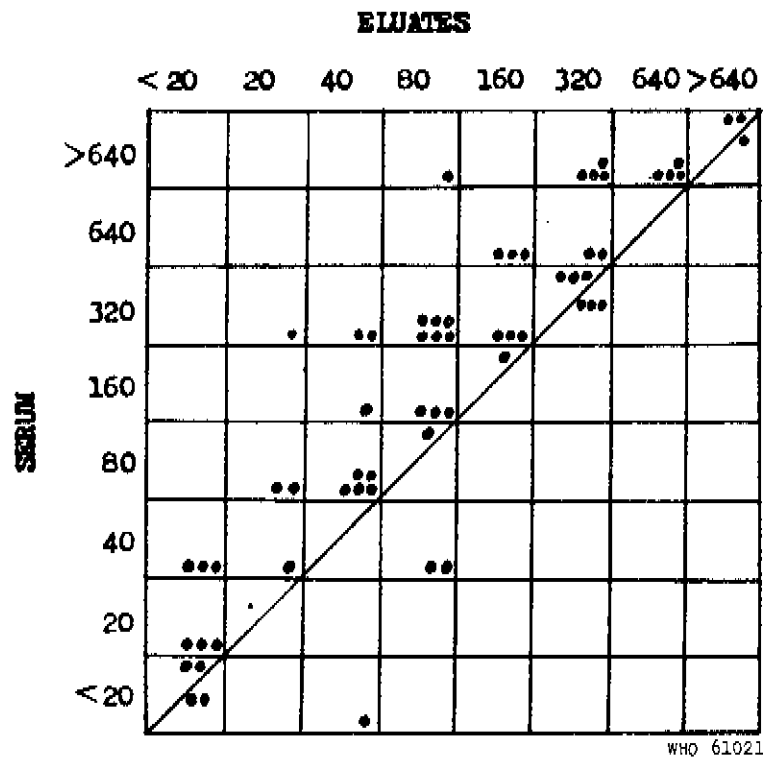


FIG. 2

POLIOVIRUS ANTIBODIES TYPE 2 IN 62 SERA FROM NIGERIA



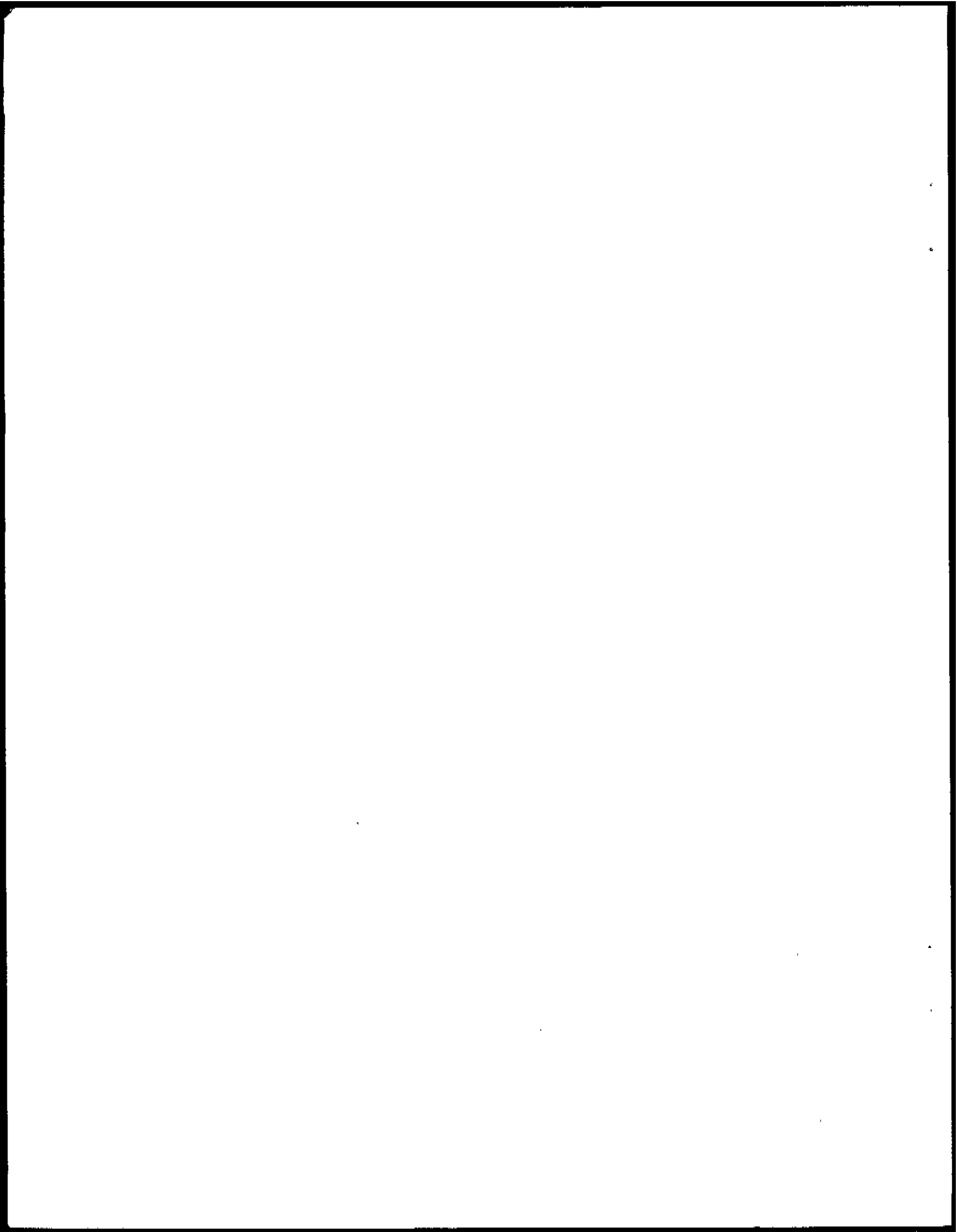


FIG. 3

POLIOVIRUS ANTIBODIES TYPE 3 IN 62 SERA FROM NIGERIA

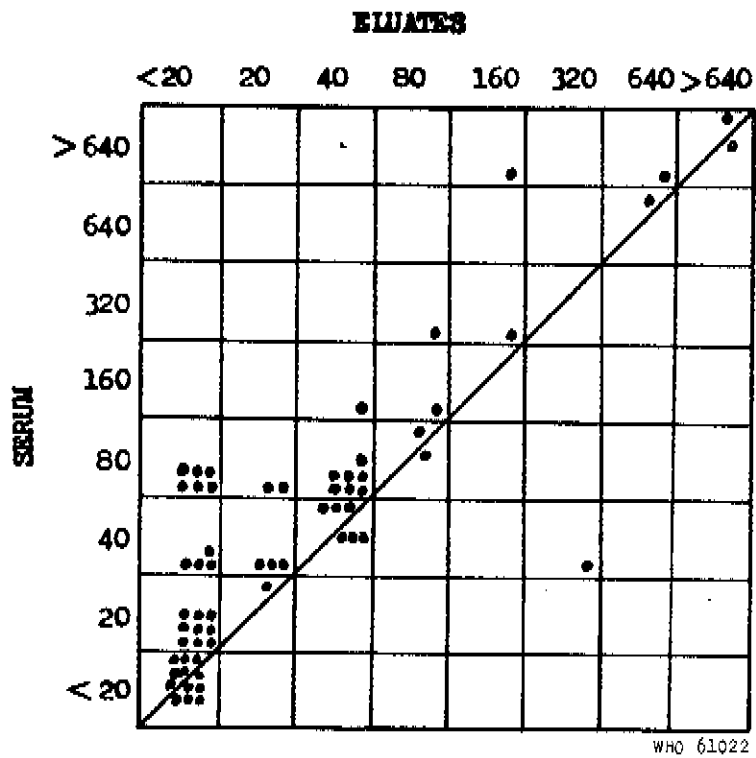


FIG. 4

		Rondelles											
		<10	10	20	40	80	160	320	640	1280	2560		
17 sera	2560						1					Antibodies to B. pertussis	
	1280					1	1	1					
	640					1	1						
	320	1		1	1	1							
	160				2								
	80	1		2	1								
	40												
	20												
	10												
	<10	1											

Blood and rondelles taken on 19.6.64, examined on 22.6.64

Initial serum dilution 1:5
Rondelles, dilution 1:10

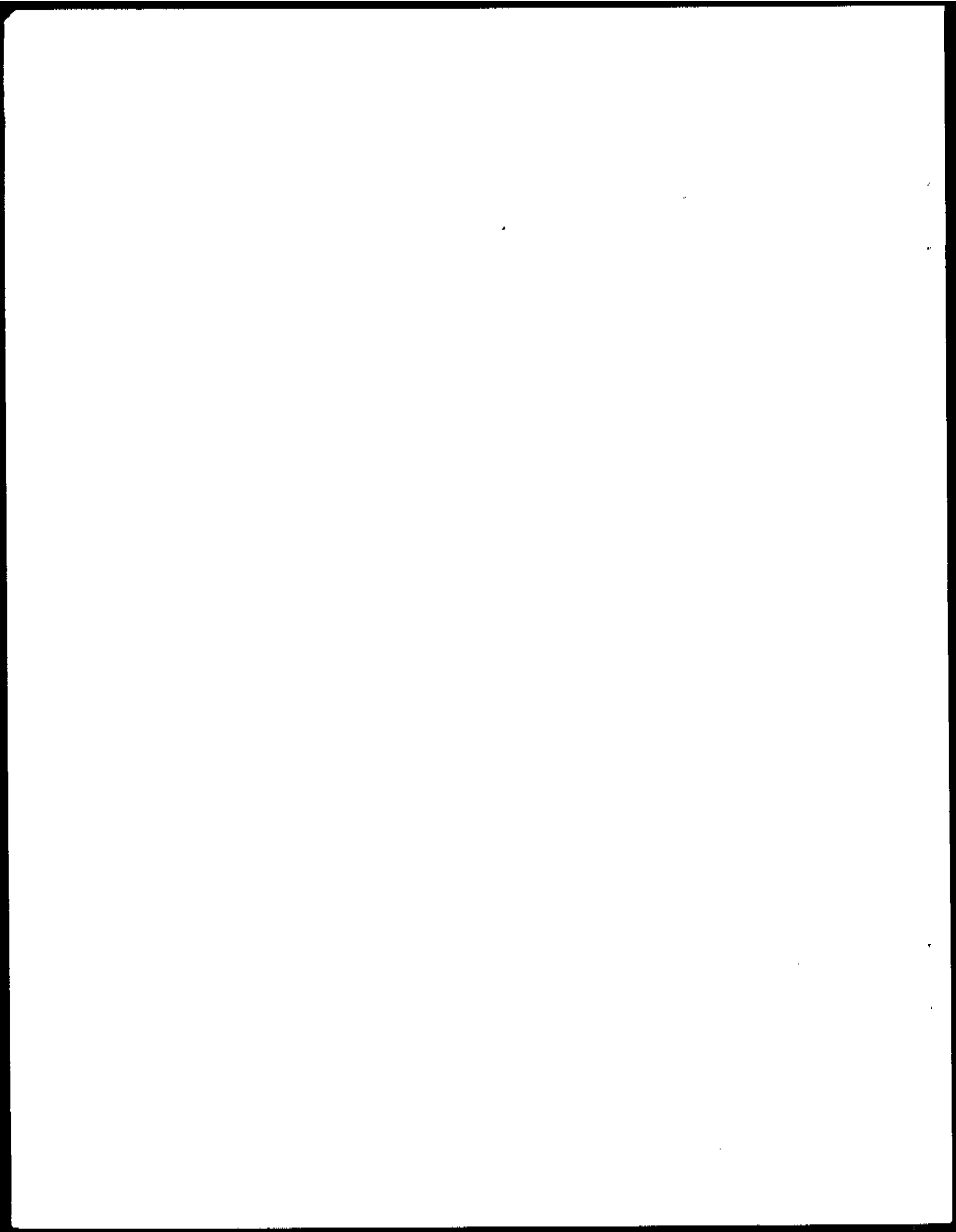


FIG. 5

Rondelles

	<10	10	20	40	80	160	320	640	1280
20 sera									
1280									
640						4			
320				1	1	1			
160			1	1					
80			2						
40		1	1						
20	2	1							
10									
<10	4								

Antibodies to *B. pertussis*

Blood and rondelles taken on 10.6.64, examined on 25.6.64

Initial serum dilution 1:5
Rondelles, dilution 1:5

WHO 61024

FIG. 6

Rondelles

	<10	10	20	40	80	160	320	640	1280
15 sera									
1280								2	3
640									
320							3		
160						2			
80				1	1				
40				2					
20		1							
10									
<10									

Antibodies to *B. pertussis*.

Blood and rondelles taken on 17.6.64, examined on 23.6.64.

Initial serum dilution 1:5
Rondelles, dilution 1:2,5

WHO 61025