

# A collaborative study of cytomegalovirus antibodies in mothers and young children in 19 countries\*

U. KRECH,<sup>1</sup> & J. TOBIN<sup>2</sup>

*Regional variations in the incidence of cytomegalovirus (CMV) infections in mothers and young children were investigated by testing cord blood and serum samples from infants and children up to four years old for the presence of CMV antibodies in 19 different regions of the world. The samples were tested by both the local virus laboratory and the reference laboratory, using the same batch of complement-fixing CMV antigen and techniques which were validated by comparison of the titres recorded for samples of coded sera sent to each laboratory. The incidence of CMV antibodies varied from 44–100% in mothers and from 3–95% in young children. The number of children with CMV antibodies increased with age in five areas; suggesting that there was some child to child transmission of CMV infection between children in these regions. In the other regions, the absence of any significant age-related increase indicated that the main pathway of CMV infection in early life was by transmission from mothers to their infants. The significance of these findings is discussed.*

The high prevalence of cytomegalovirus (CMV) complement-fixing antibodies throughout the world indicates that CMV infections are very common, although the patterns of infection may vary in different regions. It has been difficult to compare the results of previous studies because of variations in the reagents and techniques employed by the different investigators. This study was designed to produce comparable data from different regions of the world on the incidence of CMV complement-fixing antibodies in mothers and their children in the first four years of life. The results were then analysed to compare the pattern of CMV infections in different regions and to identify the mode of transmission in infants and young children.

## MATERIALS AND METHODS

### *Sera*

Blood samples were taken from umbilical cords (maternal) and newborn babies in the local maternity hospital, and from infants and young children admitted to the local hospital because of illnesses other than

those due to congenital abnormalities. The serum samples were normally divided into aliquots, one being evaluated by the local laboratory and the other by the reference laboratory at St Gallen, Switzerland. However, specimens from 5 participating centres were tested only by the reference laboratory, and the specimens from 2 other areas were tested only in the local laboratory, whose results had agreed well with those from St Gallen. Approximately 100 cord bloods and between 110 and 300 serum samples from children were tested in each location.

### *Reagents*

Lyophilized complement-fixing CMV antigen was prepared by the reference laboratory and supplied to all collaborating investigators. In addition, each laboratory was provided with samples of three different, coded, lyophilized human sera and was asked to test them for CMV complement-fixing antibodies. Most laboratories used a locally adapted technique based on a method published by the American Public Health Association (1).

## RESULTS

### *Comparative antibody titrations with coded sera*

The coded lyophilized sera were first titrated for antibody by complement fixation using the locally adapted techniques, as described in the preliminary

\* This paper reports the results of a collaborative study organized by, and supported by a grant from, the World Health Organization. The work was coordinated by Professor Dr U. Krech. The laboratories participating in the collaborative study (and the responsible officers) are listed in an annex on page 610.

<sup>1</sup> Institute for Medical Microbiology, Frobergstrasse 3, CH-9000 St Gallen, Switzerland.

<sup>2</sup> Wilhelm Dunn School of Pathology, Oxford, England.

Table 1. Cytomegalovirus complement-fixing antibodies in cord blood and children collected in different regions of the world

Region in which blood was collected <sup>a</sup>		Cord Blood		Age of children (months)											
				4-6		7-12		13-24		25-36		37-48		4-48 (Total)	
		No. positive/No. tested	% positive	No. positive/No. tested	% positive	No. positive/No. tested	% positive	No. positive/No. tested	% positive	No. positive/No. tested	% positive	No. positive/No. tested	% positive	No. positive/No. tested	% positive
Oxford	A	47/107	43.9	0/22	0.0	1/21	4.8	0/33	0.0	0/19	0.0	3/46	6.5	4/141	2.8
Albany	A	53/120	44.2	7/50	14.0	8/50	16.0	8/77	10.4	missing		6/58	10.3	29/235	12.3
	B	48/105	45.7	8/50	16.0	9/46	19.5	7/61	11.4			8/50	16.0	32/207	15.4
Manchester	A	67/109	61.5	8/32	25.0	1/29	3.4	3/33	9.1	4/35	11.4	8/32	25.0	24/161	14.9
St Gallen	A/B	58/100	58.0	10/60	16.6	10/64	15.6	5/51	9.8	16/51	31.3	12/48	25.0	53/274	19.3
Lyons	A	51/100	51.0	7/56	12.5	9/58	15.5	13/60	21.6	16/81	19.7	21/79	26.5	66/334	19.7
	B	60/98	61.2	8/55	14.5	8/56	14.2	15/59	25.4	20/80	25.0	22/72	30.5	73/322	22.6
Los Angeles	A	71/99	71.7	19/99	19.2	12/99	12.2	12/98	12.2	13/81	16.0	12/96	12.5	68/473	14.3
	B	74/97	76.2	22/86	25.5	14/86	16.2	14/80	17.5	16/81	19.7	11/91	12.1	77/424	18.2
Freiburg (in Breisgau)	A	72/94	76.6	34/67	50.7	7/20	35.0	10/26	38.5	3/8	37.5	5/16	31.2	59/137	43.0
	B	66/90	73.3	13/47	27.7	2/20	10.0	5/26	19.2	1/8	12.5	2/16	12.5	23/117	19.7
Anchorage	B	103/125	82.4	3/21	14.3	5/20	25.0	9/34	26.5	27/43	62.8	25/40	62.5	69/158	43.7
Trinidad	A	100/101	99.0	3/13	23.1	16/28	57.1	20/44	45.5	16/27	59.2	9/19	47.3	64/131	48.9
	B	80/84	95.2	3/13	23.1	11/24	45.8	14/36	38.8	8/19	42.1	9/17	52.9	45/109	41.3
Buenos Aires	A	104/105	99.0	11/32	34.4	23/46	50.0	18/39	46.1	12/23	52.5	2/3	66.6	66/143	46.2
	B	102/107	95.3	10/31	32.2	18/46	39.1	17/37	45.9	11/22	50.0	2/3	66.6	58/137	42.3
Bratislava	A	91/98	92.8	5/21	23.8	19/39	48.7	11/27	40.7	10/19	52.6	7/10	70.0	52/116	44.8
	B	93/105	88.6	7/19	36.8	20/38	52.6	11/26	42.3	10/18	55.5	8/11	72.7	56/112	50.0
Rome	A			11/17	64.7	8/33	24.2	20/39	51.3	29/46	63.0	16/26	61.5	84/161	52.2
	B	92/94	97.8	13/24	54.2	13/37	35.1	26/49	53.1	27/43	62.8	23/41	56.1	102/194	52.6
Hong Kong	A	96/100	96.0	21/49	42.8	24/52	46.2	27/50	54.0	27/49	55.1	31/50	62.0	129/250	51.6
	B	98/100	98.0	17/44	38.6	22/50	44.0	28/50	56.0	25/44	56.8	36/46	78.3	128/234	54.7
Mostar	B	118/129	91.5	6/16	37.5	24/41	58.5	26/48	54.2	19/30	63.3	17/20	85.0	92/155	59.4
Sendai	A	93/100	93.0	26/44	59.1	26/39	66.6	21/33	63.6	14/24	58.3	30/55	54.5	117/195	60.0
	B	91/96	94.8	25/38	65.8	22/35	62.8	22/28	78.6	12/20	60.0	34/53	64.1	115/174	66.1
Fiji	A	93/93	100.0	14/23	60.8	24/35	68.5	45/59	76.3	15/21	71.4	13/17	76.5	111/145	76.6
	B	67/68	98.5	14/22	63.6	20/32	62.5	36/45	80.0	11/17	64.7	8/13	61.5	89/129	69.0
Vellore	A	99/100	99.0	28/45	62.2	51/65	78.4	49/61	80.3	36/48	75.0	50/63	79.3	214/282	75.9
	B	96/97	98.9	25/40	62.5	38/58	65.5	41/53	77.3	31/43	72.1	41/53	77.3	176/247	71.3
Ibadan	B	111/115	96.5	70/75	93.3 <sup>b</sup>	29/41	70.7	51/60	85.0	27/31	87.1	24/26	92.3	131/158	82.9
Entebbe	B	121/121	100.0			24/26	92.3	28/28	100.0	24/27	88.8	52/53	98.1	128/134	95.5

<sup>a</sup> A — results obtained locally; B — results obtained in St Gallen.

<sup>b</sup> Data given for age group 0-4 years; data for infants 4-6 months of age are not available.

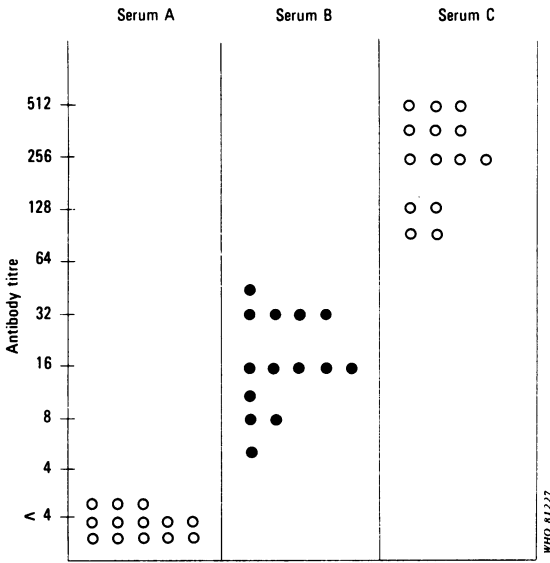


Fig. 1. Cytomegalovirus complement-fixing antibody titres in coded serum samples (A, B, and C). One result was not reported for serum A.

study (2). The results are summarized in Fig. 1. All the laboratories found the negative serum A to be without antibodies, and the titre for serum B to be lower than that for serum C. Although the actual values varied, the ratio of the titres for sera B and C was consistent in all laboratories. The results demonstrate a high degree of uniformity; for example, the standard deviation of the titres recorded for serum B was so small that the threshold titre value of 4 was more than two standard deviations from the data range. Therefore, it was regarded as unlikely that any of these laboratories would record false negative results (if their methods remained constant).

*Comparison of antibody titres obtained by the local and reference laboratories*

The data obtained by the local and reference laboratories, on the presence of CMV antibodies in cord and children's blood, are given in Table 1. For 5 regions (including that of the reference laboratory), data were available only from the reference laboratory. A good correlation was obtained between the results of the local laboratories and those of the reference laboratory, with the exception of Freiburg. The results from this laboratory were all approximately 20% higher than the corresponding figures recorded in St Gallen. In the case of Albany, the local results were all lower than those from the reference laboratory, but they were so similar that the statistical probability that both laboratories would produce the same result was 94%.

In the other regions the largest discrepancy, 49% as against 41% positive, was recorded in the data from Trinidad.

Overall, there was good agreement between the results of the reference and local laboratories, the proportions found positive, of the total number of sera tested, being 40.2% and 40.7%, respectively.

*Changes in the frequency of antibody-positive children with age*

In all areas the percentage of subjects positive for antibody was smaller in the first year of life than among the mothers (Fig. 2). The difference was significant in most cases.

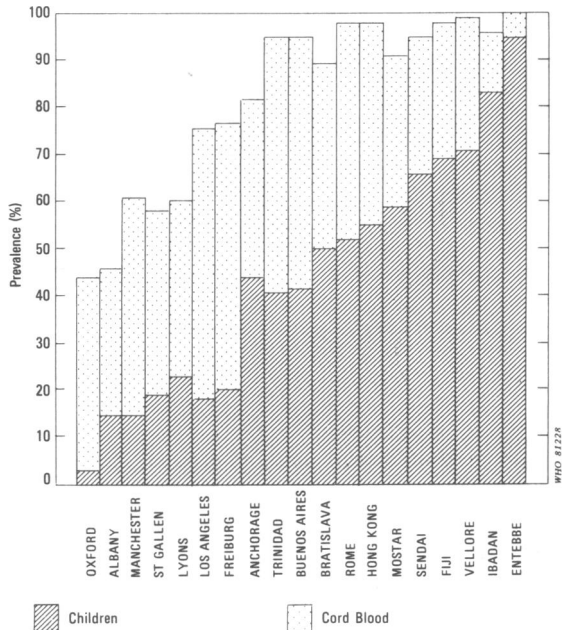


Fig. 2. Cytomegalovirus complement-fixing antibody titres in serum from cord blood and from infants and children aged 4-48 months.

In some regions, the percentage of antibody positive children stayed fairly constant during their first four years of life, while in other areas the frequency increased during infancy and early childhood.

Thus, the centres can be grouped into three categories (Table 2): group A, those centres in which the prevalence of positivity was low in children aged 4-6 months and did not increase significantly in children up to 4 years of age; group B, those centres in which the level of positivity in children aged 4-6 months was high, but nevertheless did not increase significantly in

Table 2. Prevalence of CMV complement-fixing antibody carriers in different areas, grouped according to the level of incidence at birth and its variation within the first four years

Group	Region	Approximate percentage of antibody carriers in age groups 4-48 months
A. Low antibody prevalence after birth with no significant increase in the first four years of life	Oxford	3
	Albany	15
	Los Angeles	15
	Freiburg (in Breisgau)	20
B. High antibody prevalence after birth with no significant change during the first four years of life	Fiji	70
	Sendai	70
	Vellore	70
	Ibadan	90
	Entebbe	95
C. Low antibody prevalence after birth with different degrees of increase during the first four years of life (approximate range given)	Manchester	5-25
	St Gallen <sup>a</sup>	10-30
	Lyons	10-30
	Anchorage	15-60
	Trinidad <sup>a</sup>	20-60
	Rome	25-65
	Buenos Aires	30-65
	Bratislava	30-70
	Hong Kong	40-80
Mostar	40-85	

<sup>a</sup> Regression analysis indicated that all the areas in group C showed a statistically significant trend ( $P < 0.05$ ) except for St Gallen ( $P = 0.2$ ) and Trinidad ( $P = 0.5$ ).

<sup>b</sup> See Table 1 for details.

children up to 4 years old; and group C, those areas where the proportion of antibody-positive children did increase during the first four years of life, thus implying exposure to CMV infections between 6 and 48 months of age.

These increases in the antibody positive frequency in group C were statistically significant ( $P < 0.05$ ) for seven of the regions (Table 2).

#### *Relation between CMV antibodies in mothers (cord blood) and samples from children*

Results obtained from cord blood and children between 6 months and 4 years of age are given in Table 1 and Fig. 2. The prevalence of antibody-positive mothers varied from 44% in Oxford to 100% in Entebbe and Fiji. However, the prevalence of antibody-positive children between 6 months and 4 years of age varied from 3% in Oxford to 95% in Entebbe. A prevalence of positivity among mothers of approximately 60% or less was associated with a low infection rate in their offspring, whereas a prevalence of over 85% was associated with a high prevalence of early childhood infection, ranging from 43% to 95%. How-

ever, in two areas high frequencies of 76-77% of antibody-positive mothers were associated with a low prevalence of childhood infection, as judged by the percentage of antibody-positive children in these areas.

#### DISCUSSION

The data collected, on the incidence of CMV antibodies in mothers and in children from 6 months to 4 years of age, indicated that CMV infections occur frequently in all the areas tested. In some European and North American centres, approximately half to two-thirds of the mothers had been infected at some stage, but in other areas the frequency approached 100%.

The development of CMV antibodies in response to CMV infections after birth, showed different patterns in the regions investigated. The prevalence of infection in infancy was low (<26%) in areas where the level of maternal infection was low or moderate (<60%). In places where the prevalence in adults approached 100%, that in infants varied from over 40% to 70%, except in the 2 African areas where it was between 87 and 95%. Two areas, Los Angeles and Freiburg, had a low level of infection in children although the frequency of maternal infection was over 75%. However, there was no region in which a high prevalence of infection was observed in young children whose mothers showed only a low or moderate rate of antibody-positivity. In the majority of areas (groups A and B) there was little evidence of transmission of infection from child to child during the first four years of life; this supports the hypothesis that most CMV infections in the first year of life are transmitted from mother to infant (3). However, in seven regions there was an increase in the frequency of infection of young children with age (Table 2) that was presumably due to child to child transmission. The different patterns may be explained by the differences in child/mother contact, such as a longer period of breast feeding, that may increase the frequency of mother to child transmission in early infancy.

Additional evidence indicating that mothers are the main source of infection in infancy, comes from observations in St Gallen (4), Manchester, and Singapore (5) that there is very little virus circulation after the breast-feeding period until the children reach puberty. In these areas, very few cases of CMV infection were found in the age groups between 2 and 15 years. The incidence of CMV infections in laboratory personnel handling, or exposed to, contaminated material does not differ significantly from that in other hospital personnel (6). This also supports the hypothesis that transmission of CMV is more likely to occur by close personal contact than through contact

with contaminated specimens. Transmission among primary school children seems to be low (6).

Numazaki et al. (3) related the high incidence of mother to child transmission in Japan to an increase in the cervical excretion of the virus during the latter stages of pregnancy. This conclusion is supported by the work of Alexander (7) in Taiwan. Stagno et al. (8), working in southern USA, considered the increase in cervical excretion in late pregnancy to be the result of a suppression of the normal level of excretion in early pregnancy. However, the occurrence of cervical excretion in the United Kingdom appears to be much less (12).

It has been suggested that reactivation of viraemia might be related to infection at an early age and be less common after primary infection in adult life (9). This observation could explain the results from Los Angeles and Freiburg where a low level of infection in the first years of life was accompanied by a high prevalence of antibody in the child-bearing population.

Therefore, children in Western Europe and parts of the USA would be expected to have relatively little infection in the early years of life; in contrast, in Eastern Europe, Africa, Asia, and South America high rates of cervical excretion may cause early infection in succeeding generations.

Intrauterine infection rates in developed countries have been estimated to be between 0.25 and 1.0%, but in areas where 90–100% of adult females have CMV antibodies these rates may exceed 2% (10). Secondly, in a recent study, which was part of Phase III of the WHO CMV project, the rate of intrauterine CMV-infection was found to be about 1.4% in populations in which the prevalence of CMV antibodies was almost 100%. These results were obtained in Abidjan and the Ivory Coast, and suggest that this increase in the prevalence of intrauterine infection in antibody carriers is due to reinfection or reactivation of a previous infection (11).

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#### RÉSUMÉ

##### ÉTUDE COLLECTIVE SUR LES ANTICORPS ANTI-CYTOMÉGALOVIRUS CHEZ LES MÈRES ET LES JEUNES ENFANTS DANS 19 PAYS

On a étudié dans 19 régions du monde les variations régionales de l'incidence des infections à cytomégalo-virus (CMV) chez des mères et de jeunes enfants en recherchant la présence d'anticorps anti-CMV dans le sang du cordon ombilical et dans des échantillons de sérums prélevés sur des nourrissons et de jeunes enfants âgés de moins de 4 ans. Les échantillons ont été soumis à des tests dans les laboratoires virologiques locaux et dans le laboratoire de référence à St-Gall (Suisse), qui ont utilisé le même lot d'un antigène CMV fixant le complément et des techniques dont on a établi la valeur en comparant les titres relevés dans les échantillons de sérum humain codés qui avaient été envoyés à chaque laboratoire.

Les données recueillies indiquent que les infections à CMV se produisent fréquemment dans toutes les régions étudiées. Dans certains centres d'Europe et d'Amérique du Nord, environ la moitié et parfois même les deux tiers des mères ont été infectées à un moment donné, mais ailleurs il arrive que la fréquence approche de 100%.

La production d'anticorps anti-CMV après une infection à CMV postnatale a suivi différents schémas dans les régions étudiées. La prévalence de l'infection chez les nourrissons est faible (< 26%) dans les régions où le niveau de l'infection maternelle est bas ou modéré (< 60%). Dans les endroits où

la prévalence approche de 100% chez les adultes, chez les nourrissons elle varie de plus de 40% à 70%, sauf dans les deux zones africaines où elle se situe entre 87% et 95%. Dans deux zones, Los Angeles et Fribourg, il existe un faible niveau d'infection chez les enfants, bien que la fréquence de l'infection maternelle dépasse 75%. Cependant, dans aucune région on n'a observé une forte prévalence de l'infection chez de jeunes enfants dont les mères présentent une séropositivité seulement faible ou modérée. Dans la plupart des zones (groupes A et B) il n'est pas évident qu'il y ait une transmission de l'infection d'enfant à enfant pendant les quatre premières années de vie; cette observation semble confirmer l'hypothèse que la plupart des infections à CMV dans la première année de vie sont transmises par la mère au nourrisson. Toutefois, dans sept régions la fréquence de l'infection augmente avec l'âge chez les jeunes enfants, ce qui est vraisemblablement dû à une transmission d'enfant à enfant. Les différents schémas peuvent s'expliquer par des contacts mère/enfants différents, par exemple une longue période d'allaitement maternel qui peut augmenter la fréquence de la transmission de mère à enfant dans la première enfance. La signification de ces observations est examinée.

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## Annex I

## LIST OF PARTICIPATING LABORATORIES

- M. Aymard and Agnès Calvet, Laboratoire de Virologie, Université Claude Bernard, F-69373 Lyons, France
- Maria Isabel Berria, National Microbiology Institute, Buenos Aires, Argentina
- W. K. Chang, Virus Unit, Medical and Health Department, Queen Mary Hospital, Hong Kong
- Rudolf Deibel, Virus Laboratories, New York, Department of Health, Albany, New York 12201, USA
- J. H. S. Gear, The South African Institute for Medical Research, Johannesburg, South Africa
- Barbara Hull, Caribbean Epidemiology Centre, Port of Spain, Trinidad, West Indies
- Malati Jadhav, Enterovirus Laboratory, Christian Medical College, Vellore-4, India
- U. Krech, Institut für medizinische Mikrobiologie des Kantons St Gallen, CH-9000 St Gallen, Switzerland
- Pauli Leinikki, Department of Virology, University of Helsinki, Helsinki 29, Finland
- J. A. R. Miles, University of Otago, Dunedin, New Zealand (results from Suva, Fiji)
- Michael R. Moser, Alaska Activities, Bureau of Epidemiology, Anchorage, Alaska 99501, USA
- Yoshio Numazaki, Virus Laboratory, Sendai National Hospital, Sendai 983, Japan
- A. O. Osoba, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria
- H. Schmitz, Hygiene-Institut der Universität, D-78 Freiburg/Breisgau, Germany
- V. Schwanzer, Research Institute of Epidemiology and Microbiology, Bratislava, Czechoslovakia
- A. G. Senyakazana, East African Virus Research Institute, Entebbe, Uganda
- J. O'Hara Tobin, Public Health Laboratory, Radcliffe Infirmary, Oxford, England (results from Oxford and Manchester)
- Harry T. Wright, Jr., University of Southern California, School of Medicine, Los Angeles, California 90054, USA
- M. Zurovec, Regional Public Health Services, Mostar, Yugoslavia