

RESULTS

3.1. Geographical, Age, Sex and Race Distribution of Persons from Whom Sera Were Obtained

Malaysia is divided into two regions; West and East Malaysia. There are 12 states in West Malaysia: Perlis, Kedah, Pulau Pinang, Perak, Wilayah Persekutuan, Selangor, Melaka, Negeri Sembilan, Johor, Terengganu, Kelantan and Pahang; and 2 (Sabah and Sarawak) in East Malaysia. The 900 sera were collected from all 14 states in Malaysia (Table 1). There were 75 sera for every age group (0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-15 and 15-20 years).

Four hundred and ninety eight (55.3%) sera were from males while 402 (44.7%) were from females (Table 2). Slightly more sera were collected from males compared to females in all age groups except for the last age group (15-20 years). 490 (54.4%) were collected from Malays, 250 (27.8%) from Chinese, 67 (7.4%) from Indians and 93 (10.3%) from Others (indigenous groups such as Ibans, Kadazans, Bidayahs, Bidayus and Orang Asli) (Table 3). The racial breakdown roughly corresponds with the racial composition in the country (Population census: Malays = 55%, Chinese = 30%, Indians = 5% and others = 10%; Information Malaysia Yearbook, 1995).

Table 1. Distribution of 900 Sera By Age and Place of Collection

Age group (year)	WP	SEL	Perlis	P.PIN	KED	PER	PAH	TER	KEL	N.SEM	MEL	JOH	SAB	SAR	Total
0-1	28	8	1	0	9	2	1	2	0	3	0	8	10	3	75
1-2	21	15	0	0	9	5	1	1	0	2	3	11	2	5	75
2-3	31	18	0	1	4	1	2	0	0	4	0	3	9	2	75
3-4	29	7	0	1	4	3	6	1	0	1	1	8	8	6	75
4-5	22	8	0	0	5	5	2	1	0	0	3	11	9	9	75
5-6	34	9	0	0	4	6	2	0	0	0	4	3	8	5	75
6-7	23	8	0	1	0	5	2	4	0	4	2	12	7	7	75
7-8	24	5	1	0	6	3	9	7	0	0	0	5	10	5	75
8-9	26	12	0	0	5	6	3	2	1	2	1	3	2	12	75
9-10	25	17	1	0	4	1	7	3	0	2	4	2	1	8	75
10-15	34	10	0	1	1	1	5	2	2	3	3	8	0	5	75
15-20	14	28	1	0	0	2	2	0	2	1	1	10	12	2	75
Total	311	145	4	4	51	40	42	23	5	22	22	84	78	69	900

Note :

WP - Wilayah Persekutuan

SEL - Selangor

P.PIN - Pulau Pinang

KED - Kedah

PER - Perak

PAH - Pahang

TER - Terengganu

KEL - Kelantan

N. SEM - Negeri Sembilan

MEL - Melaka

JOH - Johor

SAB - Sabah

SAR - Sarawak

Table 2. Distribution of Sera by Age and Sex

Age group (year)	Male		Female		Total
	No.	%	No.	%	
0 - 1	42	56.0	33	44.0	75
1 - 2	42	56.0	33	44.0	75
2 - 3	39	52.0	36	48.0	75
3 - 4	41	54.7	34	45.3	75
4 - 5	39	52.0	36	48.0	75
5 - 6	40	53.3	35	46.7	75
6 - 7	51	68.0	24	32.0	75
7 - 8	42	56.0	33	44.0	75
8 - 9	43	57.3	32	42.7	75
9 - 10	38	50.7	37	49.3	75
10 - 15	47	62.3	28	37.3	75
15 - 20	34	45.3	41	54.7	75
Total	498	55.3	402	44.7	900

Table 3. Distribution of Sera by Age and Race

Age Group (year)	Malay		Chinese		Indian		Others		Total
	No.	%	No.	%	No.	%	No.	%	
0 - 1	41	54.7	18	24.0	6	8.0	10	13.3	75
1 - 2	43	57.3	21	28.0	3	4.0	8	10.7	75
2 - 3	41	54.7	17	22.7	7	9.3	10	13.3	75
3 - 4	46	61.3	15	20.0	8	10.7	6	8.0	75
4 - 5	44	58.7	14	18.7	8	10.7	9	12.0	75
5 - 6	41	54.7	20	26.7	3	4.0	11	14.7	75
6 - 7	42	56.0	22	29.3	5	6.7	6	8.0	75
7 - 8	39	52.0	21	28.0	3	4.0	12	16.0	75
8 - 9	40	53.3	20	26.7	7	9.3	8	10.7	75
9 - 10	28	37.3	38	50.7	5	6.7	4	5.3	75
10 - 15	44	58.7	26	34.7	4	5.3	1	1.3	75
15 - 20	41	54.7	18	24.0	8	10.7	8	10.7	75
Total	490	54.4	250	27.8	67	7.4	93	10.3	900

3.2. EPSTEIN-BARR VIRUS (EBV)

3.2.1. EBV Immunofluorescence Assay (IFA)

Actively growing P3HR-1 cells form clumps of dividing cells (Figure 1), which become single after 3 days of induction by TPA and NBA (Figure 2). After IFA, wells containing at least 20% fluorescing cells are scored positive (Figures 3 and 5). Cell and serum controls were performed using the EBV-negative BJAB cell line (Figure 4) and an IgG-VCA negative serum (Figure 6) respectively.

3.2.2a. Prevalence of IgG-VCA

A 1:10 serum dilution was used. 822 (91.3%) sera showed positive reactivity to VCA-EBV (Table 4). 53.3% of sera from children below one year of age were seropositive. Seroconversion percent increased to 77.3% in the second age group (1-2 years old) and reached 90.7% in the third age group (2-3 years old). Percent of EBV seropositive sera in the fourth age group (3-4 years old) increased slightly to 94.7% and the percentage then fluctuated between 93.3% to 97.3% before reaching 100% by the age group of 9-10 years old. All the sera collected from persons more than 9 years of age were seropositive for EBV (Table 4 and Figure 7).

3.2.2b. Titres of IgG-VCA

IgG-VCA titres ranged from 1:10 to 1:1280 with geometric mean titre (GMT) from 23 in the 0-1 year age group to 78 in the 6-7 age group, after which it leveled off to figures between 30 and 40 (Table 5 and Figure 8).

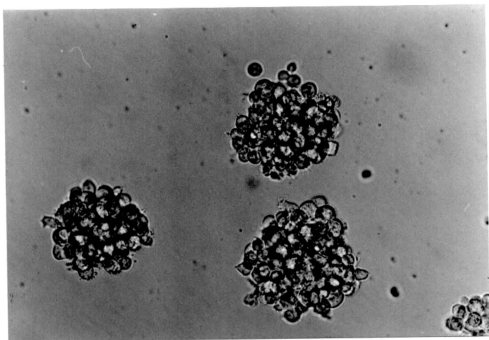


Figure 1: Clumps of healthy P3HR-1 cells before induction. (Magnification X200)

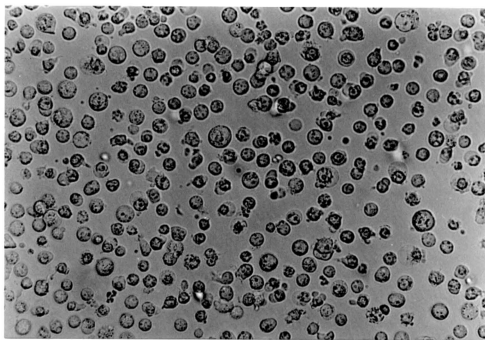


Figure 2: TPA and NBA-induced P3HR-1 cells appear in single cells form. (Magnification X200)

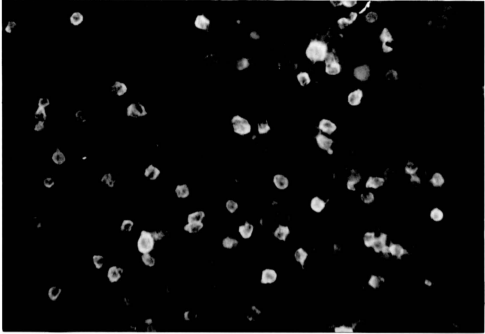


Figure 3: VCA expression in induced P3HR-1 cells detected by control positive serum by IFA. (Magnification X200)

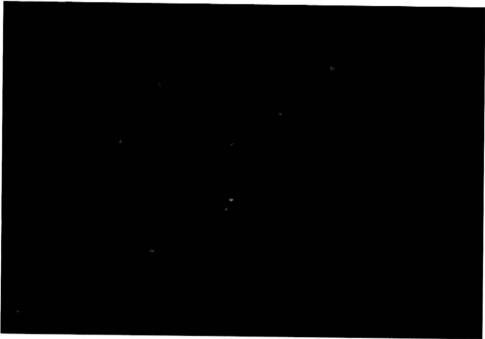


Figure 4: Negative immunofluoresence staining of BJAB cells (EBV negative cell control). (Magnification X200)

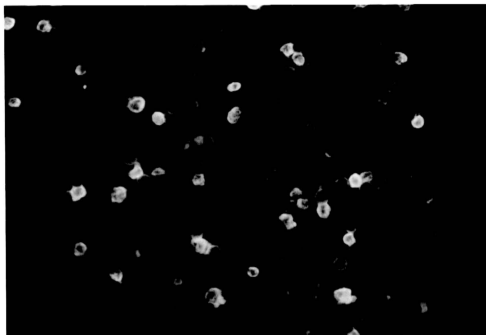


Figure 5: IgG-VCA immunofluorescence of a positive test serum.
(Magnification X200)

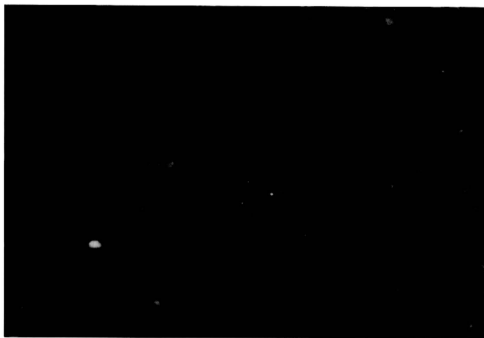


Figure 6: IgG-VCA immunofluorescence of a negative test serum.
(Magnification X200)

Table 4. EBV Seroprevalence (IgG-VCA positive) in Different Age Groups

Age group (year)	No. of positive sera	% Positive
0 - 1	40	53.3
1 - 2	58	77.3
2 - 3	68	90.7
3 - 4	71	94.7
4 - 5	73	97.3
5 - 6	73	97.3
6 - 7	70	93.3
7 - 8	73	97.3
8 - 9	71	94.7
9 - 10	75	100
10 - 15	75	100
15 - 20	75	100
Total	822	91.3

Figure 7. EBV Seroprevalence (IgG-VCA positive) in Different Age Groups

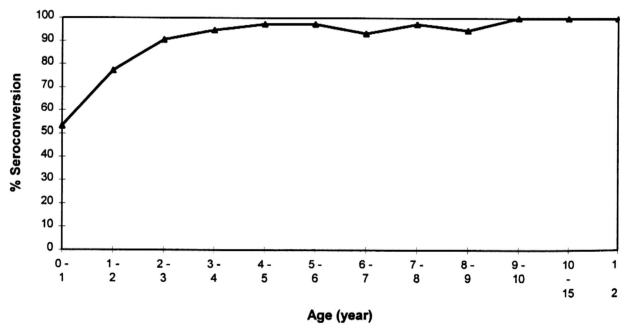
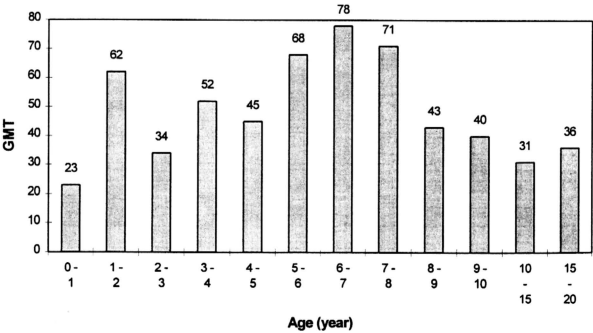


Table 5. Titres of IgG-VCA by Age

Age Group (year)	Titres								GMT
	10	20	40	80	160	320	640	1280	
0 - 1	2	10	11	8	8	0	1	0	23
1 - 2	2	8	20	21	2	1	0	4	62
2 - 3	10	15	30	9	3	0	1	0	34
3 - 4	4	10	23	24	8	2	0	0	52
4 - 5	8	4	34	22	4	1	0	0	45
5 - 6	10	4	13	24	11	10	1	0	68
6 - 7	10	1	8	21	23	7	0	0	78
7 - 8	8	1	15	30	10	8	1	0	71
8 - 9	16	6	19	19	8	2	1	0	43
9 - 10	5	20	31	12	4	3	0	0	40
10 - 15	10	26	24	13	2	0	0	0	31
15 - 20	8	20	27	18	0	2	0	0	36
Total	93	125	255	221	83	36	5	4	-

Figure 8. GMT of IgG-VCA by Age



3.2.3. EBV Seroprevalence (IgG-VCA positive) in Different States

When divided into the states from where the sera were obtained, the percentage of seropositives ranged from 75% to 100% (Table 6). The actual numbers per age group are very small in some of the states (Table 7), the data in Table 7 was therefore pooled, dividing the country into 4 regions: West Coast of West Malaysia (Wilayah Persekutuan, Selangor, Perak, Pulau Pinang, Kedah and Perlis), East Coast of West Malaysia (Pahang, Terengganu and Kelantan), South West Malaysia (Melaka, Negeri Sembilan and Johor) and the 2 states of East Malaysia (Sabah and Sarawak) (Table 8). In general, the EBV seroconversion profiles between regions were similar and no significant difference was found ($\chi^2 = 3.372$, degree of freedom = 3, $p > 0.05$. Table 9).

Table 6. Distribution of EBV Seropositive (IgG-VCA) Sera

State	Number of Positive Sera	% Positive
Wilayah Persekutuan	285	91.6
Selangor	141	97.2
Perlis	3	75.0
Pulau Pinang	4	100
Kedah	39	76.5
Perak	38	95.0
Pahang	39	92.9
Terengganu	22	95.7
Kelantan	5	100
Negeri Sembilan	18	81.8
Melaka	22	100
Johor	72	85.7
Sabah	69	88.5
Sarawak	65	94.2
Total	822	91.3

Table 7. Distribution of EBV Seropositive (IgG-VCA) Sera by Age and Place of Collection

Age group (year)	WP	SEL	Perlis	P.PIN	KED	PER	PAH	TER	KEL	N.SEM	MEL	JOH	SAB	SAR
0-1	15	7	0	0	1	2	1	1	0	2	0	4	4	3
1-2	16	14	0	0	5	4	1	1	0	1	3	8	1	4
2-3	28	16	0	1	4	1	2	0	0	3	0	3	8	2
3-4	29	7	0	1	4	3	4	1	0	1	1	7	8	5
4-5	22	8	0	0	5	5	2	1	0	0	3	9	9	9
5-6	33	9	0	0	4	6	2	0	0	0	4	3	7	5
6-7	22	8	0	1	0	5	2	4	0	3	2	10	7	6
7-8	24	5	1	0	6	2	8	7	0	0	0	5	10	5
8-9	23	12	0	0	5	6	3	2	1	2	1	3	2	11
9-10	25	17	1	0	4	1	7	3	0	2	4	2	1	8
10-15	34	10	0	1	1	1	5	2	2	3	3	8	0	5
15-20	14	28	1	0	0	2	2	0	2	1	1	10	12	2
Total	285	141	3	4	39	38	39	22	5	18	22	72	69	65

Note :

WP - Wilayah Persekutuan	KED - Kedah	TER - Terengganu	MEL - Melaka	SAR - Sarawak
SEL - Selangor	PER - Perak	KEL - Kelantan	JOH - Johor	
P.PIN - Pulau Pinang	PAH - Pahang	N. SEM - Negeri Sembilan	SAB - Sabah	

Table 8. Distribution of EBV seropositive (IgG-VCA) Sera By Age Group and Regions

Age group (year)	West Coast of West Malaysia		East Coast of West Malaysia		South West Malaysia		East Malaysia	
0-1	*25/48	52.1%	2/3	66.7%	6/11	54.5%	7/13	53.8%
1-2	39/50	78%	2/2	100%	12/16	75%	5/7	71.4%
2-3	50/55	90.9%	2/2	100%	6/7	85.7%	10/11	90.9%
3-4	44/44	100%	5/7	71.4%	9/10	90%	13/14	92.9%
4-5	40/40	100%	3/3	100%	12/14	85.7%	18/18	100%
5-6	52/53	98.1%	2/2	100%	7/7	100%	12/13	92.3%
6-7	36/37	97.3%	6/6	100%	15/18	83.3%	13/14	92.9%
7-8	38/39	97.4%	15/16	93.8%	5/5	100%	15/15	100%
8-9	46/49	93.9%	6/6	100%	6/6	100%	13/14	92.9%
9-10	48/48	100%	10/10	100%	8/8	100%	9/9	100%
10-15	47/47	100%	9/9	100%	14/14	100%	5/5	100%
15-20	45/45	100%	4/4	100%	12/12	100%	14/14	100%
Total	510/555	91.9%	66/70	94.3%	112/128	87.5%	134/147	91.2%

* Number of positive sera / Total sera Percentage of positive (%)

Table 9. χ^2 Table of EBV Seropositive (IgG-VCA) Sera In Different Regions

Region	Positive	Negative	Total
West Coast of West Malaysia	510	45	555
East Coast of West Malaysia	66	4	70
South West Malaysia	112	16	128
East Malaysia	134	13	147
Total	822	78	900

$\chi^2 = 3.372$, degree of freedom = 3, $p > 0.05$.

3.2.4. EBV Seroprevalence (IgG-VCA) in Male and Female

There were 452 sera (out of 498) in the male group and 370 sera (out of 402) from the female group which tested positive for IgG antibodies against VCA-EBV (Table 10). The EBV seroconversion patterns were similar in male and female groups (Figure 9), with no significant difference in seroprevalence between them ($\chi^2 = 0.4581$, $p > 0.05$. Table 11).

Male and female groups have similar IgG-VCA titres (Figure 10) and there is no significant difference between male and female groups in the GMT of IgG-VCA (Z-test; $Z = 0.3535$, $p > 0.05$. Table 12).

Table 10. EBV Seroprevalence (IgG-VCA) in Male and Female

Age group (year)	Male		Female	
0 - 1	*22/42	52.4%	18/33	54.5%
1 - 2	32/42	76.2%	26/33	78.8%
2 - 3	36/39	92.3%	32/36	88.9%
3 - 4	39/41	95.1%	32/34	94.1%
4 - 5	37/39	94.9%	36/36	100%
5 - 6	39/40	97.5%	34/35	97.1%
6 - 7	47/51	92.2%	23/24	95.8%
7 - 8	41/42	97.6%	32/33	97.0%
8 - 9	40/43	92.5%	31/32	96.9%
9- 10	38/38	100%	37/37	100%
10 - 15	47/47	100%	28/28	100%
15 - 20	34/34	100%	41/41	100%
Total	452/498	90.4%	370/402	92.5%

* Number of positive sera / Total sera Percentage of positive (%)

Figure 9. Percentage of EBV Seropositive (IgG-VCA) Sera in Male and Female

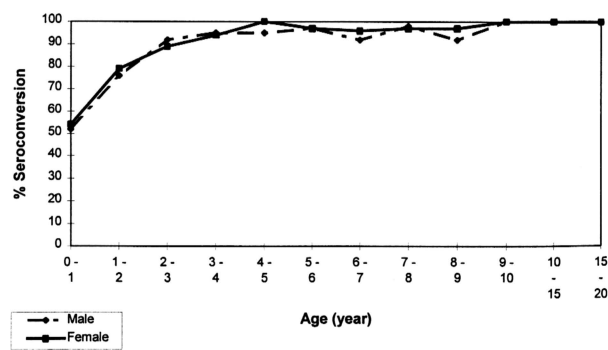


Table 11. χ^2 Table of EBV Seropositive Frequency in Male and Female

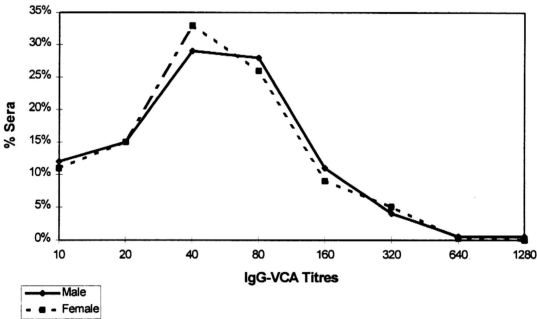
Sex	No. of positive sera	No. of negative sera	Total
Male	452	46	498
Female	370	32	402
Total	822	78	900

$\chi^2 = 0.4581, p>0.05$

Table 12. Titres of IgG-VCA in Male and Female

Sex	Titres								GMT
	10	20	40	80	160	320	640	1280	
Male	53 (12%)	69 (15%)	133 (29%)	125 (28%)	48 (11%)	16 (4%)	4 (0.5%)	4 (0.5%)	49
Female	40 (11%)	56 (15%)	122 (33%)	96 (26%)	35 (9%)	20 (5%)	1 (0.3%)	0 (0%)	48
Total	93	125	255	221	83	36	5	4	-

Figure 10. Distribution of IgG-VCA Titres in Male and Female



3.2.5. Seroprevalence (IgG-VCA) of EBV in Ethnic Groups

Table 13 shows the distribution of EBV-IgG positive sera in different ethnic groups. Generally, the EBV seroconversion pattern was similar among the ethnic groups, which showed 100% seroconversion by the age of more than 9 years. 448 of 490 sera from Malay (91.4%), 230 of 250 sera from Chinese (92.0%), 62 of 67 sera from Indians (92.5%) and 82 of 93 sera from Others (88.2%) were positive for anti-VCA IgG antibodies. There was no significant difference in EBV seropositive frequency among the ethnic groups ($\chi^2=1.4094$, degree of freedom=3, $P>0.05$. Table 14).

The IgG-VCA titres were separated into the corresponding racial groups and the GMT tabulated in Table 15. GMT in the Chinese was 56, the highest among the 4 groups. This was followed by Malay (47), Indian (44) and Others (40). GMT between group pairs were compared by Z-test. GMT in the Chinese was significantly higher than the Malays ($Z= 2.291$, $p<0.01$) and Others ($Z= 2.624$, $p<0.01$) but not significantly different from GMT in the Indians ($Z= 1.715$, $p>0.05$). GMT in the Others was not significantly different from GMT in Malays ($Z= 1.311$, $p>0.05$) or Indians ($Z= 0.5718$, $p>0.05$).

Table 13. Distribution of EBV Seropositive (IgG-VCA)Sera by Age and Ethnic Groups

Age group (year)	Malay		Chinese		Indian		Others	
0 - 1	*21/41	51.2%	9/18	50.0%	5/6	83.3%	5/10	50.0%
1 - 2	36/43	83.7%	16/21	76.2%	2/3	66.7%	4/8	50.0%
2 - 3	36/41	87.8%	15/17	88.2%	7/7	100%	10/10	100%
3 - 4	42/46	91.3%	15/15	100%	8/8	100%	6/6	100%
4 - 5	43/44	95.5%	13/14	92.9%	8/8	100%	9/9	100%
5 - 6	41/41	100%	20/20	100%	2/3	66.7%	10/11	90.9%
6 - 7	40/42	95.2%	21/22	95.5%	4/5	80.0%	5/6	83.3%
7 - 8	37/39	94.9%	21/21	100%	3/3	100%	12/12	100%
8 - 9	39/40	97.5%	18/20	90.0%	6/7	87.5%	8/8	100%
9 - 10	28/28	100%	38/38	100%	5/5	100%	4/4	100%
10 - 15	44/44	100%	26/26	100%	4/4	100%	1/1	100%
15 - 20	41/41	100%	18/18	100%	8/8	100%	8/8	100%
Total	448/490	91.4%	230/250	92.0%	62/67	92.5%	82/93	88.2%

* Number of positive sera / Total sera Percentage of positive (%)

Table 14. χ^2 Table of EBV Seropositive Sera in Different Races

Race	No. of positive	No. of negative	Total
Malay	448	42	490
Chinese	230	20	250
Indian	62	5	67
Others	82	11	93
Total	822	78	900

$\chi^2 = 1.4094$, degree of freedom = 3, $p > 0.05$

Table 15. Titres of IgG-VCA in Different Ethnic Groups

Ethnic Group	Titres								GMT
	10	20	40	80	160	320	640	1280	
Malay	49	72	149	117	37	17	3	4	47
Chinese	16	34	71	59	35	13	2	0	56
Indian	9	11	16	17	6	3	0	0	44
Others	19	8	19	28	5	3	0	0	40
Total	93	125	255	221	83	36	5	4	-

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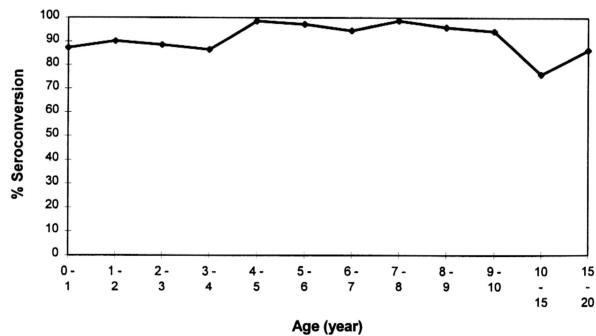
3.3. ELISA Results for IgG-VCA

There were 862 sera tested for IgG-VCA by ELISA. A total of 787 (91.3%) sera were seroreactive against VCA-EBV by ELISA. The EBV seroconversion percentage start at 87.3% in the first age group (0-1 year old). The percent of seroconversion maintained between 86.5% to 90.1% in three subsequent age groups before reaching 98.6% in the 4-5 years group. The high percentage of seroconversion remained in the subsequent 5 groups before dropping to 76.0% in the 10-15 years group. The percentage increased again to 86.4% in the last age group (15-20 years) (Table 16 and Figure 11).

Table 16. Seroprevalence of EBV (IgG-VCA) by ELISA

Age group (year)	No. of positive sera	% Positive
0 - 1	62	87.3
1 - 2	64	90.1
2 - 3	54	88.5
3 - 4	64	86.5
4 - 5	73	98.6
5 - 6	72	97.3
6 - 7	70	94.6
7 - 8	74	98.7
8 - 9	72	96.0
9 - 10	68	94.4
10 - 15	57	76.0
15 - 20	57	86.4
Total	787	91.3

Figure 11. Seroprevalence of EBV (IgG-VCA) by ELISA



3.4. Comparison of EBV Seroreactivity by IFA and ELISA

Of 862 sera tested by IFA and ELISA, 737 (85.5%) were positive for IgG-VCA in both methods (Table 17). There were 50 (5.8%) sera which were apparently positive for IgG-VCA in ELISA but not in IFA. In contrast, 47 (5.5%) sera shown to be positive for IgG-VCA in IFA were not detected in the ELISA.

EBV seroconversion profiles by both methods (IFA and ELISA) are compared in Table 18 and Figure 12.

Table 17. Serological Study of EBV IgG-VCA Antibodies by IFA and ELISA

	IFA positive	IFA negative	Total
ELISA positive	737	50	787
ELISA negative	47	28	75
Total	784	78	862

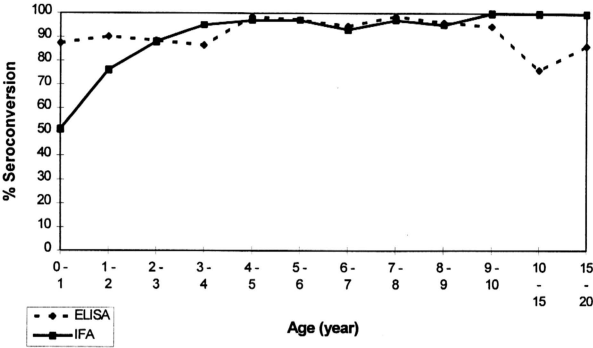
Table 18. Comparison of EBV Seroprevalence (IgG-VCA) by IFA and ELISA

Age group (year)	IFA		ELISA	
0 - 1	*36/71	50.7%	*62/71	87.3%
1 - 2	54/71	76.1%	64/71	90.1%
2 - 3	54/61	88.5%	54/61	88.5%
3 - 4	70/74	94.6%	64/74	86.5%
4 - 5	72/74	97.3%	73/74	98.6%
5 - 6	72/74	97.3%	72/74	97.3%
6 - 7	69/74	93.2%	70/74	94.6%
7 - 8	73/75	97.3%	74/75	98.7%
8 - 9	71/75	94.7%	72/75	96.0%
9 - 10	72/72	100%	68/72	94.4%
10 - 15	75/75	100%	57/75	76.0%
15 - 20	66/66	100%	57/66	86.4%
Total	784/862	91.0%	787/862	91.3%

* Number of positive sera / Total sera

Percentage of positive (%)

Figure 12. Seroprevalence of EBV (IgG-VCA) by IFA and ELISA



3.5 HUMAN HERPESVIRUS 6 (HHV-6)

3.5.1. HHV-6 Infection of Human Cord Blood Mononuclear Cells (HCBMC)

Freshly activated HCBMC (Figure 13) infected by HHV-6 showed distinct cytopathic effects (CPE) by becoming irregularly shaped, larger, balloon-like and refractile by day 4 of virus infection (Figure 15 and 17). CPE appeared much faster and in a bigger proportion of HCBMC upon co-cultivation with passaged infected cells (Figure 14, 15, 16 and 17).

3.5.2. Electron Microscopy of HHV-6 Infected HCBMC

Under the transmission electron microscope, extracellular particles which fitted the description of HHV-6 particle were observed (Figure 18). The diameter of the extracellular particles were 150-180nm. The size and morphology of these particles were similar to the virus described by Salahuddin *et al.* (1986).

3.5.3. Confirmation of HHV-6 Infection in HCBMC

HHV-6 infection in HCBMC was confirmed by using OHV-3 monoclonal antibody and positive control serum. The pattern of immunofluorescence staining in fixed infected cells varied from punctate nuclear and cytoplasmic staining to diffuse staining of the entire cell (Figure 19 & 20).

Serum and cell controls were performed using an HHV-6 IgG negative serum (Figure 21) and an uninfected HCBMC (Figure 22) respectively.

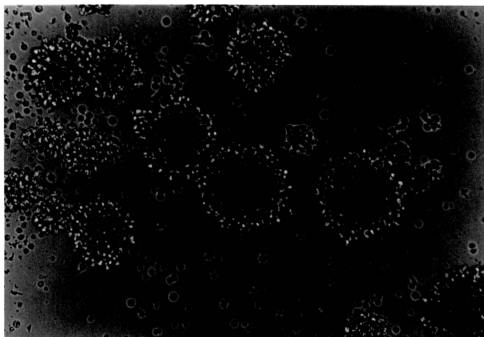


Figure 13: Activated human cord blood mononuclear cells on the third day of culture.
(Magnification X200)

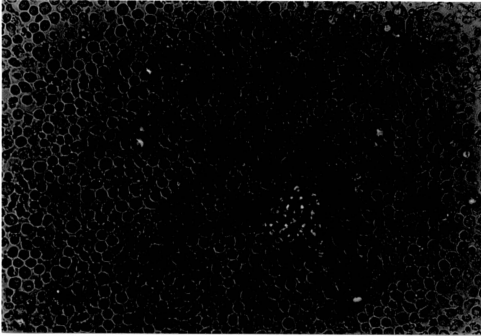


Figure 14: Human cord blood mononuclear cells at 48 hours post-infection by HHV-6.
No distinct CPE observed. (Magnification X 200)

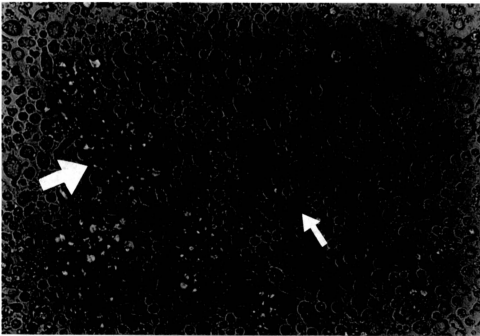


Figure 15: 96 hours post-infection by HHV-6. Some cells remained in clumps (big arrow). CPE observed in a majority of the single cells (small arrow).
(Magnification X 200)

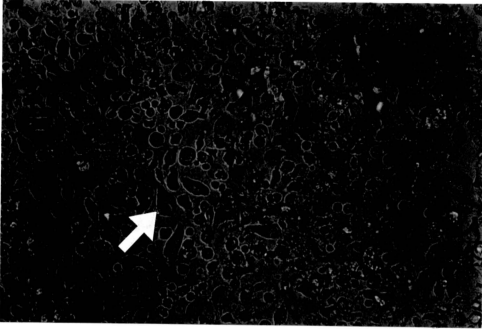


Figure 16: Human cord blood mononuclear cells after 48 hours of co-cultivation with passaged HHV-6 infected cells. Note cells with CPE (arrowed). (Magnification X200)

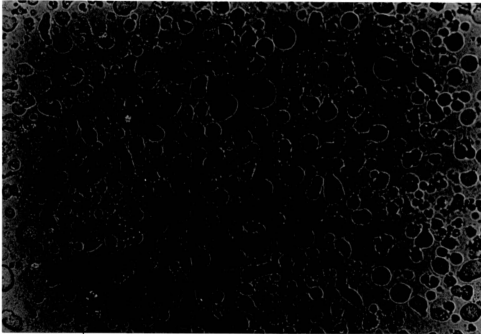


Figure 17: Human cord blood mononuclear cells after 96 hours of co-cultivation with passaged HHV-6 infected cells. (Magnification X200).

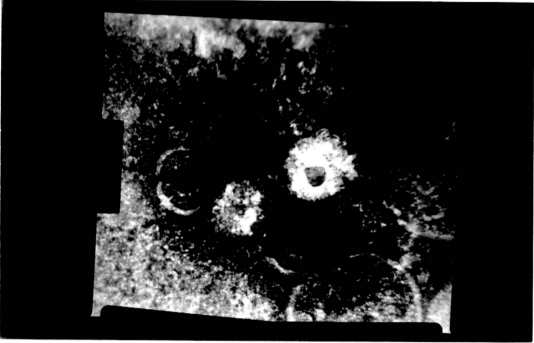


Figure 18: Electron micrograph of extracellular presumptive HHV-6 particle.

— Bar = 100 nm

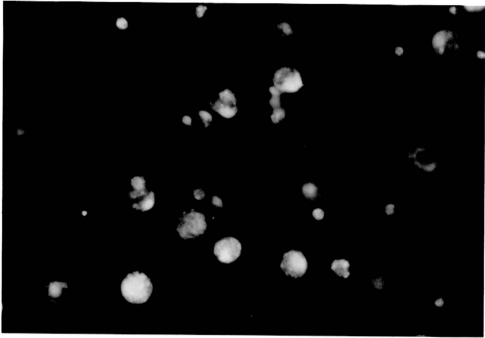


Figure 19: Immunofluorescence of HHV-6 infected human cord blood mononuclear cells showing punctate nuclear and cytoplasmic staining to OHV-3 monoclonal antibodies. (Magnification X200)

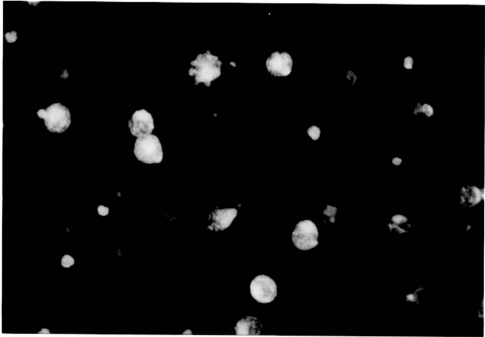


Figure 20: Immunofluorescence staining of an HHV-6 antibodies positive serum on HHV-6 infected human cord blood mononuclear cells. (Magnification X200)

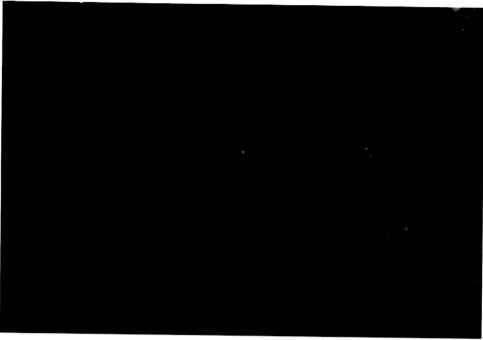


Figure 21: IFA of an HHV-6 antibodies negative serum on HHV-6 infected human cord blood mononuclear cells. (Magnification X200)

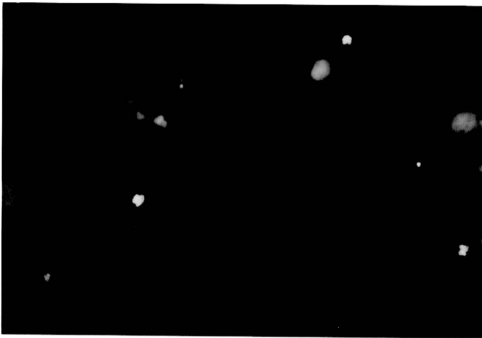


Figure 22: IFA of the same serum as in Figure 20 on an uninfected human cord blood mononuclear cells. (Magnification X 200)

3.5.4a. HHV-6 Seroprevalence in Different Age Groups

Table 19 shows the seroprevalence of HHV-6 in each age group from birth to 20 years. IgG was detected in 815 of the sera tested for seroreactivity against HHV-6, giving seropositive rate of 90.6%. HHV-6 seroconversion occurred very early in life, whereby 77.3 % of children at 0 -1 year seroconverted. From 77.3 % in children aged below 2 years, the seropositive percentage increased to 88% in 2-3 years group and subsequently fluctuated between 86.7% to 97.3% (Figure 23).

3.5.4b. Titres of IgG Anti-HHV-6 in Different Age Groups

Titres of IgG against HHV-6 ranged from 1:10 to 1:1280, and the GMT ranged from 71 to 284 (Table 20 and Figure 24). End point titre of 1:320 was the most common titre, found in 169 sera (20.7%) of the 815 HHV-6 IgG positive sera. 1:10 was the least common titre, found in 8 sera (9.8%). From a GMT of 185 in the 0-1 age group, it rose to GMT=284 in the 1-2 age group, and then fluctuated in the following age groups before gradually declining to a GMT of 71 in the age group of 15-20 years (Figure 24).

Table 19. Ig-G anti-HHV-6 in Different Age Groups

Age group (year)	No. of positive sera	% Positive
0 - 1	58	77.3
1 - 2	58	77.3
2 - 3	66	88.0
3 - 4	65	86.7
4 - 5	73	97.3
5 - 6	70	93.3
6 - 7	71	94.7
7 - 8	71	94.7
8 - 9	73	97.3
9 - 10	72	96.0
10 - 15	68	90.7
15 - 20	70	93.3
Total	815	90.6

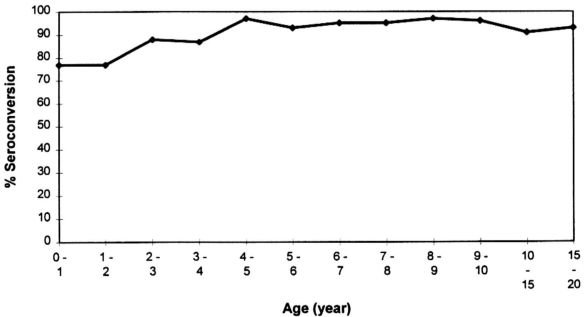
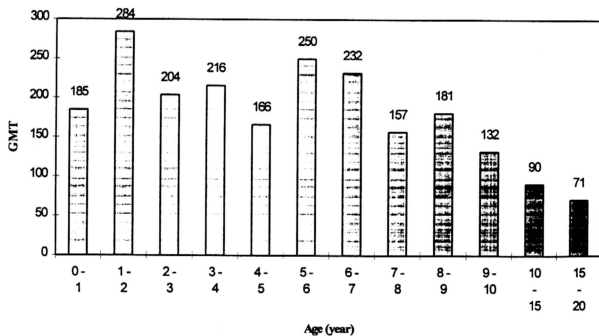
Figure 23. Ig-G anti-HHV-6 in Different Age Groups

Table 20. Distribution of IgG anti-HHV-6 Titres by Age

Age Group (year)	Titres								GMT
	10	20	40	80	160	320	640	1280	
0 - 1	3	4	6	8	7	13	8	9	185
>1 - 2	1	4	1	5	10	9	22	6	284
>2 - 3	0	7	8	5	12	15	7	12	204
>3 - 4	0	4	13	2	15	6	13	12	216
>4 - 5	0	4	8	19	11	14	14	3	166
>5 - 6	0	2	2	16	9	15	22	4	250
>6 - 7	0	3	4	12	9	25	12	6	232
>7 - 8	0	10	0	18	14	14	13	2	157
>8 - 9	0	3	4	21	9	22	13	1	181
>9 - 10	0	9	4	25	6	19	6	3	132
>10 - 15	2	16	5	14	15	8	8	0	90
>15 - 20	2	10	24	14	7	9	3	1	71
Total	8	76	79	159	124	169	141	59	-

Figure 24. GMT of IgG anti-HHV-6 by Age

3.5.5. HHV-6 Seroprevalence in Different Regions

The HHV-6 seroprevalence is tabulated with respect to the state from which the sera were collected (Table 21 and 22). Due to the small sample sizes of sera from some states (notably Penang, Perlis and Kelantan), the data were pooled into 4 regions (Table 23) as was done in Table 8 for EBV seroprevalence. HHV-6 seroconversion profile between regions were similar and χ^2 -test showed no significant difference between the HHV-6 seropositive in the different regions ($\chi^2 = 0.9256$, $p > 0.05$. Table 24).

Table 21. HHV-6 Seroprevalence in Different States

State	Number of Positive Sera	% Positive
Wilayah Persekutuan	287	92.3
Selangor	135	93.1
Perlis	4	100
Pulau Pinang	2	50.0
Kedah	42	82.3
Perak	37	92.5
Pahang	39	92.9
Terengganu	22	95.7
Kelantan	5	100
Negeri Sembilan	20	90.9
Melaka	18	81.8
Johor	74	88.1
Sabah	67	85.9
Sarawak	63	91.3
Total	815	90.6

Table 22. Distribution of HHV-6 Seropositive Sera by Age and Place of Collection

Age group (year)	WP	SEL	Perlis	P.PIN	KED	PER	PAH	TER	KEL	N.SEM	MEL	JOH	SAB	SAR
0-1	21	7	1	0	6	1	1	2	0	3	0	6	7	3
1-2	14	12	0	0	6	4	1	1	0	2	2	9	2	5
2-3	27	16	0	0	3	1	2	0	0	4	0	3	8	2
3-4	28	5	0	0	4	3	5	1	0	1	0	6	7	5
4-5	22	8	0	0	5	5	2	1	0	0	3	11	7	9
5-6	33	9	0	0	3	6	2	0	0	0	3	3	6	5
6-7	23	8	0	1	0	4	2	4	0	4	2	11	6	6
7-8	23	5	1	0	5	3	8	6	0	0	0	5	10	5
8-9	26	12	0	0	5	6	3	2	1	2	1	3	2	10
9-10	24	16	1	0	4	1	6	3	0	2	4	2	1	8
10-15	33	10	0	1	1	1	5	2	2	2	2	6	0	3
15-20	13	27	1	0	0	2	2	0	2	0	1	9	11	2
Total	287	135	4	2	42	37	39	22	5	20	18	74	67	63

Note :

WP - Wilayah Persekutuan
 SEL - Selangor
 P.PIN - Pulau Pinang
 KED - Kedah
 PER - Perak
 PAH - Pahang
 TER - Terengganu
 KEL - Kelantan
 N. SEM - Negeri Sembilan
 MEL - Melaka
 JOH - Johor
 SAB - Sabah
 SAR - Sarawak

Table 23. HHV-6 Seroprevalence in 4 Different Regions

Age group (year)	West Coast of West Malaysia		East Coast of West Malaysia		South West Malaysia		East Malaysia	
0-1	*36/48	75%	3/3	100%	9/11	81.8%	10/13	76.9%
1-2	36/50	72%	2/2	100%	13/16	81.2%	7/7	100%
2-3	47/55	85.5%	2/2	100%	7/7	100%	10/11	90.9%
3-4	40/44	90.9%	6/7	85.7%	7/10	70%	12/14	85.7%
4-5	40/40	100%	3/3	100%	14/14	100%	16/18	88.9%
5-6	51/53	96.2%	2/2	100%	6/7	85.7%	11/13	84.6%
6-7	36/37	97.3%	6/6	100%	17/18	94.4%	12/14	85.7%
7-8	37/39	94.9%	14/16	87.5%	5/5	100%	15/15	100%
8-9	49/49	100%	6/6	100%	6/6	100%	12/14	85.7%
9-10	46/48	95.8%	9/10	90%	8/8	100%	9/9	100%
10-15	46/47	97.9%	9/9	100%	10/14	71.4%	3/5	60%
15-20	43/45	95.5%	4/4	100%	10/12	83.3%	13/14	92.9%
Total	507/555	91.4%	66/70	94.3%	112/128	87.5%	130/147	88.4%

* Number of positive sera / Total sera Percentage of positive (%)

Table 24. χ^2 Table of HHV-6 Seroprevalence in 4 Different Regions

Region	Positive	Negative	Total
West Coast of West Malaysia	507	48	555
East Coast of West Malaysia	66	4	70
South West Malaysia	112	16	128
East Malaysia	130	17	147
Total	815	85	900

$\chi^2 = 3.370$, degree of freedom = 3, $p > 0.05$.

3.5.6. HHV-6 Seroprevalence in Male and Female

89.5% (443 out of 498) of sera from males and 92.5% (372 out of 402) of sera from females were positive for IgG against HHV-6 (Table 25). The seroconversion patterns were similar in males and females (Figure 25) with the χ^2 -tested showing no significant difference between the seropositive frequencies in the sexes. ($\chi^2 = 3.3388$, $p > 0.05$. Table 26).

Males and females have similar IgG-HHV-6 titres profiles (Figure 26) and there was no significant difference between male and female groups in the GMT of IgG-HHV-6 (Z-test; $Z = 0.3581$, $p > 0.05$. Table 27).

Table 25. HHV-6 Seroprevalence in Male and Female

Age group (year)	Male		Female	
0 - 1	*32/42	76.2%	*26/33	78.8%
1 - 2	32/42	76.2%	26/33	78.8%
2 - 3	34/39	87.2%	32/36	88.9%
3 - 4	35/41	85.4%	30/34	88.2%
4 - 5	37/39	94.9%	36/36	100%
5 - 6	37/40	92.5%	33/35	94.3%
6 - 7	47/51	92.2%	24/24	100%
7 - 8	41/42	97.6%	30/33	90.9%
8 - 9	41/43	95.3%	32/32	100%
9- 10	36/38	94.7%	36/37	97.3%
10 - 15	40/47	85.1%	28/28	100%
15 - 20	31/34	91.2%	39/41	95.1%
Total	443/498	89%	372/402	92.5%

* Number of positive sera / Total sera Percentage of positive (%)

Figure 25. HHV-6 Seroprevalence in Male and Female

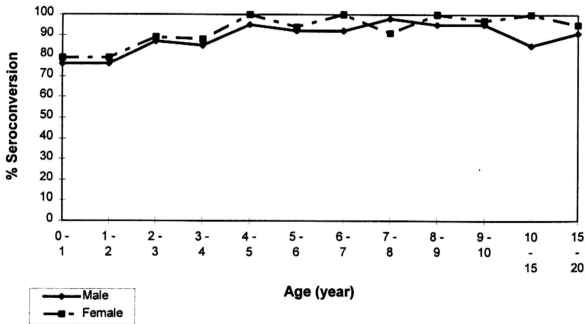


Table 26. χ^2 Table of HHV-6 Seropositive Sera in Male and Female

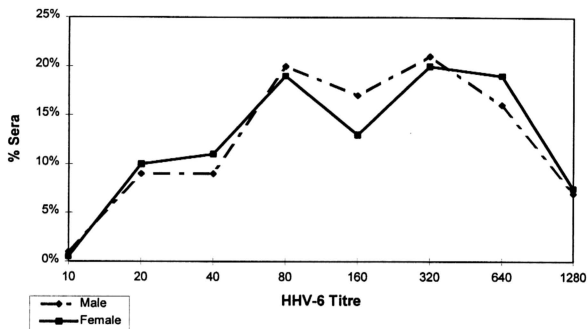
Sex	No. of positive sera	No. of negative sera	Total
Male	443	55	495
Female	372	30	402
Total	815	85	900

$$\chi^2 = 3.3388, p > 0.05$$

Table 27. IgG anti-HHV-6 Titres in Male and Female

Sex	Titres								GMT
	10	20	40	80	160	320	640	1280	
Male	6 (1%)	39 (9%)	39 (9%)	88 (20%)	76 (17%)	93 (21%)	71 (16%)	31 (7%)	165
Female	2 (0.5%)	37 (10%)	40 (11%)	71 (19%)	48 (13)	76 (20%)	70 (19%)	28 (7.5%)	169
Total	8	76	79	159	124	169	141	59	-

Figure 26. IgG-HHV-6 Titres in Male and Female



3.5.7. Seroprevalence of IgG-HHV-6 by Age and Ethnic Groups

The Chinese have the highest seroprevalence of 92.0% (230 out of 250 sera tested) while the lowest was in Others with 88.2% (82 of 93) (Table 28). The number of IgG HHV-6 positive sera was shown to be not significantly different among the ethnic groups by χ^2 -test ($\chi^2 = 1.3189$, degree of freedom=3, $p > 0.05$. Table 29).

Ninety of the 442 sera from Malays had a titre of 1:80, 87 at 1:320 and 73 at 1:160. On an average, the Malays had the lowest GMT (154.8) compared to other ethnic groups (Table 30), but the mean was not significantly different from the Chinese ($Z = 1.4195$, $p > 0.05$), Indians ($Z = 1.672$, $p > 0.05$) or Others ($Z = 0.9070$, $p > 0.05$).

Sixty two of 230 sera from Chinese had a titre of 1:320, 45 had a titre of 1:80 and 38 sera were at 1:640. GMT of the HHV-6 antibodies in Chinese was 178, which was higher than the Malay group, slightly more than the Others group and less than the Indian group. However there was also no significant difference shown by the Z-test when the GMT was compared to Indian ($Z = 0.8647$, $p > 0.05$) and Others group ($Z = 0.0479$, $p > 0.05$).

Fourteen of 61 sera from the Indian group had a titre of 1:640 followed by 12 sera with titre of 1:80, 10 sera with titre of 1:320 and 9 sera were at 1:1280. The Indian group had the highest GMT (210) but it was not significantly different compared to Others group ($Z = 0.7828$, $p > 0.05$).

Table 28. IgG anti-HHV-6 Seroprevalence by Age and Ethnic Groups

Age group (year)	Malay		Chinese		Indian		Others	
0 - 1	*30/41	73.2%	15/18	83.3%	5/6	83.3%	8/10	80.0%
1 - 2	31/43	72.1%	18/21	85.7%	2/3	66.7%	7/8	87.5%
2 - 3	35/41	85.4%	15/17	88.2%	7/7	100%	9/10	90.0%
3 - 4	38/46	82.6%	15/15	100%	7/8	87.5%	5/6	83.3%
4 - 5	44/44	100%	14/14	100%	8/8	100%	7/9	77.8%
5 - 6	39/41	95.1%	18/20	90.0%	3/3	100%	10/11	90.9%
6 - 7	41/42	97.6%	22/22	100%	3/5	60.0%	5/6	83.3%
7 - 8	38/39	97.4%	19/21	90.5%	3/3	100%	11/12	91.7%
8 - 9	40/40	100%	19/20	95.0%	6/7	85.7%	8/8	100%
9 - 10	26/28	92.9%	37/38	97.4%	5/5	100%	4/4	100%
10 - 15	42/44	95.5%	22/26	84.6%	4/4	100%	0/1	0.00%
15 - 20	38/41	92.7%	16/18	88.9%	8/8	100%	8/8	100%
Total	442/490	90.2%	230/250	92.0%	61/67	91.0%	82/93	88.2%

* Number of positive sera / Total sera Percentage of positive (%)

Table 29. χ^2 Table of HHV-6 Seropositive Sera in Different Ethnic Groups

Race	No. of positive	No. of negative	Total
Malay	442	48	490
Chinese	230	20	250
Indian	61	6	67
Others	82	11	93
Total	815	85	900

$\chi^2 = 1.3189$, degree of freedom = 3, $p > 0.05$

Table 30. Titres of IgG-HHV-6 in Different Ethnic Groups

Ethnic	Titres								GMT
	10	20	40	80	160	320	640	1280	
Malay	1	48	46	90	73	87	69	28	155
Chinese	6	14	21	45	27	62	38	17	178
Indian	1	5	5	12	5	10	14	9	210
Others	0	9	7	12	19	10	20	5	177
Total	8	76	79	159	124	169	141	59	-

3.6. Serum IgG Against EBV and HHV-6

Of 900 sera tested for IgG against EBV and HHV-6, 757 (84.1%) sera had IgG against EBV-VCA and HHV-6. Sixty five sera were positive for only EBV-VCA, and 58 sera were positive only to HHV-6. Only 20 sera (2.2%) were negative for both viruses (Table 31). Seroconversion profiles for both viruses are portrayed in Figure 27.

3.7. EBV and HHV-6 Seroreactivity in Newborns and Babies

Although 75 sera in the first age group (0-1 year old) were available for testing, there was no detail on the actual age in month. We were interested to determine when seroconversion occurred in very young age. So, 60 sera from newborns were collected from Clinical Diagnostic Lab (CDL), UHKL for detection of maternal antibodies against EBV and HHV-6. All the newborns were less than 1 month of age. There were 36 sera collected from Malays, 18 from Chinese and 6 sera from Indians. Out of the 60 sera, 33 were from males and 27 from females (Table 32). 59 (98.3%) of the 60 newborns sera were seropositive against VCA-EBV and 57 sera (95%) were positive against HHV-6 (Table 33). Thus maternal antibodies to EBV and HHV-6 are present in nearly all newborns.

Another 9 sera were collected from 6 male and 3 female babies aged 5-11 months. 4 sera were seropositive against VCA-EBV, the babies were at ages of 5, 7, 9 and 11 months. 4 HHV-6 seropositive were detected among 9 sera, 2 from 8-month-old, 1 each from 10-month-old and 11-month-old. The 11 months old baby had seroconverted

with respect to both EBV and HHV-6. Though the sample size is very small, maternal antibodies against EBV and HHV-6 are likely to have diminished by 5 months and seroconversion begins soon after (Table 34).

Table 31. Serum IgG Against EBV-VCA and HHV-6

EBV IgG/VCA	HHV-6 IgG		Total
	Positive	Negative	
Positive	757	65	822
Negative	58	20	78
Total	815	85	900

Figure 27. Seroconversion Patterns for EBV and HHV-6

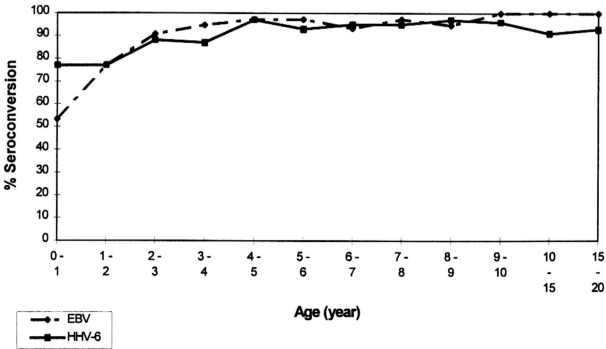


Table 32. Sex and Race Distribution of Newborn Babies from Whom Sera were Obtained.

Race	Male	Female	Total
Malay	20	16	36
Chinese	10	8	18
Indian	3	3	6
Total	33	27	60

Table 33. Serum reactivity to EBV-VCA and HHV-6 in Newborn Babies

Reactivity to :	No. of Positive	No. of Negative	% of Positive
VCA-EBV	59	1	98.3
HHV-6	57	3	95.0

Table 34. EBV and HHV-6 Seroreactivities in 9 Sera from Babies

Number	Age (Month)	Sex	Race	EBV Seroreactivity	HHV-6 Seroreactivity
Z1	5	Male	Malay	Negative	Negative
Z2	5	Female	Chinese	Positive	Negative
Z3	6	Female	Malay	Negative	Negative
Z4	7	Male	Chinese	Positive	Negative
Z5	8	Male	Malay	Negative	Positive
Z6	8	Male	Malay	Negative	Positive
Z7	9	Male	Malay	Positive	Negative
Z8	10	Female	Malay	Negative	Positive
Z9	11	Male	Malay	Positive	Positive