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Prevalence of viral infections in clinical specimens at Virology Laboratory Unit during the year 1998 to 2004

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Objective : *To investigate the prevalence of viral infections in clinical samples sent to Virology Laboratory Unit, King Chulalongkorn Memorial Hospital*

Design : *Chronological retrospective and descriptive study*

Setting : *Virology Laboratory Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.*

Subjects : *All clinical samples sent to Virology Laboratory Unit, from 1998 to 2004*

Methods : *All samples requested for viral antibody detection assays were performed by means of enzyme-linked immunosorbent assay (ELISA), whereas viral antigen detection of respiratory syncytial virus (RSV) was determined by immunofluorescent assay, and rotavirus antigen detection was done by immunochromatography.*

Results : *From 1998 to 2004, the mean prevalence of CMV, EBV, HSV and VZV infection were 90.09, 84.60, 64.44 and 77.48 %, respectively. As for viral examthem such as rubella and measles infections, their mean prevalence were 81.01 and 61.53 %. Moreover, the highest incidence of RSV was found in September which was in the rainy season, while rotavirus in January, the winter.*

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Conclusion : *Although the data obtained from laboratory investigation might not be a good representative figure of prevalence of the population, at least it is useful for estimating the situation of viral infections that occurred each year which also included seasonal spreading.*

Keywords : *Prevalence, Viral infection, Virology Laboratory Unit.*

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รุ่งทิพย์ ธรรมบวร, วนิดา มั่งมี, ลักคณา ธรรมโชติรุจน์, เอกสิทธิ์ โกวิทย์ดำรงค์, ภาวพันธ์ ภัทรโกศล. ความชุกของการติดเชื้อไวรัสในตัวอย่างสิ่งส่งตรวจที่ห้องปฏิบัติการไวรัสวิทยา ระหว่างปี ค.ศ. 1998 ถึง 2004. จุฬาลงกรณ์เวชสาร 2550 เม.ย; 51(4): 229 - 39

วัตถุประสงค์ : *เพื่อศึกษาหาความชุกของการติดเชื้อไวรัสที่เกิดขึ้นในตัวอย่างส่งตรวจที่ห้องปฏิบัติการไวรัสวิทยา โรงพยาบาลจุฬาลงกรณ์*

รูปแบบการวิจัย : *แบบพรรณนา โดยศึกษาข้อมูลย้อนหลังเป็นช่วงเวลาต่อเนื่อง*

สถานที่ทำการศึกษา : *ห้องปฏิบัติการไวรัสวิทยา ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10330, ประเทศไทย*

ประชากรที่ศึกษา : *ตัวอย่างที่ส่งตรวจทั้งหมดที่ห้องปฏิบัติการไวรัสวิทยา ระหว่างปี ค.ศ. 1998 ถึง 2004*

วิธีการศึกษา-วัดผล : *ตัวอย่างที่ได้รับการร้องขอให้ตรวจหาแอนติบอดีจำเพาะต่อการติดเชื้อไวรัสตรวจด้วยวิธี enzyme-linked immunosorbent assay (ELISA) ส่วนการตรวจหาแอนติเจน เช่น respiratory syncytial virus (RSV) ทำการตรวจด้วยวิธี immunofluorescent assay และการตรวจหาแอนติเจนของไวรัสโรตา ทำโดยวิธี immunochromatography*

ผลการศึกษา : *ค่าเฉลี่ยความชุกของการติดเชื้อ CMV, EBV, HSV และ VZV ระหว่างปี ค.ศ. 1998 ถึง 2004 คือ ร้อยละ 90.09, 84.60, 64.44 และ 77.48 ตามลำดับ ส่วนไวรัสที่เป็นสาเหตุก่อโรคไข้ออกผื่นเช่น rubella และ measles พบว่ามีค่าเฉลี่ยความชุกร้อยละ 81.01 และ 61.53 นอกจากนี้การตรวจหาอุบัติการณ์การติดเชื้อ RSV พบได้มากในเดือนกันยายน ซึ่งเป็นช่วงฤดูฝน ส่วนการตรวจพบ rotavirus พบได้สูงสุดเดือนมกราคม ซึ่งเป็นช่วงฤดูหนาว*

วิจารณ์และสรุปผล : *แม้ว่าข้อมูลที่นำมาวิเคราะห์เป็นข้อมูลที่ได้จากการตรวจทางห้องปฏิบัติการ ซึ่งอาจเป็นตัวแทนของความชุกและอุบัติการณ์ของประชากรได้ไม่ดีนัก แต่อย่างไรก็ตามข้อมูลดังกล่าวก็สามารถประมาณสถานการณ์ของการติดเชื้อไวรัสในแต่ละปี รวมทั้งยังสามารถแสดงช่วงระบาดของไวรัสบางชนิดได้*

คำสำคัญ : *ความชุก, การติดเชื้อไวรัส, ห้องปฏิบัติการไวรัสวิทยา*

The Virology Laboratory Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University is responsible for routine laboratory diagnosis that serves all physicians of King Chulalongkorn Memorial Hospital and other neighboring healthcare institutions. From 1998 to 2004, our laboratory unit received many clinical specimens for diagnostic testing which included conventional methods, such as viral antibody detection, viral antigen detection and viral isolation, and molecular detection of viral genome both qualitative and quantitative. All data were collected and here, we demonstrated the usefulness of some data by summarizing (Table 1) and analyzing in order to determine the incidence and prevalence of viral infections. In addition, seasonal epidemiology of some viruses might be able to be illustrated.

Table 1. Number of clinical specimens sent to the Virology Laboratory Unit from 1998 to 2004.

Test Year	1998	1999	2000	2001	2002	2003	2004
Antibody detection							
CMV-IgG	620	674	730	614	537	531	403
CMV-IgM	645	685	541	602	534	539	403
EBV-IgG	*	*	119	152	132	195	175
EBV-IgM	*	*	145	185	173	200	244
HSV-IgG	356	341	373	277	210	152	164
HSV-IgM	359	334	330	307	237	185	153
VZV-IgG	*	*	*	130	232	292	189
VZV-IgM	*	*	*	6	15	15	11
Rubella IgG	1418	1610	1538	1560	2085	2258	2593
Rubella- IgM	297	297	206	225	151	175	124
Parvo B 19 IgG	*	*	5	6	16	17	50
Parvo B 19 IgM	*	*	4	10	17	19	55
Measle-IgG	*	*	*	26	18	39	34
Measle-IgM	*	*	*	23	23	41	36
Antigen detection							
RSV Ag**	122	*	21	63	130	218	206
Rotavirus Ag	111	31	104	95	142	155	185
Total	**3928**	**3972**	**4116**	**4281**	**3652**	**5031**	**5035**

* Not yet start service

** Stop service between September 1998-September 2000

Materials and Methods

Clinical specimens

The specimens were from both the Out-patient and In-patient departments of King Chulalongkorn Memorial Hospital from 1998 to 2004. They were sent to the Virology Laboratory Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Few samples were from nearby hospitals. The serum sample was used for viral antibody detection, stool for rotavirus antigen detection, nasopharyngeal suction for determining respiratory syncytial virus (RSV) infected cells.

Viral antibody detection

Most of the tests are based on enzyme-linked immunosorbent assay (ELISA), cytomegalovirus (CMV) IgG and IgM, herpes simplex virus (HSV) IgG and IgM, rubella IgG and IgM (Cobas cores, Roche Diagnostic Company, USA), HSV IgM, varicella zoster virus (VZV) IgG and IgM, measles IgG and IgM (Human, Germany), Epstein-Barr virus (EBV) IgG and IgM, parvovirus B19 IgG and IgM (Novatec, Germany). All assays were performed according to the manufacturers' recommendations.

Viral antigen detection

As for RSV, the infected cells were determined by direct immunofluorescent assay (IFA) using viral specific antibody conjugated with Fluorescene Thiocyanate (FITC). Rotavirus antigen was determined by immunochromatography using rapid strip test from Newmarget, United Kingdom.

Table 2. The number of samples with positive specific IgG antibody and the estimated prevalence of each viral infection from 1998 to 2004.

Viral Type	No of positive IgG/Total samples (% Prevalence)							Total
	1998	1999	2000	2001	2002	2003	2004	(%)
CMV	535/620 (86.29)	612/674 (90.80)	650/730 (89.04)	561/614 (91.36)	488/537 (90.87)	488/531 (91.90)	379/415 (91.32)	3713/4121 (90.09)
EBV	NA	NA	90/119 (75.63)	136/152 (89.47)	116/132 (87.87)	163/195 (83.58)	149/175 (85.14)	654/773 (84.60)
HSV	219/356 (61.51)	216/341 (63.34)	245/373 (65.68)	175/277 (63.17)	152/210 (72.38)	97/152 (63.81)	103/164 (62.80)	1207/1873 (64.44)
VZV (P)	NA	NA	NA	86/130 (66.15)	59/73 (80.82)	117/141 (82.98)	51/60 (85.0)	313/404 (77.48)
VZV (NHMS)	NA	NA	NA	NA	144/159 (90.56)	144/151 (95.36)	120/129 (93.02)	408/439 (92.93)
Parvo B19	NA	NA	2/5 (40.00)	3/6 (50.00)	7/16 (43.75)	2/17 (11.76)	19/50 (38.00)	33/94 (35.10)
Rubella	1112/1418 (78.42)	1286/1610 (79.88)	1178/1538 (76.60)	1180/1560 (75.64)	1714/2085 (82.20)	1863/2258 (82.50)	2249/2593 (86.73)	10582/13062 (81.01)
Measles	NA	NA	NA	15/26 (57.69)	13/18 (72.22)	19/39 (48.71)	25/34 (73.52)	72/117 (61.53)

Results

Presence of specific IgG antibody in an individual means that person should have either present infection or past infection. Therefore, the prevalence of specific infectious agents could be determined. Moreover, the incidence of infection also could revealed by the presence of specific IgM antibody.

Herpesviruses infection

Herpesviruses in this study include CMV, EBV, HSV, and VZV. The clinical criteria of all samples was unable to define except for VZV. The samples of VZV were composed of two groups, patients (P) and normal healthy 5th year medical students (NHMS). Among the patients, the prevalence range of CMV infection between 1998 and 2004 was 86.29 - 91.90 %, EBV infection was 75.63 - 89.47%, HSV infection was 61.51 - 72.38 %, and VZV infection was 66.15 - 85 %. The means average of those viruses were 90.09, 84.60, 64.44 and 77.48 %, respectively. The prevalence of VZV infection was found higher in NHMS group (92.93 %) with the range between 90.52-93.02 % (Table 2). Although the incidence of infected cases of CMV, EBV, HSV and VZV were varied from year to year, the estimated average incidences of them were 8.4, 13.51, 5.24 and 17.02 %, respectively (Table 3).

Table 3. The number of samples with positive specific IgM antibody and the estimated incidence of each viral infection from 1998 to 2004.

Viral Type	No of positive IgM/Total samples (% Incidence)							Total
	1998	1999	2000	2001	2002	2003	2004	(%)
CMV	58/645 (8.99)	62/685 (0.90)	54/541 (9.98)	37/602 (6.14)	44/534 (8.23)	57/539 (10.57)	22/429 (5.12)	334/3975 (8.4)
EBV	NA	NA	17/145 (11.72)	26/185 (14.05)	30/173 (17.34)	21/200 (10.50)	34/244 (13.93)	128/947 (13.51)
HSV	3/359 (0.83)	3/334 (0.89)	5/330 (1.51)	39/307 (18.84)	17/237 (7.17)	13/185 (7.02)	20/153 (13.07)	100/1905 (5.24)
VZV (P)	NA	NA	NA	3/6 (50)	2/15 (13.33)	1/15 (6.66)	2/11 (18.18)	8/55 (17.02)
Parvo B19	NA	NA	1/4 (25)	0/10 (0)	9/17 (52.94)	2/19 (10.52)	8/55 (14.54)	20/105 (19.04)
Rubella	2/297 (0.67)	4/297 (1.34)	15/206 (7.28)	8/225 (3.55)	7/151 (4.63)	1/175 (0.57)	4/124 (3.22)	41/1475 (2.77)
Measles	NA	NA	NA	13/23 (56.52)	12/23.00 (52.17)	22/41 (53.65)	21/36 (58.33)	68/123 (55.25)

Viral exanthem infections

Rubella virus, parvovirus B19 and measles virus were agents that cause exanthem manifestation. Here, we demonstrated that the prevalence of rubella infection from all cases was ranged from 75.64 to 86.73 % with the average prevalence of 81.01 % (Table 2). Rubella samples were mainly from the antenatal care (ANC) and check-up clinics. Since the rubella IgG screening test is required for person who applies for visa, then all samples were presumably from healthy people. However, there were some samples that cannot be definitely classified. Therefore, the prevalence of rubella can be devided into at least 3 groups, i.e., normal adults and probably infected children and unkown. It is clearly shown that most of samples came from normal adults (>90 %). Among those adults, the prevalence of rubella infection between the year 2002 and 2004 was range from 82.75 % to 86.93 % with the mean average of 84.78 % while children, that was range from 36.56 % to 86.08 % with the mean average of 64.68 % (Table 4). The mean incidence of rubella infection between the adult and children groups was also determined according to the presence of IgM, only 1.43 % (4/280) was demonstrated in adult group whereas 5.71 % (8/140) was found in children group.

Due to small number of specimens in each year of parvovirus B19 and measles, their prevalence could not be determined. However, if total number of cases were used, the prevalence of parvovirus B19 infection would be 35.10 %. Similarly, that of measles would be 61.53 % (Table 2).

Considering of the data of IgM positive for parvovirus B19 and measles, the incidence of measles infection was higher than that of parvovirus infection: 55.25 % *vs.* 19.04 % (Table 3). In contrast to rubella infection, the incidence was very low (2.77 %).

RSV and Rotavirus infections

Beside the incidence determining by using the presence of IgM, direct viral antigen detection could also be used. The incidence of RSV infection was shown in Figure 1. Since the service was stopped for a while during September 1998 to August 1999,

Table 4. The prevalence of rubella during the year 2002 to 2004.

Group/Year	2002		2003		2004		(%) Mean
	No. Pos/Total (%)		No. Pos/Total (%)		No. Pos/Total (%)		prevalence
	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Adult	1680/1992	1/109	1761/2128	0/81	2096/2411	3/90	5537/6531
	(84.34)	(0.92)	(82.75)	(0)	(86.93)	(3.33)	(84.78)
Children	34/93	6/42	72/97	1/82	68/79	1/16	174/269
	(36.56)	(14.29)	(74.23)	(1.22)	(86.08)	(6.25)	(64.68)
Unknown	0/0	0/0	30/33	0/12	85/103	0/18	115/136
	(0)	(0)	(90.91)	(0)	(82.53)	(0)	(84.56)
Total	**1714/2085**	**7/151**	**1863/2258**	**1/175**	**2249/2593**	**4/124**	**5826/6936**
	(82.21)	**(4.63)**	**(82.50)**	**(0.57)**	**(86.73)**	**(3.22)**	**(84.00)**

the data from the year 2000 to 2004 were analyzed. Although RSV infection seemed to presence through out the year, the incidence peak started from July and declined in October which is the raining season of Thailand (Figure 1).

In addition, rotavirus infection seemed to present for a whole year, the incidence of rotavirus infection, in contrast to RSV, was found high during October to January (Figure 2) which is the winter of Thailand.

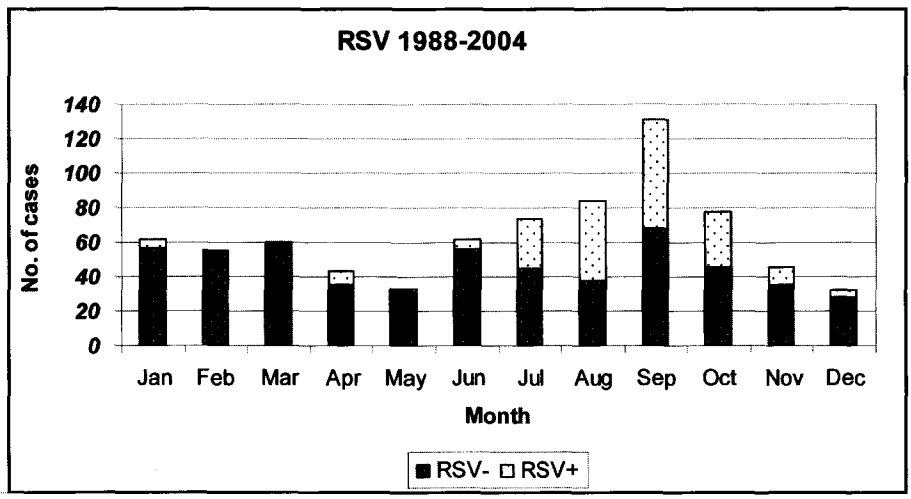

Figure 1. The incidence of RSV infection during from 1998 to 2004.

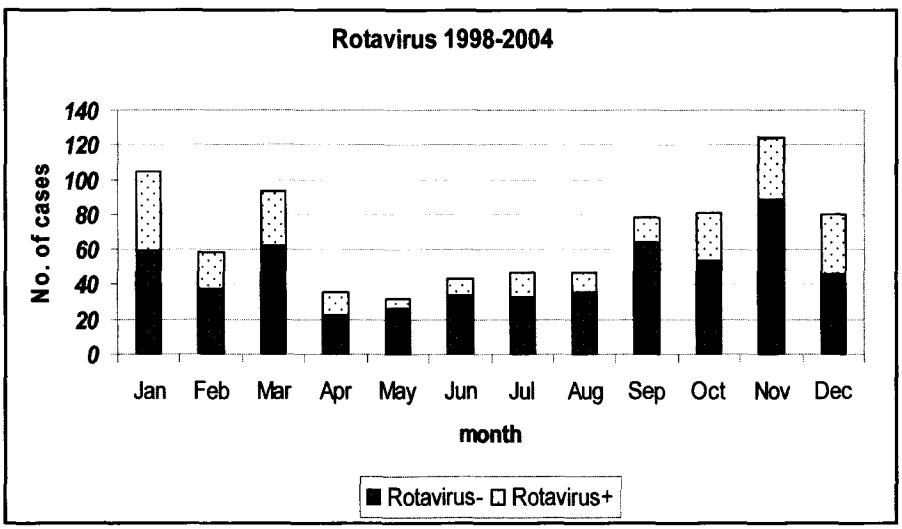

Figure 2. The incidence of rotavirus infection from 1998 to 2004.

Discussion

The prevalence of CMV, EBV and HSV in normal Thai population were previously reported as 97, 97.6, and 92 %, respectively. [1-3] Moreover, we previously showed that the prevalence determined by retrospective analysis from laboratory data might be lower than the real figure due to bias in the studied population.[4] When the information of prevalence previously observed from 1993 to 1997 [4] was compared to that of 1998 to 2004 of the same laboratory, it indicated that the prevalence of infection did not change much (CMV, 84.76 % *vs.* 90.09 %; EBV, 81.25 % *vs.* 84.60 %; HSV, 66.74 % *vs.* 64.44 %). In contrast to the incidence of those viral infections, they clearly showed an increase in the incidence of EBV and HSV infection while that of CMV seemed to be reduced (CMV, 11.57 % *vs.* 8.4 %; EBV, 7.16 % *vs.* 13.51%; HSV, 1.05 % *vs.* 5.24 %). Interestingly, as for HSV infection, the incidence rose from 0.38 % in 1998 to 18.84 % in 2001. The reason for this phenomenon was hardly explained. Most samples were from newborns, and the common route of HSV transmission is during delivery, this is suggestive that the transmission is direct from their mothers who probably have genital HSV infection. The incidence of HSV genital infection might increase due to an intervention method to control STD in Thailand which is to come into account and orogenital sex is probably more frequently practiced as a consequence of safe sex programs in the HIV campaign. [5]

Recently, Kowitdamrong E *et al.* 2005 reported the prevalence of VZV infection was 84 % in young adults aged 20 - 24 years old and 100 % in >25 years old.[6] In our study, the prevalence of NHMS group was 92.93 % (Table 2) which can represent Thai young adults aged between 20 - 25 years old. In the group suspected of VZV infection (P), the prevalence was 77.48 %. There was a number of studies that showed the prevalence of VZV infection among the Thais was 64 to 100 %. [7-10] This variations are probably due to the studied population, geographic distribution and timing. Interestingly, the incidence of VZV infection was only 17.02 % (Table 3). Since the clinical information is limited, we are not sure whether all these samples were obtained from patients who had clinical appearance of VZV symptoms or only they were only exposed people.

In these present data, the mean prevalence of rubella infection was 81.01 % (Table 2). Among those samples, more than 90% were from normal adults which were those who enrolled for check-up program for traveling aboard and pregnancy clinic. When grouping between normal adults and suspected infected children was done, the mean prevalence of the adults did not change (84.78 %, Table 4). This information may represent Thai adult population. Although the variation of the prevalence and incidence of rubella infection in children group were observed, the incidence of rubella infection was greater than adult group as expected (Table 4). The samples were all from newborn cases suggesting congenital rubella infection was low comparing to 36.7 % in 1980, reported by Tantivanich S *et al.* [11] The low incidence might be due to rubella vaccine program. Although rubella vaccine programs were started in Thailand since 1986, only girls were targeted.[12] Later in 1997, under the expanded program on immunization (EPI) of MMR the vaccine was begun and applied to all Thai children.

The prevalence of parvovirus B19 infection in Thailand was 10.94 % and 20.16 %, previously reported by Bhattarakosol P *et al.* [13] and Poovorawan Y *et al.* [14], respectively. Here, the prevalence was 35.10 % and the incidence was 19.04 %. All samples were from patients with clinically matched to parvovirus B19 infection. The number of suspected cases each year was quite small which was related to the prevalence figure.

As for measles, surprisingly, although measles vaccine was included in EPI since1984 and measles vaccine coverage was 96 % (data from EPI/ CDC/MOPH in 2003), the prevalence of immunity against measles was merely 61.53 %. This might be resulted from either primary vaccine failure or secondary vaccine failure. In 2001, Saipan P *et al.* [15] reported that measles antibody level decreased to the lowest level at 3-5 years after primary immunization (4 - 6 years of age). Also, in 2003, Ariyasriwatana C [16] reported that after measles was included in EPI, the group of children infected with measles changed from under 5 years to the age group of 5 - 9 years. Therefore, the second dose of measles vaccine should be administered to all children at the age of 4 - 6 years according to the current EPI recommendation in order to increase seroprotection in these children. Finally, the number of measles cases in this report may be lower than the fact because most cases of measles can be clinically diagnosed without any need of laboratory confirmation.

In addition, our information could reveal the prevalence and seasonal distribution of RSV as well as rotavirus. The high incidence of RSV was found in the rainy season (August to September) corresponding to previous reports of Siritantikorn S *et al.* [17] and Bhattarakosol P *et al.* [18] Wet and cool weather might be a good support for viable virions to stay longer in the environment. In contrast to rotavirus infection, it commonly occurred in winter season so the disease was named after as "winter gastroenteritis" but in tropical countries including Thailand, the incidence of rotavirus infection, in contrast to RSV, was found through a whole year with high incidence during October to February, the winter of Thailand. [19]

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