Acute Viral Lower Respiratory Infections in Children in a Rural Community in Thailand

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Abstract

The present study was conducted as a population based cohort in a rural community of Amphoe Takhli, Nakhon Sawan province for the determination of the prevalence of acute viral lower respiratory infection (ALRI) in pediatric cases under 5 years of age from November 1998 to February 2001. There were 472 ALRI episodes during the study period; and there were 5 cases who contracted ALRI twice. The etiologic agents were determined by indirect immunofluorescence (IIF) test using specific monoclonal antibodies for the staining of exfoliated cells in nasopharyngeal aspirate (NPA) samples. The slides of fixed cells were prepared by Takhli Hospital and posted in ambient temperature to the Virology Laboratory, Siriraj Hospital where they were stained and examined. Among 472 episodes of ALRI, 170 (36.0%) viral agents were found. Viral agents were associated with 41.4 per cent of all pneumonic cases. Respiratory syncytial virus (RSV) was the most common virus observed in the present study; and it was also the most common virus associated with pneumonia and bronchitis. RSV subgrouping was performed directly in NPA samples by IIF test using a panel of subgroup specific monoclonal antibodies. RSV subgroup B predominated over subgroup A in the first study year, and it was *vice versa* in the second year. Overall, more cases of subgroup B were found which was in contrast to what the authors had reported in the previous study. Prevalence of RSV was seasonal dependent, the epidemic was seen during the rainy season with peaks in August or September of each year. As the method of viral identification was limited to IIF only, therefore, fewer viruses were detected. Parainfluenza viruses were detected as the second most common viral agent, the viruses spread during early summer with peaks in February or March of each year. However, its association with croup could not be demonstrated which may be due to the insensitivity of

IIF in the diagnosis of non-RSV infection. Nevertheless, in terms of feasibility to investigate the disease in a rural area, IIF is economic, convenient and rapid; and gives enough information for the nationwide plan of a health care development system.

Key word : Viral Acute Lower Respiratory Infection, Rural Community

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Acute lower respiratory infection (ALRI) is an important cause of morbidity and mortality in children world-wide. Data from both developing and developed countries show that the majority of respiratory tract infections in children are caused by viruses such as the respiratory syncytial virus (RSV), parainfluenza types 1 (PF1), 2 (PF2) and 3 (PF3), influenza virus types A (fluA) and B (fluB), and adenovirus[1,2]. Among these viruses, RSV plays the most important role in both lower and upper respiratory tract infections in children under 5 years of age[3,4]. For the last two decades, RSV has been recognized as an important cause of acute viral respiratory infection in elderly persons[5-7].

A variety of methods are used for the diagnosis of respiratory virus infections, i.e. the isolation of viruses in cell culture, viral antigen detection by the immunofluorescence (IF) technique or enzyme immunoassays and antibody detection by the serological test[8]. Recently, the method of genome detection by PCR or RT PCR has been introduced for the investigation of respiratory viral infection, the advantage may be its application in the diagnosis of adult cases, the age-group with less virus shedding[9], or in the establishment of multiplex PCR for the diagnosis of multiple viral agents in a single reaction. In pediatric cases, plenty viral antigens are present in the respiratory specimens, thus, making it easier to detect by the IF technique which is less expensive. On the other hand, IF is less dignosis of sensitive in the infection in respiratory specimens from adults.

It is generally accepted that the diagnosis of RSV is more successful with viral antigen detection by IF than the virus isolation method, while the virus isolation method is slightly more sensitive than IF in the diagnosis of other respiratory viruses[7].

Based on the authors' previous experience on the proficiency test, slides of exfoliated respiratory cells fixed in precooled acetone could be transported in ambient temperature without deteriortion in quality of the viral antigen when stained by IF. The method is applied to the present study in which the slides of fixed respiratory cells were posted from a rural hospital to a laboratory in Bangkok where the slides were stained and examined, The authors' herein, determined the incidence of acute lower respiratory infection caused by respiratory viruses, i.e. RSV, parainfluenza types 1, 2 and 3, adenovirus and influenza virus types A and B in pediatric patients under five years old who were admitted to Takhli Hospital, Nakhon Sawan province by using the IF method. The study will provide additional information needed for further development of prevention and control measures of viral respiratory infections in Thailand.

MATERIAL AND METHOD

Subjects

The subjects enrolled in this study came from a population based cohort in a rural community of Amphoe Takhli, Nakhon Sawan province which is located in central Thailand, 240 km north of Bangkok. The subjects were under 5 years old and were

hospitalized in Takhli Hospital with the diagnosis of ALRI including pneumonia, bronchitis, croup and acute bronchiolitis as defined by the WHO standard protocol. There were 472 episodes from 467 cases (202 females and 265 males) of ALRI occurring in the cohort period from November 1998 to February 2001. The subjects had mean age ± SD of 20 ± 14 months (range 11 days to 5 years).

Specimen collection and processing

Nasopharyngeal aspirate (NPA) specimens were collected in mucus traps and processed for preparing the slides of cell deposit at Takhli Hospital. Briefly, NPA samples collected in normal saline were mixed thoroughly and washed 3 times with phosphate buffered saline (PBS) by spinning at 1,000 rpm for 5 minutes. The pellets of exfoliated cells from NPA were dropped onto glass slides, air dried and fixed in pre-cooled acetone at -20°C for 10 minutes. The processed slides of cell deposits could be kept at 4°C for one week before being mailed in ambient temperature to the Virology Laboratory, Siriraj Hospital for staining by indirect immunofluorescence (IIF).

Indirect immunofluorescence

Reagent

Immunofluorescence kits for diagnosis of respiratory viruses were purchased from Chemicon International Inc, Temecula, CA, USA. The kit was composed of a vial of pooled monoclonal antibodies to RSV, fluA, flu B, PF1, PF2, PF3 and adenovirus, each individual vial of monoclonal antibody to the virus mentioned above and a vial of fluorescein isothiocyanate (FITC) conjugated anti-mouse immunoglobulin.

Staining procedure

The fixed cell deposits on microscopic glass slides were covered with mouse monoclonal antibody specific to respiratory viruses for 30 minutes at 37°C in a moist chamber. After a rinse and soak in PBS for 10 minutes, the slides were repeatedly stained with FITC conjugated anti-mouse immunoglobulin for 30 minutes at 37°C before rinsing and soaking in PBS, and then followed by the final rinsing with distilled water. The stained slides were air-dried and examined under fluorescence microscope (Nikon, Tokyo, Japan). The fluorescent positive cells appeared apple green with a specific pattern of localization of viral antigen in the infected cells.

The fixed slides were screened for the presence of a viral antigen by staining with pooled antivirus monoclonal antibodies. If a positive result was obtained, the additional slides would be stained with individual monoclonal antibody in order to identify specific viral antigen. Thus, the specific viral etiology could be diagnosed.

RSV subgrouping

Additional slides of RSV positive NPA specimens were further characterized for RSV subgroup by a panel of 3 mouse monoclonal antibodies specific to F protein; 92-11c for subgroup A epitope, 102-10b for subgroup B epitope and 131-2a which reacts to common epitopes of both subgroups. These monoclonal antibodies were kindly provided by Dr. Larry J. Anderson, the Center for Disease Control, Atlanta, USA.

Subgroup specific infection could be identified if the NPA sample reacted either with subgroup A or B monoclonal antibody only. If the sample reacted to both subgroup specific mononoclonal antibodies it would be identified as RSV A/B.

RESULTS

Among 472 episodes of ALRI, 170 (36.0%) viral agents were found, i.e. 122 (25.8%) RSV, 13 (2.8%) fluA, 6 (1.3%) PF1, 1 (0.2%) PF2, 21 (4.4%) PF3 and 4 (0.8%) adenovirus. There were 3 (0.6%) samples which were reactive as tested with pooled monoclonal antibodies, but did not react with any single monoclonal antibody tested separately. Therefore, they were diagnosed as unidentified viruses (Table 1). These data implicated that RSV was the most common viral agent found followed by PF3 and other viruses. From November 1998 to February 2001, there were 5 cases who contracted ALRI twice. Of these 5 cases, one developed RSV associated pneumonia followed by PF1 associated bronchitis 5 months later; three cases had one episode of viral infection among two episodes of ALRI; and no viral agent could be found in both ALRI episodes of the fifth case.

The incidence of pneumonia was slightly higher than that of bronchitis in the present cohort. i.e. 232 *vs* 214 episodes, respectively. Thus, both diseases accounted for 94.5 per cent of all ALRI episodes. Viral agents were associated with 41.4 per cent of all pneumonic cases, while 30.4 per cent was associated with bronchitis (Table 2). RSV again was the most common agents found in both diseases.

Table 1. Incidence of acute respiratory virus infections in children with ALRI at Takhli Hospital.

Study periods	Number of ALRI episodes	Frequency of virus detected															
		RSV	%	FluA	%	PF1	%	PF2	%	PF3	%	Ad	%	Unidentitied	%	Total	%
Nov 98 - Oct 99	215	77	35.8	10	4.7	0	0	0	0	10	4.7	1	0.5	3	1.4	101	47.0
Nov 99 - Oct 00	206	43	20.9	2	1.0	6	2.9	1	0.5	6	2.9	0	0	0	0	58	28.2
Oct 00 - Feb 01	51	2	3.9	1	2.0	0	0	0	0	5	9.8	3	5.9	0	0	11	21.6
Total	472	122	25.8	13	2.8	6	1.3	1	0.2	21	4.4	4	0.8	3	0.6	170	36.0

Table 2. Incidence of acute respiratory virus infections by clinical symptom.

Symptom	Number of ALRI episodes	Frequency of virus detected															
		RSV	%	FluA	%	PF1	%	PF2	%	PF3	%	Ad	%	Unidentitied	%	Total	%
Bronchitis	214	40	18.7	7	3.3	4	1.9	0	0	9	4.2	3	1.4	2	0.9	65	30.4
Pneumonia	232	79	34.1	4	1.7	2	0.9	0	0	10	4.3	0	0	1	0.4	96	41.4
Croup	18	0	0	2	11.1	0	0	1	5.6	1	5.6	1	5.6	0	0	5	27.8
Bronchiolitis	8	3	37.5	0	0	0	0	0	0	1	12.5	0	0	0	0	4	50.0
Total	472	122	25.8	13	2.8	6	1.3	1	0.2	21	4.4	4	0.8	3	0.6	170	36.0

Regarding RSV subgroup prevalence, subgroup B was predominate over subgroup A during the first year of study; while it was *vice versa* during the second year. However, RSV subgroup B was found in a higher frequency than subgroup B in overall episodes (Table 3).

Prevalence of RSV and PF was seasonal dependent (Fig. 1 and 2). Peak of RSV was seen during the rainy season, i.e. August and September; meanwhile, PF viruses as a whole had its peak in early summer, i.e. March and February of the consecutive study years. The incidence of other viruses was too small to see the seasonality.

DISCUSSION

Several studies have been conducted on acute respiratory infections in pediatric cases in Thailand since 1973[3,10-16]. However, information obtained from the first study was limited to acute upper respiratory infection only[10]. The studies on ALRI were carried out later in several major hospitals of the country: Ramathibodi Hospital from 1986 to 1987, Childrens' Hospital from 1988 to 1989, Siriraj Hospital from 1989 to 1991 and Srinagarind Hospital, Khon Kaen from 1992 to 1994. Nevertheless, the present study is different from the previous ones in such a way that it was the only population-based cohort study done in Thailand. The subjects of all the other studies were pediatric patients under 5 years of age who were diagnosed with ALRI. The viral etiology agents in those studies varied from 35.1 per cent (26 of 24 cases) of Srinagarind Hospital to 52.6 per cent (210 of 399 cases) of Siriraj Hospital[3,11-16]; and it was 36.0 per cent (Table 1) in the present study. Interestingly, all studies confirmed that RSV was the most common and usually accounted for more than 50 per cent of all viral agents observed.

RSV is the most important cause of serious ALRI in infants, young children and the elderly. The virus exists as a single serotype but has two antigenic subgroups i.e., A and B, or 1 and 2 which are 25 per cent related antigenically overall. F proteins are 50 per cent related, while G proteins are 1-7 per cent related among the RSV strains of both subgroups. Therefore, antigenic dimorphism is most evident with monoclonal antibodies against the G glycoprotein [17]. RSV subgroup A predominates over subgroup B overall in outbreaks worldwide; but predomination of subgroup B over subgroup A was occasionally observed in some seasons including the present study [14,18-20]. In general, circulation of one subgroup was usually followed by the other subgroup in the consecutive season. There were 18 per cent of 122 RSV positive samples which could not be grouped in this study; the figure was slightly higher than that of 13.7 per cent of 139 samples which the authors had reported previously as the same panel of monoclonal antibodies was employed[14]. Inclusion of monoclonal antibodies to other epitopes into the panel may be required for characterization of the ungrouped RSV samples. Regarding disease severity, it can not be concluded which subgroup elicits more serious symptoms or both subgroups have no difference in disease severity at all[17,18,21,22]. On the other hand, it has been accepted that serious outcome of RSV infection is related to age and other host risk factors[23-25].

RSV outbreaks in Thailand occur during the rainy season with peaks varying from August to October, while in a temperate climate the outbreaks usually begin each fall and peak in winter[11,14,26].

It has been shown that the second most common viral agent in pediatric ALRI is the PF viruses also shown in Thailand. Among the 4 types of the virus, type 4 produced mild symptoms, while types 1, 2 and 3 are of medical importance[7,11,14]. In the Western world, PF1 was found in a higher frequency than PF3[7], while previous studies in Thailand

Table 3. Incidence of RSV subgroup during the studied period.

Months	Total	Number of RSV subgroup found					
		RSV A	%	RSV B	%	RSV A/B	%
Nov 98 - Oct 99	77	5	6.5	51	66.2	21	27.3
Nov 99 - Oct 00	43	30	69.8	12	27.9	1	2.3
Oct 00 - Feb 01	2	1	50.0	1	50.0	0	0
Total	122	36	29.5	64	52.5	22	18.0

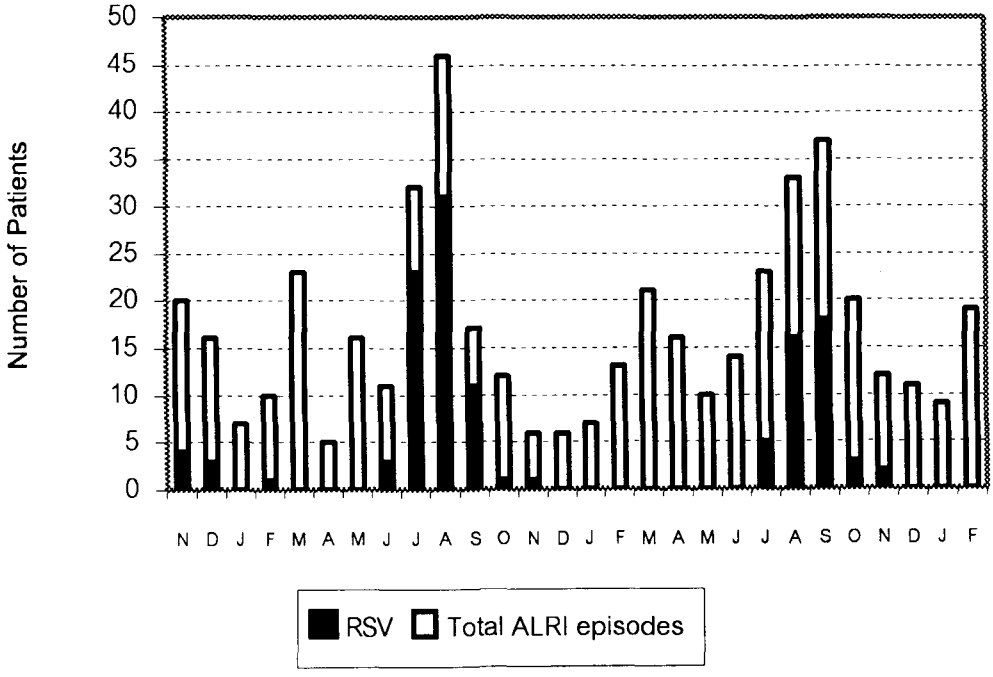

Fig. 1. Seasonality of RSV, November 1998 to February 2001.

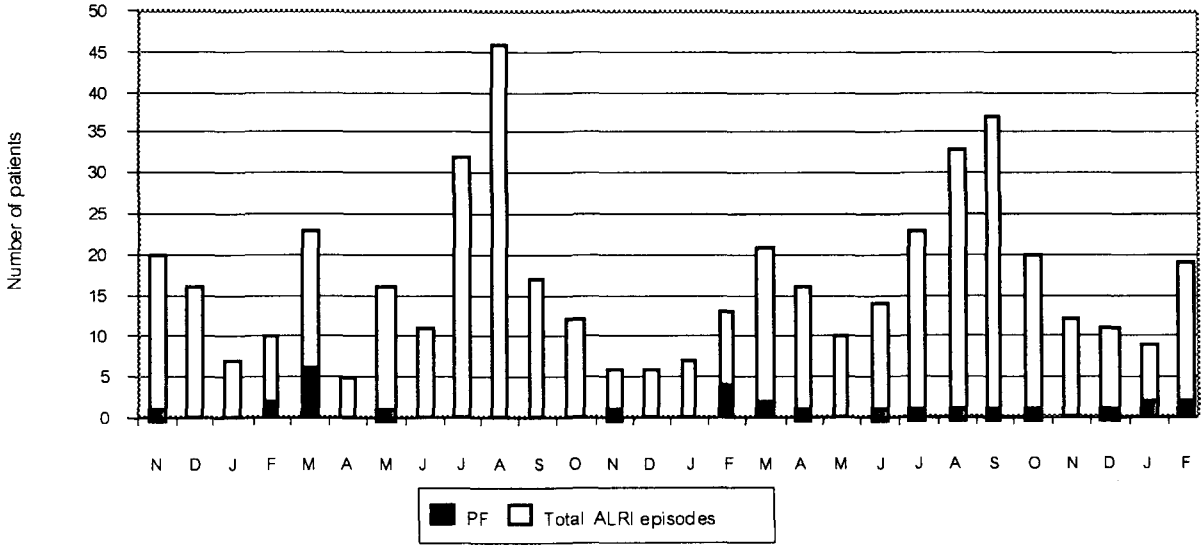

Fig. 2. Seasonality of Parainfluenza viruses, November 1998 to February 2001.

together with the present study showed that PF was much more common[11,14]. The authors' previous studies at Ramathibodi and Siriraj Hospital showed that RSV played the most important role in pneumonia and bronchitis, while parainfluenza viruses were the most common in croup[11,15]. The findings for RSV are repeatable, nevertheless, the role of PF in croup could not be demonstrated in present study. However, all of the authors' studies agreed that PF viruses in Thailand are common in the late cool season or early summer with a peak prevalence in February or March.

The diagnostic method used in this study was confined only to IIF, the test may be suitable for RSV investigation, but may not be sensitive enough for diagnosis of other non-RSV infections which produced fewer virus infected cells. The authors also found 3 NPA samples which were positive as tested by pooled monoclonal antibodies, but negative when each virus specific monoclonal antibody was used. The number of virus infected cells in these samples may be too few to distribute equally in all cell deposits, thus, misdiagnosis could occur in IIF. The authors previously reported that a combination of different investigation methods would increase the detection rate of all kinds of respiratory viruses including RSV infections[3,27].

Where laboratory facilities are limited, the authors' present study has demonstrated that IIF is a suitable method to investigate RSV infection, especially in term of transportation of fixed slides of cell deposits from a rural community to a Virology Laboratory in ambient temperature. The system is convenient, gives enough information for the study and is of low cost. IIF is feasible to support the country to set a nationwide plan which will lead to the prevention and control of RSV in Thailand.

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การติดเชื้อไวรัสในทางเดินหายใจส่วนล่างในผู้ป่วยเด็กในชนบทไทย

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การศึกษานี้มีวัตถุประสงค์เพื่อหาอุบัติการณ์ของไวรัสก่อโรคระบบทางเดินหายใจส่วนล่างในเด็กเล็กอายุต่ำกว่า 5 ปี ในช่วงเวลาพฤศจิกายน 2542 – กุมภาพันธ์ 2544 โดยเป็นการดำเนินการแบบ cohort study ที่อำเภอตาคลี จังหวัดนครสวรรค์ ในช่วงระยะเวลาดังกล่าวมีโรคระบบทางเดินหายใจส่วนล่างเกิดขึ้นทั้งหมด 472 ครั้ง และมีผู้ป่วย 5 ราย ที่เกิดโรคซ้ำ การวินิจฉัยไวรัสก่อโรคทำโดยวิธีอิมมูนเรืองแสง และใช้โมโนโคลนัลแอนติบอดีย์จำเพาะในการย้อมเซลล์ที่อยู่ในสิ่งดูดจากนาโสฟาริงซ์ของผู้ป่วย โดยห้องปฏิบัติการของโรงพยาบาลตาคลีจะเป็นผู้เตรียมสไลด์ของเซลล์ และส่งมาทางไปรษณีย์ด่วนเพื่อทำการย้อมที่ห้องปฏิบัติการไวรัสวิทยาของโรงพยาบาลศิริราช ในจำนวนการติดเชื้อระบบทางเดินหายใจส่วนล่าง 472 ครั้ง พบว่าเกิดจากไวรัส 170 ครั้ง (ร้อยละ 36.0) โรคปอดบวมร้อยละ 41.4 มีสาเหตุเกิดจากไวรัส เชื้อไวรัสที่พบบ่อยที่สุดในการศึกษานี้คือ เชื้อ respiratory syncitial virus (RSV) และเชื้อนี้ยังพบได้บ่อยที่สุดในโรคปอดบวม และหลอดลมอักเสบ ได้ทำการศึกษา RSV subgroup โดยย้อมเซลล์จากนาโสฟาริงซ์ด้วยโมโนโคลนัลแอนติบอดีย์ที่จำเพาะต่อ RSV subgroup A หรือ subgroup B พบว่าเชื้อ subgroup B มีจำนวนมากกว่า subgroup A ในปีแรก และ subgroup A พบได้บ่อยกว่าในปีที่สอง แต่ในจำนวนเชื้อ RSV ทั้งหมดของการศึกษานี้ ส่วนใหญ่เป็น subgroup B ซึ่งต่างไปจากการศึกษาอื่น ๆ ที่ผ่านมา RSV พบมากในฤดูฝน อุบัติการณ์สูงสุดเกิดขึ้นในเดือนสิงหาคม หรือกันยายนของแต่ละปี เนื่องจากการศึกษาครั้งนี้ใช้วิธีอิมมูนเรืองแสงเพียงวิธีเดียวจึงอาจทำให้วินิจฉัยเชื้อไวรัสได้น้อยกว่าความเป็นจริงโดยเฉพาะเชื้อที่อยู่ในกลุ่ม non RSV เช่น พบว่าเชื้อ parainfluenza virus มีมากเป็นอันดับสอง รองจาก RSV และพบบ่อยในต้นฤดูร้อนช่วงเดือนกุมภาพันธ์หรือมีนาคม แต่ก็ไม่พบความเกี่ยวข้องระหว่าง parainfluenza virus และโรค croup ซึ่งอาจเป็นเพราะพบเชื้อ parainfluenza virus ค่อนข้างน้อยกว่าที่เคยรายงานมาก่อน อย่างไรก็ดีวิธีอิมมูนเรืองแสงจัดเป็นวิธีที่สะดวก ประหยัด ให้ผลเร็ว เหมาะที่จะนำไปใช้ศึกษาเชื้อไวรัสก่อโรคระบบทางเดินหายใจส่วนล่างในชนบท และให้ข้อมูลเพียงพอสำหรับการวางแผนสาธารณสุขในการป้องกันโรค

คำสำคัญ : การติดเชื้อไวรัสในทางเดินหายใจส่วนล่าง, ชนบท

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