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Short Communication

An Outbreak of Respiratory Infection due to Respiratory Syncytial Virus Subgroup B in Ankara, Turkey

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SUMMARY: During the outbreak, from 16 January 2002 to 3 March 2002, nasopharyngeal secretions obtained from 35 pediatric patients under 2 years of age and suffering from acute respiratory disease were tested by VIDAS respiratory syncytial virus (RSV) assay (an automated enzyme-linked fluorescent immunoassay) and reverse transcription-polymerase chain reaction (RT-PCR). RSV antigen was detected in 16 specimens by VIDAS RSV assay, and 15 of these were confirmed by the RT-PCR. A total of 18 samples were found to be positive by RT-PCR. RSV subgroup B was identified by further restriction fragment length polymorphism analysis using *Ava*II and *Bgl*II endonucleases in 17 of 18 (94%) RT-PCR positive samples. These findings indicated that RSV subgroup B was highly dominant during an outbreak of RSV infection among children in Ankara. To our knowledge, this is the first outbreak due to dominant RSV subgroup B documented in Turkey.

Respiratory syncytial virus (RSV) is a major cause of serious respiratory tract infection in infants and the most common cause of nosocomial diseases in pediatric wards (1,2). Infections associated with RSV have a worldwide distribution, and they occur in epidemics with various degrees of severity each year (3-5). RSV is divided into two distinct major antigenic groups, RSV subgroups A and B virus (5,6). The relative frequency of RSV subgroups A and B is variable among annual outbreaks in all parts of the world. In any particular season, subgroups RSV A and B may be co-circulating though one type may be dominant (more frequently subgroup A) (5,7,8). The attempt to discriminate between RSV subgroups is of immediate relevance for analyzing the epidemiology and immunology of infection and for providing crucial information for vaccine development efforts.

Little information regarding the prevalence and antigenic characteristics of RSV epidemics in Turkey is available in literature in English, and subgrouping of the isolates was not performed in a previous study (9). This is probably because of the difficulties in detecting RSV by culture, which requires trained and experienced personnel, is time-consuming, labor-intensive, and may require several days to weeks to complete.

Currently, the expanded use of new techniques such as direct antigen detection methods and reverse transcription-polymerase chain reaction (RT-PCR) has facilitated the local identification of etiologic agents, allowing the comparison of clinical and epidemiological features with biological agent characteristics (5,6,8,10).

The aim of this study was to provide the first information about circulation of RSV subgroups and epidemiological aspects of RSV infections in children under the age of 2 years admitted to the pediatric ward of a tertiary hospital in Ankara. We used the VIDAS RSV assay (BioMéreiux, Marcy-L'Étoile, France) for RSV antigen detection, and the RT-PCR method with further restriction fragment length polymorphism (RFLP) analysis for simultaneous detection and subgrouping of RSV in clinical respiratory specimens.

The VIDAS RSV assay had been performed for routine diagnostic purposes in a virology laboratory. From the winter season of 2001 and into that of 2002, no RSV was detected before 16 January 2002 by routine testing. After the VIDAS RSV positive result on this day, subsequent analyses were performed with inclusion of RT-PCR for all samples. On 3 March 2002, the last positive results were obtained by both assays. No RSV was detected after this time. The time period from 16 January through 3 March 2002 was defined as RSV outbreak.

Nasopharyngeal secretions (NPS) collected from children with acute respiratory tract infections were submitted to the Division of Virology in the Department of Microbiology and Clinical Microbiology at Gulhane Military Medical Academy in Ankara during the winter seasons of 2001 and 2002. The specimens were collected by physicians or nursing personnel from infants and children (21 females and 14 males, under 2 years of age, 23 of whom were hospitalized during sampling) with acute respiratory illnesses and transferred in sterile screw-capped tubes to the laboratory with a request for a rapid test for RSV. All of these NPSs were tested by both VIDAS RSV assay and RT-PCR for the presence of RSV at the virology laboratory.

VIDAS RSV assay was performed upon receipt of sample, and an aliquot of original specimen was stored at −70°C for batch testing with RT-PCR later. Test procedures were performed according to the manufacturer's instructions (BioMéreiux). RT-PCR to detect RSV in samples was performed using previously reported procedures (10). The group-specific primers (Table 1) used for RSV subgroups A and B (P1-P2 and P3) were chosen from reported sequences of the L-polymerase gene (10). The three primers, P1, P2, and P3, were mixed in the same RT-PCR mixture for the simultaneous diagnosis of any RSV infection in sample

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Table 1. Primers from the L-polymerase gene of the RSV genome used in the RT-PCR assay

Primers	Sequence (5′-3′)
P1, (+) sense primer	ACA ACA GAT CTC AGC AAA T
P2, (–) sense primers	CTA TTG CTT GAT TGT CAC C
P3, (–) sense primers	CTA TTG CTT GAT TGT CTC C

Table 2. The results of VIDAS and RT-PCR assays from patients with RSV infection

RT- PCR	VIDAS		Total
	Positive	Negative	
Positive	15	3	18*
Negative	1	16	17
Total	16	19	35

*Of them, 17 were identified as RSV subgroup B by further RFLP analysis.

extracts. To monitor the PCR, a number of negative controls (uninfected HEp-2 cells) and positive controls (RSV A2 and B2 strains, respectively, propagated on HEp-2 cells) were included in each run. The PCR products (355 bp) were analyzed and visualized as described elsewhere (10). In PCR-RFLP analysis, a 355-bp segment was purified by use of the Gel Extraction Purification kit (Bio-Rad Laboratories, Hercules, Calif., USA) and subsequently digested with the restriction enzymes *Ava*II and *Bgl*II in accordance with the manufacturer's instructions. Finally, the digestion products were again analyzed and visualized as above.

Of the total of 35 specimens tested, 15 were found to be positive by both VIDAS RSV assay and RT-PCR method, and 16 specimens were negative by both assays (Table 2). On the other hand, four discrepant results were obtained. Of these, three specimens were negative by VIDAS assay and positive by RT-PCR, whereas only one specimen was positive by VIDAS assay and negative by RT-PCR.

Seventeen of 18 (94%) RT-PCR positive specimens were cleaved by both *Ava*II and *Bgl*II endonucleases and produced digestion products of bp 263 and 92, and bp 283 and 66, respectively. This cleavage pattern is specific for RSV B. The remaining specimen was only digested by *Ava*II enzyme and yielded a product of bp 263 and 92, which was specific for RSV subgroup A. Thus, RFLP data allowed simple distinctions between RSV subgroups A and B. This study indicated that almost all (94%) cases of RSV infections occurring among children in Ankara, Turkey, during the winter seasons

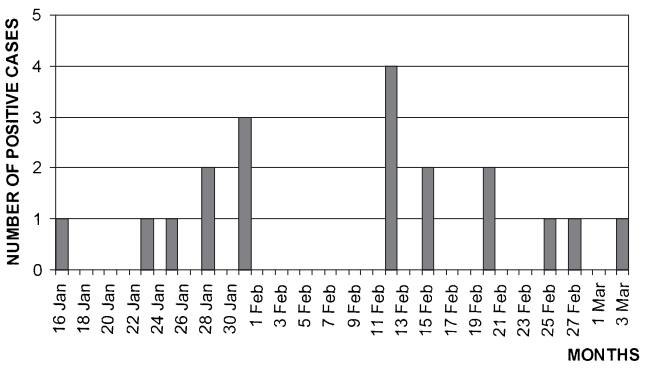

Fig. 1. Number of all positive cases of RSV infection identified on each day of the epidemic.

of 2001-2002 were caused by RSV subgroup B. The number of positive cases of RSV infection identified per day of the outbreak were used to construct an epidemic curve (Fig. 1).

Two antigenic subgroups of RSV, represented by subgroups A and B, have been described on the basis of their respective reactions to monoclonal antibodies (5,6). The relative frequency of RSV subgroups A and B is variable among annual outbreaks in all parts of the world. In the same season, both subgroups may be circulating, and one type may clearly be dominant. Subgroup A has been dominant in more epidemics than subgroup B for most years (5,7,8). A study performed in Austria and Croatia in the period from 1988 to 1994 showed that RSV strains of both antigenic subgroups were circulating in this part of Europe (4). In the UK, from 1988 through 1999, subgroup A isolates were dominant in eight epidemics, subgroup B only in three epidemics (5). Although the incidence and prevalence of human RSV infection together with its geographic and temporal distribution were studied in several reports (4,5,11), little information regarding the prevalence and antigenic characteristics of RSV epidemics in Turkey is available in the literature in English. Subgrouping of Turkish isolates was not performed in a previous study (9).

Although subgroup A viruses have been reported to be the dominant strain in outbreaks in other countries, our PCR-RFLP data showed that RSV subgroup B (94%) was the prevalent strain detected in the respiratory specimens obtained from patients with acute respiratory disease due to RSV during the period from January through March 2002, in Ankara, Turkey. The occurrence of one RSV subgroup A infection during the same period may be explained by the possibilility of a co-circulating type A strain. These findings were consistent with previous reports expressing epidemiological data on RSV infections. To our best knowledge, this is the first study documenting an RSV outbreak of subgroup B dominance in Turkey.

Overall, the positive diagnosis of respiratory illness due to RSV infection was achieved by either VIDAS (16/35) or RT-PCR (18/35) methods during the outbreak. Fifty-four percent (19/35) of the studied patients were diagnosed as RSV-positive.

In conclusion, the subgroup B was found to be dominant in the outbreak presented in this study. The significance of this finding is unclear and should be clarified with additional large-scale studies.

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