Detection and Serotyping of Human Adenoviruses from Patients with Influenza-Like Illness in Mongolia

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SUMMARY: Human adenoviruses (HAdVs) are responsible for approximately 5%–10% of acute respiratory infections. The serotypes of commonly detected respiratory HAdV in Asian countries are diverse. However, there are no well-documented reports of circulating HAdV serotypes in Mongolia. Between January 2010 and May 2011, 1,950 influenza-negative samples from patients with influenza-like illness, including eye swabs from patients with eye symptoms, were screened for HAdV, and 40 samples (2.1%) were positive for HAdVs. Among these 40 samples, 31 samples were positive for the hexon gene used in phylogenetic analysis, as determined by PCR. We identified 7 different serotypes. We constructed the phylogenetic trees of HAdV-B7 and HAdV-B3, the 2 most commonly detected serotypes in this study. All detected HAdV-B7 and -B3 Mongolian strains had identical sequences. HAdV-D8, known to be associated with epidemic keratoconjunctivitis (EKC), was detected from nasopharyngeal and eye swabs. There was no difference between the amino acid sequences of the hexon and fiber genes that may affect tissue tropism in Mongolian strains and those in EKC-causing strains.

INTRODUCTION

Human adenoviruses (HAdVs) are non-enveloped, double-stranded DNA viruses belonging to the genus Mastadenovirus and the family Adenoviridae (1). There are 52 serotypes that have been classified into 7 species (A–G) (2,3). Recently, full genome sequencing of HAdVs was performed, and some novel HAdVs (HAdV-D53, -D54, -B55, -D56, -C57, and -D58) were discovered through phylogenetic analysis (4–9). HAdVs cause variable serotype-specific diseases, such as acute respiratory infection, conjunctivitis, and gastroenteritis, and are responsible for approximately 5%–10% of acute respiratory infections (1). HAdV-C1, -C2, -B3, -E4, -C5, -C6, and -C7 are the most common serotypes that cause respiratory infections while HAdV-D8 is the one of the major causes of epidemic keratoconjunctivitis (EKC) (1).

The circulating respiratory HAdV serotypes vary both geographically and temporally among Asian countries. For example, HAdV-B3 and -B7 were the most commonly encountered serotypes in surveillance conducted from 1991 to 2007 in Korea (10), and HAdV-C2 and -B3 were common in Japan between 2000 and 2007 (11). HAdV-C, especially HAdV-C1 and -C2, were common in Malaysia between 1999 and 2005 (12), whereas HAdV-B3 was common in Taiwan in 2004 and 2005 (13). However, there are no available data on currently circulating HAdV serotypes in Mongolia. Between January 2010 and May 2011, HAdVs were detected in patients with influenza-like illness (ILI), and phylogenetic analysis of the hexon gene from these HAdVs was performed to determine which serotypes were circulating in Mongolia. To our knowledge, this is the first report describing the serotypes of HAdVs in Mongolia.

MATERIALS AND METHODS

From January 2010 to May 2011, a total of 6,774 nasopharyngeal or eye swabs were collected from patients who visited outpatient clinics and hospitals in Mongolia and were clinically diagnosed with ILI within 3 days of disease onset. ILI was defined as sudden onset of fever (>38.0°C) with cough or sore throat in the absence of other diagnoses. Nasopharyngeal swabs were collected from patients with ILI whereas eye swabs were collected from patients with ILI and eye symptoms, such as tear shedding, eyelid swelling, and festering. The swabs were placed into 2 ml of Hanks (-) balanced salt solution (pH 7.2) with bovine serum albumin (0.5%), penicillin (500 U/ml), streptomycin (500 µg/ml), and amphotericin B (Fungizone) (2.5 µg/ml). The specimens were transported under refrigeration with ice packs to the National Influenza Center, National Center of Communicable Diseases in Ulaanbaatar within 48 h of collection.

At least 22 samples in each month were randomly selected from among those samples that were collected from patients <4 years or >60 years of age and tested negative for influenza virus using real-time reverse transcription polymerase chain reaction (rt-RT PCR) (14). Selected samples were screened for HAdVs either using
MEGA 5 software (20).

constructed by the maximum likelihood method using
The sequences were aligned, and phylogenetic trees were
determined as described previously (19).

fiber gene-specific PCR was also performed, and se-
quences of these genes with the reference strains in
ships of these sequences with the reference strains in
mine serotypes according to the phylogenetic relation-
for HAdV-B7 and -B3 were constructed in order to
Mongolian strains were located in the same cluster, and all
strains of both HAdV-B7 and -B3 shared identical sequences within each serotype. When Mon-
golian strains were clustered with other HAdV-B7 strains that

RESULTS

A total of 6,774 samples were tested using rt-RT
PCR for influenza viruses, and 6,193 samples were
negative. Among the 1,950 samples randomly selected
and screened for HAdVs using either multiplex rt-PCR
or immunofluorescence assay, 40 (2.1%; 95% confi-
dence interval, 1.5–2.7) were HAdV-positive. There
were 31 HAdV-positive samples with hexon genes that
were amplified by the first or nested PCR.

As a result of phylogenetic analysis of the 31 HAdVs,
7 different serotypes belonging to 3 species of HAdVs
were detected. Patient information and the serotyping
results of the 31 samples used for serotyping are summa-
rized in Table 1. Among them, 4 samples were eye swab
samples from patients with ILI and eye symptoms (1
HAdV-C6, 1 HAdV-B7, and 2 HAdV-D8). The most
common serotypes among patients with ILI were
HAdV-B7, followed by HAdV-B3 and -D8, although
there was no statistically significant difference in the
frequency of these serotypes ($P = 0.175$). Among these
31 samples, there were 2 cases of co-infection with other
respiratory viruses (1 with HAdV-B7 and influenza A
virus, and 1 with HAdV-D8 and human metapneumovi-
rus). The frequency distribution of the 31 HAdV-positi-
cases by month is shown in Fig. 1. Each serotype
was detected sporadically, and no apparent seasonality
was observed for any serotype.

HAdV-B7 and -B3 were the common serotypes in
Mongolia and cause severe acute respiratory infection
(21–25). Therefore, phylogenetic trees of the hexon gene
for HAdV-B7 and -B3 were constructed in order to
compare the phylogenetic relationship between Mon-
golian strains and those in other countries, which were
available in GenBank (Figs. 2A and 2B). The Mon-
golian strains were located in the same cluster, and all
Mongolian strains of both HAdV-B7 and -B3 shared
identical sequences within each serotype. When Mon-
golian HAdV-B7 and -B3 strains were compared with
those detected in other countries, Mongolian HAdV-B7
strains were clustered with other HAdV-B7 strains that

Table 1. Patient information and the results of serotyping

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Type of samples</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4 5 6 30s</td>
<td>Male</td>
<td>Female</td>
<td>Nasopharyngeal swab</td>
</tr>
<tr>
<td>C1</td>
<td>1 1 1 0 0 0 0 0</td>
<td>2 1</td>
<td>3 0</td>
<td>3 (10)</td>
</tr>
<tr>
<td>C2</td>
<td>0 4 0 0 0 0 0 0</td>
<td>1 3</td>
<td>4 0</td>
<td>4 (13)</td>
</tr>
<tr>
<td>B3</td>
<td>1 3 1 0 1 0 0 0</td>
<td>4 2</td>
<td>6 0</td>
<td>6 (19)</td>
</tr>
<tr>
<td>C5</td>
<td>1 1 1 0 0 0 0 0</td>
<td>3 0</td>
<td>3 0</td>
<td>3 (10)</td>
</tr>
<tr>
<td>C6</td>
<td>0 0 0 1 0 0 0 0</td>
<td>0 1</td>
<td>0 1</td>
<td>1 (3)</td>
</tr>
<tr>
<td>B7</td>
<td>0 2 3 1 1 1 1 0</td>
<td>5 4</td>
<td>8 1</td>
<td>9 (29)</td>
</tr>
<tr>
<td>D8</td>
<td>0 1 2 1 0 0 0 1</td>
<td>2 3</td>
<td>3 2</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Total</td>
<td>3 12 8 3 2 1 1 1</td>
<td>17 14</td>
<td>27 4</td>
<td>31</td>
</tr>
</tbody>
</table>

The sequence data obtained in this study is available
in the GenBank database under accession numbers
AB685342-AB685377.

Statistical analysis was performed by chi-square ($\chi^2$)
analysis using PASW Statistics 17.0 (International Busi-
ness Machines Corp., N.Y., USA), and $P$-values less
than 0.05 were considered to be statistically significant.

290
Fig. 1. Monthly distribution of human adenoviruses detected in Mongolia between January 2010 and May 2011.

Fig. 2. (A) The phylogenetic tree of HAdV-B7 in Mongolia. (B) The phylogenetic tree of HAdV-B3 in Mongolia.

The tree was constructed using the maximum likelihood method. The percentage of replicate trees in which associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. Strains detected in nasopharyngeal swabs in this study are denoted by ◯, and the 1 sample denoted by ● was detected in an eye swab.

were detected in other parts of the world, even though Mongolian strains formed a single cluster in the phylogenetic tree. HAdV-B7 strains, the most common serotype in Mongolia, were more closely related to strains detected before 2000 in different parts of the world. These strains were distinct from recent Asian strains detected in Korea, China, and Japan in the 2000s. In addition, HAdV-B3 strains in Mongolia were more closely associated with the strains detected in Japan, Korea, Taiwan, and Germany in the 2000s.

Among the 5 samples positive for HAdV-D8, 3 were nasopharyngeal swabs collected from patients with ILI. Generally, HAdV-D8 causes EKC, and there are a few reports of acute respiratory infection caused by HAdV-D8 (24). To determine whether Mongolian HAdV-D8 strains from patients with ILI exhibited any mutations that may alter tissue tropism, the amino acid sequences of loops 1 and 2 of the hexon gene, and those of the fiber knob and shaft domains were compared between

Japan, Korea, Taiwan, and Germany in the 2000s.
Mongolian strains and other EKC-causing strains in GenBank (HAdV-D8p, 8b, and 8e [26]) (Figs. 3A and 3B). Two strains (10_03377 and 10_04126) were detected in nasopharyngeal swabs and 2 strains (10_03019 and 10_03192) were detected in eye swabs in Mongolia. In loops 1 and 2 of the hexon protein, all HAdV-D8 strains in Mongolia, including those collected from nasopharyngeal and eye swabs, were almost identical. Only 1 strain from a nasopharyngeal swab sample (11_00695) exhibited a single amino acid substitution, as compared to other Mongolian strains. This substitution occurred between HVRs 3 and 4, where the amino acid was sub-

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**Fig. 3.** The amino acid sequence comparison between Mongolian and other epidemic keratoconjunctivitis (EKC)-causing strains. (A) The amino acid sequence comparison of loops 1 and 2 of the hexon gene between Mongolian and other EKC-causing strains. (B) The amino acid sequence comparison of the fiber knob and shaft domains between Mongolian and other EKC-causing strains.
stittuted from leucine to phenylalanine (Fig. 3A). Near-complete identity was observed in the fiber knob and shaft domains, and only 1 strain (11_00695) displayed a substitution from glutamine acid to lysine in the fiber knob domain (Fig. 3B). The effect of these substitutions in 11_00695 is not known.

**DISCUSSION**

In this study, 2.1% (95% confidence interval, 1.5–2.7) of ILI infections were caused by HAdVs. However, previous reports from Mongolia indicated that 5.4%–5.7% of ILI infections were caused by HAdVs, as determined by multiplex rt-PCR and direct immunofluorescent test (27–29). The rate of HAdVs in our study was also lower than those of studies conducted in different part of the world (5–10%). However, this data should be cautiously interpreted because each study used different enrollment criteria and detection methods.

A total of 7 different serotypes (HAdV-C1, -C2, -B3, -C5, -C6, -B7, and -D8) were detected from patients with ILI in Mongolia. HAdV-B7, followed by HAdV-B3 and -D8, were the most commonly detected serotypes. A similar distribution of serotypes was observed in Korea (10). HAdV-B3 and -B7 are likely to be associated with severe acute respiratory infection. Moreover, HAdV-D8 might be associated with serious respiratory infection, even though it is rarely detected in patients with respiratory infection (24). Our data emphasize the importance of monitoring these serotypes in Mongolia.

In general, there is no clear-cut seasonality for HAdV infection, and HAdVs are usually detected in all seasons (30–33). Our study in Mongolia also did not reveal any seasonal pattern to HAdV infection. Mongolia has an extreme continental climate with wide daily temperature fluctuations and long, cold winters and short summers. This may imply that HAdV infection is not affected by climatic factors. However, more HAdV-positive cases were detected between September and December 2010. Various serotypes were detected during this period, and this increase was not caused by an outbreak of any one serotype. In this study, an immunofluorescence assay was performed for screening between January and October 2010. Multiplex rt-PCR was performed after October 2010, which coincided with the increased incidence of positive cases. Therefore, this change in screening method may have affected the detection rate and resulted in the observed increase in the number of positive cases after October 2010.

HAdV-B3 and -B7 is known to cause severe respiratory infection (21–25). In our phylogenetic analysis, Mongolian HAdV-B7 strains formed a single cluster and were similar to strains detected before 2000. On the other hand, Mongolian HAdV-B3 strains were also clustered into a single cluster in the phylogenetic tree and were closely related to strains detected in other Asian countries in the 1990s. This may suggest that these Mongolian strains were imported from other countries more than 10 years ago and have been circulating in Mongolia since that time. However, more sequence data is needed to support this hypothesis.

HAdV-D8 was detected in patients with ILI in this study. There was only one case of co-infection with another respiratory virus, and there were 4 ILI cases that might have been caused by HAdV-D8. HAdV-D8 is one of the most important causes of EKC, and there are few reports of acute respiratory infection that was caused by HAdV-D8 (24). The sequences of loops 1 and 2 of the hexon gene and those of the fiber knob and shaft domains were compared to detect mutations that may be associated with tissue tropism and acute respiratory infection. However, Mongolian HAdV-D8 strains were similar to other EKC-causing HAdV-D8 strains, and no specific mutations were found in loops 1 and 2, which are responsible for the antigenicity to the neutralizing antibody. Similarity, no specific mutations were found in the fiber knob and shaft domains, which are responsible for receptor binding. Moreover, HAdV-D8 is reported to cause serious acute respiratory infection (24). However, in this study, there was no clinical information available regarding the severity of the acute respiratory infection caused by HAdV-D8. Further studies are needed to determine the phenotypic or genetic changes in these HAdV-D8 strains.

Restriction fragment length polymorphism (RFLP) analysis has been commonly used to identify genotypes in the epidemiological study of HAdVs (34–42). This method can divide genotypes that cannot be differentiated by sequence analysis of the hexon gene (35). Although RFLP analysis is a useful method, we were unable to analyze the samples we collected using RFLP because the concentration of DNA was too low. All of the samples used in this study were clinical samples and RFLP requires the isolation of HAdVs. Therefore, it is necessary to isolate HAdVs in order to conduct more in-depth analyses of circulating HAdVs in Mongolia.

Only 31 samples were analyzed in this study, and the study period was only 1 year. To clarify the more detailed epidemiology of HAdVs in Mongolia, a longer study period with more samples is needed. In conclusion, a diverse number of HAdVs serotypes were circulating throughout the year in Mongolia. Therefore, serotyping of HAdV is an important component of ILI surveillance and should be performed regularly.

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**Conflict of interest** None to declare.

**REFERENCES**


