ンフルエンザ罹患歴が無く，基礎免疫を有さない者の割合が高く，特に低年齢児ではそれが顕著である。また，免疫機構が未成熟な年少児では，不活化HAワクチンでは十分なプライミング効果を期待できない可能性もある[6]。

2）用法・用量（一回接種量）

わが国のHAワクチンと米国のスプリットワクチンとでは，成分組成がほぼ同一であるにもかかわらず，小児の年齢別1回接種量にかつては差異があり，わが国の低年齢児に対する1回接種量は少なかった。H1N1/09単価ワクチン，その後2010～11シーズン用3価ワクチンを用いた検討が行われ，2011～12シーズン用ワクチンから，国際標準である米国の規定と同様の1回接種量となった。上述した低年齢児における不良な免疫原性は，1回接種量が少なかったことだけが原因ではないだろうが，新しい接種量による免疫原性や臨床的予防効果のデータも集積することが必要である。

Ⅲ．不活化HAワクチンの安全性

1．高　齢　者

先に引用した研究[2]において，接種後48時間以内に発現した有害事象が解析された。全身反応としては37.5℃以上の発熱0.5～1.3%，発疹0～0.6%で，局所反応は接種部位の発赤8.8～17.6%，疼痛1.3～3.0%，腫脹2.8～6.6%であった。いずれも頻度や程度とも軽微で，重篤なものは認められなかった。

2．小　　児

小児でも接種後48時間の有害事象が観察された[4]。発熱については，37.5℃以上2.7～4.6%，38.0℃以上1.3～2.8%，39.0℃以上0.2～1.4%であった。発熱の頻度は高齢者より高いが，もともと小児は発熱性疾患に罹患する頻度が高いためであるとも考えられる。接種部位の局所反応は，発赤10.6～18.9%，硬結7.6～12.0%，腫脹6.6～11.4%で，症状はいずれも軽微であった。総合して考えると，不活化HAワクチンは，小児においても安全に接種できる製剤と考えられる。

3．H1N1/09単価ワクチン

2009年のH1N1/09単価ワクチンについては，新しい型のウイルスに対するワクチンということもあり，わが国でも多数の接種例に対する調査が実施された[7]。その結果，アナフィラキシーの発現は因果関係を確定できないものも含めて約10万接種当たり1例程度で，それ以外の重篤な有害事象の発現頻度も背景発生数（自然発生数）を上回るものではなかった。その他の全身反応，局所反応についても，程度や頻度において大きな問題となる副反応は認められなかった。

4．2012～13シーズン用ワクチン

不活化HAワクチンは，わが国でも毎年多数の者に接種され，対象は各年齢層に及ぶ。これまでに，頻度や程度の点で大きな問題となる副反応は報告されておらず，一定水準以上の安全性が確保されていると考えられるが，各種検討は継続して行われている。

2011～12シーズン用ワクチンで，保存剤としてフェノキシエタノール（phenoxyethanol，PE）を含有する製剤（化学及血清療法研究所（化血研）製）の接種を受けた者において，アナフィラキシー様症状が散発した。その頻度は10万接種当たり1例未満であり，H1N1/09単価ワクチン接種後の発生率と比較して高い値ではなかったが，3歳～12歳の小児で接種後にアナフィラキシー様症状を示す者が目立ち，各種検討が成された[8]。

不純物有無のチェック及び物理的化学的分析では，2010～11シーズン用と2011～12シーズン用の同社製剤，あるいは他社製剤との比較で明確な差は認められなかった。アナフィラキシーを発症した患者の血清中に，ワクチン抗原に対する特異的IgE抗体が検出された。ただし，どの抗原に反応するIgE抗体であるかは個人差があり，明確な傾向は認められなかった。

アナフィラキシーを呈した者の血液を用いた好塩基球活性化試験では，PE含有製剤の添加でCD203cの発現量が増加する症例があった。一方，チメロサール含有製剤では，CD203c発現の増強は認められなかった。また，PE単独では，CD203c発現量の増加はなく，特異的IgE抗体も検出されなかった。これらより，PE含有製剤接種後のアナフィラキシー発症に関しては，PEとインフルエンザHAワクチン成分との相乗作用などの不明な点があることが推察された。これら検討の結果，化血研製2012～13年

表 3　2012～13年シーズン用のインフルエンザHAワクチン（予定）

	化血研	阪大微研	北里第一三共ワクチン	デンカ生研
0.5mL シリンジ	製剤なし	フルービックHAシリンジ 包装：0.5mL×2 保存剤：なし ※注射針添付 (27G×1/2サイズ)	インフルエンザHAワクチン「北里第一三共」シリンジ0.5mL 包装：0.5mL×5 保存剤：なし ※注射針添付なし	Flu-シリンジ「生研」 包装：0.5mL×2 保存剤： チメロサール 4ppm ※注射針添付なし
0.5mL バイアル	製剤なし	フルービックHA 包装：0.5mL×2 保存剤：なし	製剤なし	製剤なし
1mL バイアル	インフルエンザHAワクチン"化血研" 包装：1mL×2 保存剤： チメロサール 5ppm	ビケンHA 包装：1mL×2 保存剤： チメロサール 8ppm	インフルエンザHAワクチン「北里第一三共」1mL 包装：1mL×2 保存剤： チメロサール 5ppm	インフルエンザHAワクチン「生研」 包装：1mL×1 保存剤： チメロサール 4ppm

(2012年8月現在)（各社添付文書とHP掲載事項，および文献[1]より引用して作成）

シーズン用ワクチンは，保存剤としてチメロサールを低容量含む製剤に変更された。各社の2012～13年シーズン用インフルエンザHAワクチンを，その保存剤含有量とともに一覧表とした[1]（表3）。

Ⅳ．新型インフルエンザ対策とワクチン

新型インフルエンザ対策においても，ワクチンは大切な存在である。わが国では，「新型インフルエンザ対策行動計画」上のプレパンデミックワクチンとして，「沈降インフルエンザワクチン（H5N1株）」が製剤化され，「新型インフルエンザワクチン接種に関するガイドライン」に準拠して使用することが添付文書に記載されている。

本ワクチンは，季節性インフルエンザの予防に用いられる不活化HAワクチンと，"不活化"という点では同じであるが，HA蛋白以外も成分として含む"全粒子ワクチン"である。また，アルミニウムアジュバントを含有する。本剤は，臨床試験による免疫原性が確認されており，新型インフルエンザに対する防御あるいは症状の低減が期待できるが，臨床的な有効性はまだ評価されていない。

Ⅴ．今後の開発テーマ

1．アジュバント

アジュバントを免疫補助剤としてワクチンに添加することにより，2つのことが期待できる。抗原量を減じることができることと，より高い免疫原性を有するワクチンとなることである。免疫原性については，抗体陽転率や幾何平均抗体価の高さのみならず，交差免疫性への期待もある。例えば，わが国のH5N1全粒子ワクチンにはアルミニウムアジュバントが添加されているが，ベトナム株の2回接種を受けた者に対して約2年後に，異なるクレードに分類されるインドネシア株あるいは安徽株を1回追加接種する研究が実施された[9]。追加接種前のインドネシア株，安徽株に対する抗体価は陰性であったが，接種7日後にはいずれの株に対しても抗体反応が認められ，二次免疫応答と考えられた。この交叉免疫性がアジュバントによって導かれたものかどうかは定かでないが，パンデミック対策として本ワクチンを用いたプライミングとブースター接種が計画できる可能性が考えられる。

パンデミックが発生した2009年には，世界各国で新開発のアジュバントが添加されたインフ

ルエンザワクチンが承認された。わが国でも，国内供給量でカバーできない分を補う目的で，海外から特例承認により2種類のアジュバント入りワクチンが輸入された。しかし，実際にはごく少数例に接種されたのみで，特例承認の期間を終了し，現在では用いられていない。

一方，アジュバントについては，安全性に対する懸念事項もしばしば議論される。免疫賦活作用とともに，全身や局所の副反応を増強しないか，自己免疫疾患の発症など長期的な悪影響は無いのか，などである。今後，新開発のアジュバントについても，広く使用された際のデータ収集につとめ，エビデンスを集積していくことが大切である。

2. 経鼻ワクチン

米国で2003年に承認された低温馴化3価弱毒生ワクチンは，経鼻的に噴霧投与する。自然感染と同様の経路で，気道粘膜局所免疫の付与も期待される。その効果については，流行株とワクチン株の抗原性が一致しなくても有効との報告がある一方で，注射用不活化スプリットワクチンの方が健常成人において予防効果が高いとする研究結果[10]も報告された。今後の研究による詳細な評価が待たれる。

また，現在わが国では，動物実験で素晴らしい効果が確認された経鼻不活化ワクチン[11]の開発が進められている。

3. 細胞培養ワクチン

不活化HAワクチンは現在発育鶏卵で製造されており，鶏卵の供給量に影響されて増産能力に限界がある。H1N1/09ウイルスが出現した際に，短期間で十分な量のワクチンが供給できないことが問題となったことは記憶に新しい。

また，ヒトから分離されたインフルエンザウイルスは，鶏卵で増殖させると宿主馴化による変異が起こり，元のウイルスとは抗原性の異なるウイルスに変化する場合があるという問題点も指摘されている。これら鶏卵培養に伴う課題を克服する方策として，細胞培養により製造するインフルエンザワクチンの開発が進められている。

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Research Article

Prescription Surveillance and Polymerase Chain Reaction Testing to Identify Pathogens during Outbreaks of Infection

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Syndromic surveillance, including prescription surveillance, offers a rapid method for the early detection of agents of bioterrorism and emerging infectious diseases. However, it has the disadvantage of not considering definitive diagnoses. Here, we attempted to definitively diagnose pathogens using polymerase chain reaction (PCR) immediately after the prescription surveillance system detected an outbreak. Specimens were collected from 50 patients with respiratory infections. PCR was used to identify the pathogens, which included 14 types of common respiratory viruses and *Mycoplasma pneumoniae*. Infectious agents including *M. pneumoniae*, respiratory syncytial virus (RSV), rhinovirus, enterovirus, and parainfluenza virus were detected in 54% of patients. For the rapid RSV diagnosis kit, sensitivity was 80% and specificity was 85%. For the rapid adenovirus diagnosis kit, no positive results were obtained; therefore, sensitivity could not be calculated and specificity was 100%. Many patients were found to be treated for upper respiratory tract infections without the diagnosis of a specific pathogen. In Japan, an outbreak of *M. pneumoniae* infection began in 2011, and our results suggested that this outbreak may have included false-positive cases. By combining syndromic surveillance and PCR, we were able to rapidly and accurately identify causative pathogens during a recent respiratory infection outbreak.

1. Introduction

Japanese traditional surveillance is based on definitive diagnosis and is enforced by the infection control laws in Japan for the early detection of agents of bioterrorism and outbreaks of emerging infectious diseases. After the infectious disease is diagnosed at sentinel medical institutions, at least 10 days are required until it is announced nationwide. Therefore, a major fault of this surveillance system is the delay in disseminating information.

A surveillance system that can identify the early stages of an outbreak of infectious disease is necessary. Therefore, syndromic surveillance systems have been implemented in many countries since 1995 [1]. Syndromic surveillance monitors changes in the number of patients according to symptoms such as fever, vomiting, diarrhea, and rash for further investigations. Information regarding the identification of local infectious disease outbreaks, such as school absenteeism, emergency room visits, and prescriptions of therapeutic drug against infectious diseases, are also subjects of the survey [2]. Syndromic surveillance can offer a rapid method to detect an outbreak of infection compared with traditional surveillance; such surveillance systems are currently used worldwide [3, 4].

In some cases, an infectious outbreak can be detected on the same or the following day. Although syndromic surveillance provides rapid results, it has the disadvantage that

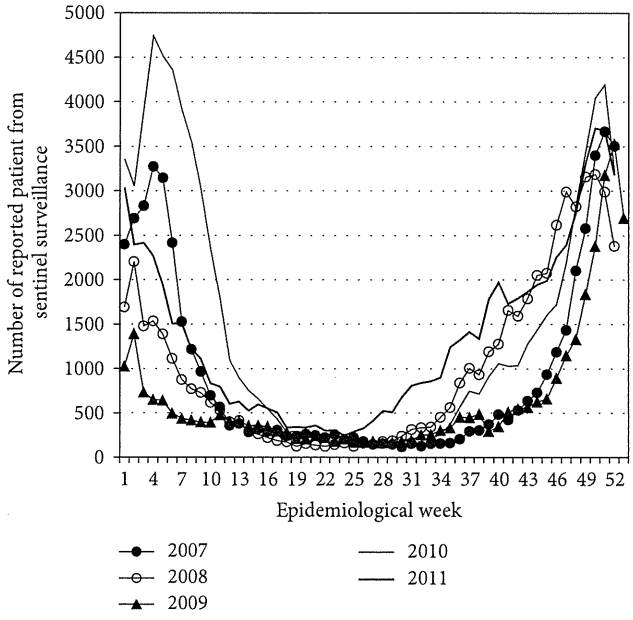

FIGURE 1: Number of reported RSV cases from sentinel medical institutions in Japan. There are approximately 500 sentinel medical institutions in Japan, which are selected from those equipped with departments of pediatrics and internal medicine and with more than 300 beds.

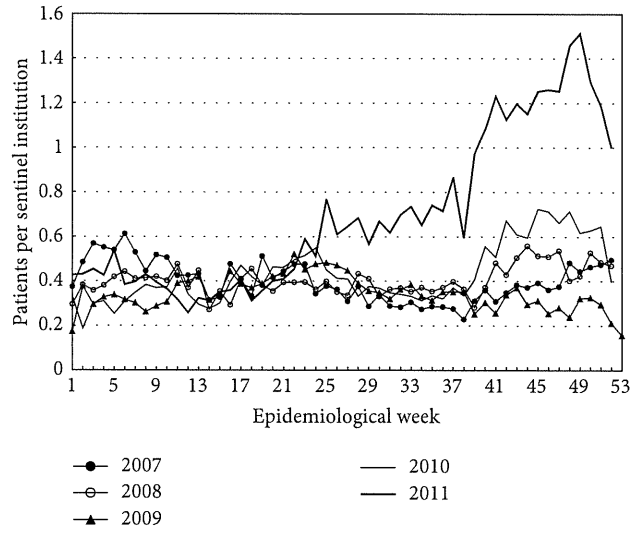

FIGURE 2: Patients per sentinel medical institution reporting *M. pneumoniae* infections.

definitive diagnoses are not considered. In other words, in general, its specificity may be lower than that of traditional surveillance systems. Laboratory testing performed on all symptomatic patients can yield a very high specificity, but is cost prohibitive, whereas laboratory testing on selected patients for syndromic surveillance can detect some specific aberrations at a lower cost, thereby overcoming the shortcomings of both systems. The current study highlights an example to further analyze this possibility.

In the fall of 2011, the number of patients with symptoms of upper respiratory tract infections markedly increased in Japan. Infectious disease weekly reports (IDWRs) (http://www.nih.go.jp/niid/ja/idwr.html in Japanese), which constitute the traditional and official Japanese sentinel surveillance system, reported a higher incidence of respiratory syncytial virus (RSV) (Figure 1) and *M. pneumoniae* infections (Figure 2). A primary feature of *M. pneumoniae* respiratory infections is the degree of the symptom worsening from mild upper respiratory tract inflammation to pneumonia. *M. pneumoniae* infection is associated with exanthem, hemolytic anemia, gastrointestinal damage, arthritis, and various neurological symptoms [5].

Outbreaks of *M. pneumoniae* persisted throughout June 2012, although it is unclear why this organism has continued to be responsible for such a widespread national outbreak in Japan since the fall of 2011 [6]. Koike et al. [7] detected only 40 patients (14.5%) among 275 suspected cases of *M. pneumoniae* infection from 2006 to 2008 in Japan. A clinical diagnosis of *M. pneumoniae* infection is difficult without laboratory confirmation. In many sentinel hospitals, the *M. pneumoniae*-specific IgM antibody rapid detection test is used, but during screening, a positive result in the test does not always indicate acute infection by this organism. We suspect that the outbreak of *M. pneumoniae* infection included false-positive cases [7, 8].

IDWRs are very important for clinicians, enabling them to identify the seasonal prevalence of known diseases. However, these reports become available after a minimum of 10 days following patient examinations. Therefore, traditional and official surveillance systems have the distinct disadvantage of being slow and are limited to reporting pathogens chosen in advance.

In the fall of 2011, by monitoring the increase in the number of combination cold medications (active ingredients: salicylamide, acetaminophen, anhydrous caffeine, and promethazine methylene disalicylate) prescribed since 2009, the prescription surveillance system detected an increase in the number of patients with symptoms of upper respiratory tract infections. On September 26, 2011, we noticed the first unusual peak and began to carefully monitor the real-time prescription surveillance system and observed a second peak on October 3, 2011 (Figure 3). However, monitoring prescriptions for combination cold medications does not lead to the identification of the pathogens responsible for the illnesses being treated. Thus, we conducted pathogen identification using the PCR method after being alerted by the prescription surveillance system on October 4, 2011 (the following day).

The purpose of the present study was to evaluate whether the PCR method triggered by the results of the prescription surveillance system can rapidly and accurately identify causative pathogens of local outbreaks of infection. Our results allowed for earlier diagnoses at medical facilities and the dissemination of this information among other institutions to avoid inappropriate use of antibiotics and instigate measures against the spread of infectious diseases.

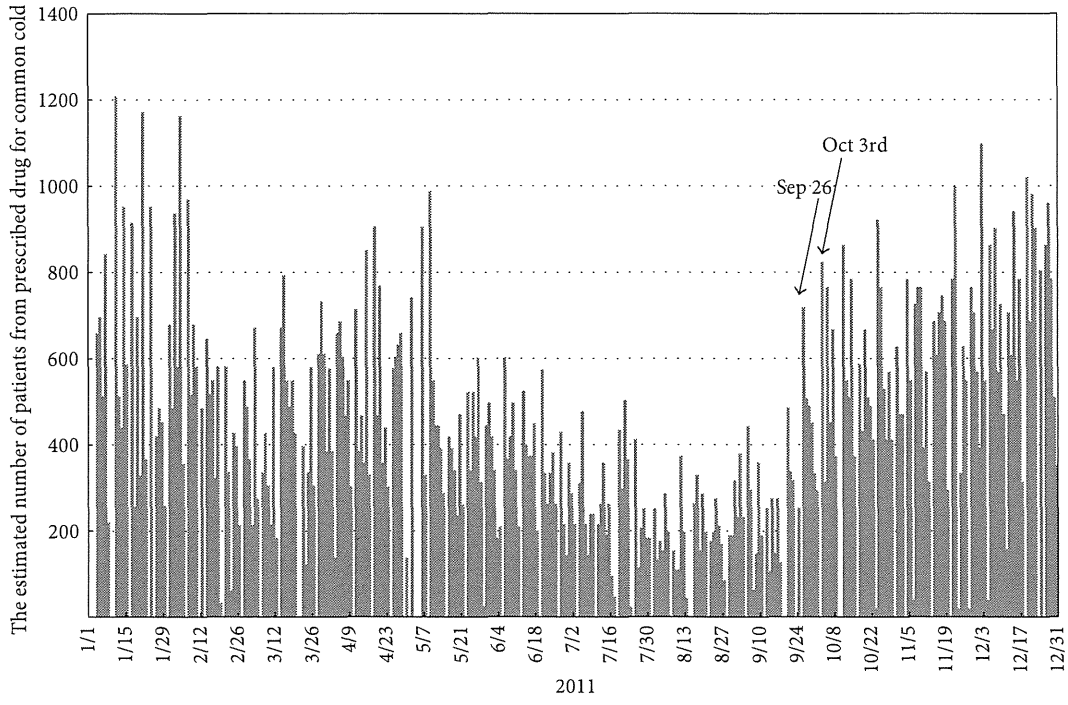

FIGURE 3: Combination cold medication prescriptions recorded by the prescription surveillance system over time. On September 26, 2011, we noticed an unusual peak and then carefully monitored the real-time prescription surveillance and found a second peak on October 3, 2011. We confirmed this abnormality and began this study on the following day (October 4, 2011).

2. Materials and Methods

2.1. Prescription Surveillance. Although very common in the US and other countries, there is no nationwide syndromic surveillance system to electronically monitor medical records in Japan. Because of the low prevalence of electronic medical records and a restrictive privacy policy, we perform prescription surveillance nationwide for syndromic surveillance by monitoring the number of prescriptions for certain types of drugs such as anti-influenza medications.

There are approximately 45,000 pharmacies that deliver almost half of the prescribed drugs nationwide and almost all record prescriptions electronically. The prescription surveillance system was developed by the Infectious Disease Surveillance Center of the National Institute of Infectious Diseases in collaboration with EM Systems Co. Ltd. (Osaka, Japan), a leading provider of prescription surveillance used by pharmacies through the Application Server Provider (ASP) system. The ASP system is very useful for syndromic surveillance because data transfer is unnecessary. Thus, it can dramatically decrease costs and maintain a high level of confidentially. Its widespread use started in April 2009, and approximately 6,300 (13%) Japanese pharmacies actively participated in the program as of October 2011.

The ASP system tracks prescription information, but patient symptoms and diagnoses are not recorded. Categories of syndromic surveillance include the type of prescribed drugs. Currently, the syndromic surveillance system monitors several types of drugs, including those for relief of fever and pain due to common colds, as well as antiviral agents, anti-influenza medications (except amantadine), and anti-varicella zoster virus (VZV) drugs. The surveillance of the last two is also classified by age: <15, 16–64, and >65 years. Data collection and analysis are automatically performed every night, and the results are available on the home page of a secure internet site early the next morning.

Monitoring the usage of anti-influenza and anti-VZV drugs is particularly useful for early detection of outbreaks of infection because these drugs are used only to treat specific viral infections.

2.2. Clinical Samples. Between October 4 and 28, 2011, 50 patients were included in the present study who either presented at a single clinic with a chief complaint of respiratory symptoms or fever or were suspected of having respiratory tract infections after being identified through the syndromic prescription surveillance system. In Japan, a rapid diagnosis kit suitable for use at outpatient clinics is currently available, and the costs are covered by the national health insurance program. The tests allow for rapid detection of infections caused by the influenza virus, RSV, and adenovirus. A total of 18 pharyngeal swabs to screen for adenovirus infections and 32 nasal swabs to screen for RSV and influenza viral infections (rapid RSV) were collected [9]. Viruses were extracted from the swabs using immunochromatography (IC) kits with approximately 500 μL of a mucolytic agent provided by the manufacturer. After the assay, approximately 200 μL of the agent remained in the IC-kit tubes. This medical waste was transferred to universal transport medium (359C; Copan Italia S.p.A, Brescia, Italy) and analyzed using

Table 1: Hyper-PCR primers.

Pathogen	Primer	Base sequence (5′-3′)	Polarity	Reference
RSV-A	RSA-F	TGC AAG CAG AAA TGG AAC AAG T	+	[14]
106 bp	RSA-R	AAT AAT GAT GCT TTT GGG TTG TTC A	−	
RSV-B	RSB-F	GATGGCTCTTAGCAAAGTCAAGTTAA	+	[15]
104 bp	RSB-R	TGTCAATATTATCTCCTGTACTACGTTGAA	−	
Parainfluenza 1	PIS1+	CCGGTAATTTCTCATACCTATG	+	[16]
317 bp	PIS1−	CCTTGGAGCGGAGTTGTTAAG	−	
Parainfluenza 3	Para3.1	CTCGAGGTTGTCAGGATATAG	+	[16]
189 bp	Para3.2	CTTTGGGAGTTGAACACAGTT	−	
Rhinovirus	SRHI-1-NIID	CGGGTAGCTTCCACCACCAGCCCTT	+	[16]
549 bp	SRHI-2	GGGACCAACTACTTTGGGTGTCCGTGT	−	
Enterovirus	entR1	ATTGTCACCATAAGCAGCCA	+	[17]
172 bp	entE2	CCTCCGGCCCCTGAATG	−	
H1N1 2009	swH1-F2	TCATGCGAACAATTCAACA	+	Present study
127 bp	swH1-R2	TGGGGCTACCCCTCTTAGTTTG	−	

real-time polymerase chain reaction (PCR) [10] and Hyper-PCR [11], which is a faster technique compared with the previously available PCR applications. Thus, we used Hyper-PCR for the applicable pathogens. The CycleavePCR respiratory infection-pathogenic virus detection kit (Takara Bio, Shiga, Japan) was used to detect 11 types of viruses: human RSV types A and B, human parainfluenza virus types 1–3, human metapneumovirus, influenza A and B viruses, human adenovirus, human bocavirus, and human rhinovirus. The Thermal Cycler Dice Real Time System II MRQ (Takara Bio) was used to detect and identify the 11 types of viruses detected by the CycleavePCR kit [10]. Hyper-PCR [11] was performed using the One Step SYBR High Speed RT-PCR Kit (Hyper-PCR) (Takara) to detect RSV types A, B, human parainfluenza virus types 1, 3, human rhinovirus, enterovirus, and influenza A (H1N1) 2009 (primers are listed in Table 1) using the Hyper-PCR MK IV PCR system (Trust Medical, Hyogo, Japan). The accuracy of the Hyper-PCR methods was confirmed by comparison with other conventional PCR methods. Conventional PCR was used to detect *M. pneumoniae* [12]. In addition, the presence of coronavirus infection was tested in patients from whom no infectious agents were detected [13].

3. Ethical Considerations

This study only collected anonymous information that cannot be associated with individual patients. Patient samples were collected during the course of medical care provided at the participating facilities, and all examinations and testing for pathogens occurred at the request of the medical facilities for the purposes of diagnosis and treatment. This study used only existing medical records and documents, and oral informed consent was obtained from all patients.

4. Results

After testing the specimens, we provided the results to a medical institution within 4 days including the conveyance period. The 50 patients tested in this study included 2 infants (1 male and 1 female, aged <1 year), 25 children (12 males and 13 females, aged 1–6 years), 10 elementary school pupils (6 males and 4 females, aged 7–12 years), 4 minors (2 males and 2 females, aged 13–18 years), 8 adults (3 males and 5 females, aged >18 years), and 1 patient (age unavailable).

Table 2 lists the pathogens detected by the PCR analysis stratified by age in the 27 patients. In children, enterovirus, rhinoviruses, RSV, and parainfluenza viruses were detected, whereas *M. pneumoniae* was detected only in elementary school pupils and minors. In the remaining 23 patients, no pathogens were detected. These 23 patients were also found to be negative for coronavirus.

PCR was used to obtain definitive viral diagnoses via rapid RSV and adenovirus diagnosis kits, and the sensitivity and specificity were calculated for these test kits. For the rapid RSV diagnosis kit, sensitivity was 80% and specificity was 85%. For the rapid adenovirus diagnosis kit, no positive results were obtained; therefore, sensitivity could not be calculated and specificity was 100%.

RSV infections were detected using the rapid diagnosis kit, but rhinovirus, enterovirus, and parainfluenza virus infections were not. The causative pathogens were unknown in many patients, although they were nevertheless treated for upper respiratory tract infections.

Evaluation of the incidence of various symptoms in patients infected with different pathogens showed that rhinoviruses were detected in nasal swab specimens more often than other viruses and patients with rhinovirus infections were less likely to present with fever (Table 3).

All RSV-positive patients were children, 80% of whom presented with coughing. All patients who were tested using the rapid adenovirus detection kit showed negative results. However, all these patients also tested negative for adenovirus using sensitive PCR tests. Thus, adenovirus was not considered to be the causative organism of this suspected outbreak.

Table 2: Numbers of pathogens detected by PCR according to age.

	Infants	Children	Elementary school pupil	Minor (junior high school student or older)	Adult
Enterovirus	0	2	1	0	1
Mycoplasma pneumoniae	0	0	1	1	0
Parainfluenza 1	0	2	0	0	1
Rhinovirus	2	9	0	0	1
Rhinovirus + parainfluenza 1	0	1	0	0	0
Rhinovirus + RSV-A	0	1	0	0	0
Rhinovirus + RSV-A and RSV-B	0	1	0	0	0
RSV-A	0	2	0	0	0
RSV-B	0	1	0	0	0

Table 3: Incidences of symptoms detected in infections according to individual pathogens ($n = 50$).

	Number of infections	Fever	Headache	Nasal discharge	Pharyngeal pain	Cough
Mycoplasma pneumoniae	2	0%	0%	0%	50%	100%
Enterovirus	4	67%	25%	75%	25%	25%
Parainfluenza 1	3	33%	33%	33%	0%	67%
Rhinovirus + parainfluenza 1	1	0%	0%	0%	0%	100%
Rhinovirus	12	20%	0%	67%	0%	83%
Rhinovirus + RSV-A	1	0%	0%	100%	0%	100%
Rhinovirus + RSV-A + RSV-B	1	100%	0%	100%	0%	100%
RSV-A	2	100%	0%	100%	0%	100%
RSV-B	1	100%	0%	0%	0%	0%
None	23	21%	13%	57%	30%	35%

5. Discussion

Here, we examined a combination of syndromic surveillance and PCR testing and showed the potential to identify pathogens during the early stage of an outbreak of respiratory infections. In the future, it would be desirable to develop an *M. pneumoniae* diagnosis kit that can diagnose pathogens from nasal or pharyngeal swabs at outpatient clinics or the bedside of patients.

In Japan, two official pathogen surveillance methods have been conducted under the infection control laws: sentinel pathogen surveillance and active surveillance. The official pathogenic surveillance has been conducted at sentinel medical institutions regardless of outbreaks. On the other hand, in patients with serious diseases, active pathogenic surveillance has sometimes been conducted on the basis of notifications by medical institutions. However, active surveillance is conducted only when an infection spreads widely enough to cause serious problems in a particular region and the surveillance of pathogens may not be timely enough to mount a response to control outbreaks. Pathogenic surveillance for all patients with signs of an infection would detect agents of bioterrorism and emerging infectious diseases; however, the cost would be prohibitive. Therefore, system coordination to perform pathogen surveillance based on early detection of outbreaks is necessary. The scheme proposed by the present study uses PCR testing triggered by detection alerts from syndromic surveillance systems. In general, syndromic surveillance offers earlier detection of infectious diseases than traditional surveillance. Moreover, if the pathogen remains unknown following bedside testing using several rapid tests or other typical examinations, the proposed scheme requires the collection of specimens as soon as possible and sending them to a laboratory for definitive diagnoses. However, it takes a few days to transfer the specimens and a few extra days for the information of the identified pathogen to be shared among medical facilities, public health centers, and local governments in the involved areas. In the proposed scheme, we can use pathogenic information to control ongoing outbreaks and, hopefully, decrease the number of potential infections.

Thus far, syndromic surveillance with pathogenic testing has been conducted by collecting samples from patients receiving telephone consultations [18] and those receiving emergency department consultations [19]. Syndromic surveillance using electronic medical records has been combined with testing for the influenza virus [20]. However, these systems have focused only on rapid testing and are mainly used for influenza monitoring [20, 21]. Therefore, syndromic surveillance trials for nonspecific pathogens using PCR for undiagnosed infectious diseases, similar to the present study, have not been performed before.

In the present study, an outbreak was detected by routine syndromic surveillance, in which samples were regionally collected for PCR analysis. These tests for viral infections allowed for differentiation between bacterial and viral infections, thus facilitating treatment without the unnecessary use of antibiotics. Although the present laboratory tests cannot be performed for all individual clinical diagnoses, the

results were immediately made available to clinicians for the treatment of other patients with similar symptoms.

The symptoms reported in the present study were rather mild; therefore, no patient required hospitalization, and no further testing was performed in undiagnosed patients. However, if severe cases were to occur, careful identification of pathogens would be desirable. Rhinoviruses were detected in nasal swab specimens more frequently than other viruses. Therefore, it is likely that children who present with nasal discharge and mild fever may be reservoirs for rhinoviruses [22]. Testing for respiratory viral infections in emergency room outpatients by PCR analysis showed that the most frequently detected viruses were picornaviruses, including rhinoviruses [23]. When children present with coughing as the main symptom, RSV should be considered as the most likely pathogen.

The finding that *M. pneumoniae* infection was not detected in infants and children, but rather in elementary school pupils and minors, was consistent with reports that *M. pneumoniae* may often cause asymptomatic infections before the age of 5 years, after which immunity decreases as children become susceptible to symptomatic *M. pneumoniae* infections [5].

In Japan, nationwide outbreaks of *M. pneumoniae* began in 2011 and continued as of January 2012, during which time *M. pneumonia*, rhinovirus, enterovirus, parainfluenza virus, and RSV have been identified. Our results suggested that this outbreak may include false-positive cases and subsequent inappropriate prescriptions of antibiotics.

An increased frequency of macrolide-resistant *M. pneumoniae* became widely reported in the Japanese media in the fall of 2011 [24]. Therefore, this news may have induced an abnormal increase in the number of patients (Figure 2). The rapid test available in Japan for *M. pneumoniae* uses sera samples [25]. Although general clinics may outsource *M. pneumoniae* antibody testing and cold hemagglutinin testing, blood testing is usually not performed in cases of mild pediatric illnesses.

In the future, it would be desirable to develop *M. pneumoniae* diagnostic kits using nasal or pharyngeal swabs at outpatient clinics or bedside. Until such kits for the diagnoses of *M. pneumoniae* and other infectious diseases are developed, syndromic surveillance with PCR testing offers a useful countermeasure against infectious outbreaks. In this study, we could not detect single infectious agents that explained the outbreaks; however, our results excluded *M. pneumoniae*.

The present study was limited to a single clinic. Therefore, further studies involving more facilities should be undertaken. It is also necessary to develop a network and sample transportation system among the facilities partaking in the syndromic surveillance system and to adequately staff laboratories with experienced technicians.

Syndromic surveillance data has been mathematically or statistically analyzed in many studies. However, when an abnormal value is reported by syndromic surveillance, there are many cases in which the pathogens cannot be identified by the calculations introduced in these articles.

6. Conclusion

When *M. pneumoniae* and RSV infections were prevalent nationwide during the fall of 2011, we observed an abnormal increase in common cold prescriptions through the Japanese surveillance system and were able to evaluate the incidence of various pathogens via PCR testing.

Authors' Contribution

The authors contributed equally to this paper.

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Novel Human Adenovirus Strain, Bangladesh

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We report a novel human adenovirus D (HAdV-65) isolated from feces of 4 children in Bangladesh who had acute gastroenteritis. Corresponding genes of HAdV-65 were related to a hexon gene of HAdV-10, penton base genes of HAdV-37 and HAdV-58, and a fiber gene of HAdV-9. This novel virus may be a serious threat to public health.

Human adenoviruses (HAdVs) are common pathogens that cause several diseases, such as pneumonia, acute gastroenteritis, and epidemic keratoconjunctivitis (*1*). HAdV infection is also associated with a serious adenovirus syndrome in immunocompromised patients after stem cell transplantation (*2*). Acute gastroenteritis causes illness and death in humans worldwide. Illness is associated with infection of enteric viruses, including rotavirus, astrovirus, norovirus, sapovirus, and adenovirus.

HAdVs are divided into 7 species (HAdV-A–G) on the basis of DNA genome homology. Most acute gastroenteritis related to HAdVs is caused by HAdV-F species (HAdV-40 and HAdV-41 (*3*,*4*). Recently, we detected HAdV-D in feces of children with diarrhea in Bangladesh (*5*). Other HAdV-D strains have also been associated with diarrhea in Kenya and Brazil (*6*,*7*).

We report a novel HAdV-D (HAdV-65) strain detected in feces of 4 children with acute gastroenteritis during October 2004–March 2005 in Bangladesh (*5*) and results of hexon, penton base, and fiber gene sequence analyses. We also report the full genome sequence of this virus, whose corresponding genes are closely related to the hexon gene of HAdV-10, penton base genes of HAdV-37 and HAdV-58, and the fiber gene of HAdV-9.

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The Study

Cloned virus (3 plaque purifications) was propagated in an A549 cell line, which was maintained in minimal essential medium supplemented with 1% fetal bovine serum (Cansera International Inc., Toronto, Ontario, Canada). Cultures were observed for 3–4 weeks for a cytopathic effect. After a cytopathic effect was observed, cell lysates were centrifuged at 1,430 × *g* for 20 min at 4°C. Supernatants were centrifuged at 72,000 × *g* for 3 h at 4°C. Pellets were resuspended in sterile water and treated with 10 μL (20 mg/mL) of proteinase K. DNA was extracted by using the phenol:chloroform:isoamyl alcohol (25:24:1) method (Invitrogen, Carlsbad, CA, USA) and precipitated with isopropyl alcohol.

PCR was performed in a total volume of 50 μL containing 20 pmol/μL of each primer, 2.5 mmol/L of dNTP, 1.25 units of GXL DNA polymerase (Takara, Shiga, Japan), and 5 μL of DNA template. After PCR products were purified by using the MinElute PCR Purification Kit (QIAGEN, Hilden, Germany), cycle sequencing was conducted by using the Genome Lab DTCS Quick Start Kit (Beckman Coulter Inc., Fullerton, CA, USA).

The complete genome of HAdV-65 was sequenced by using the primer walking method. The 5′ terminus of full-length DNA was phosphorylated with 20 units of T4 polynucleotide kinase and 0.5 μL of 100 mmol/L ATP, and ligated to a blunt *Eco*RI-*Not*I-*Bam*HI adaptor (1 pmol/μL) for 3 h at 8°C by using the DNA Ligation Mighty Mix Kit (Takara), PCRs with primer pairs containing adaptor sequences were conducted as described (*8*). DNA sequences were assembled by using the CEQ 2000XL DNA Analysis System version 4.3.9 (Beckman Coulter Inc.).

The genome of HAdV-65 was 35,172 bp. It had a GC content of 56.9%, and the inverted terminal repeat sequence of this virus was 150 bp. Phylogenetic trees were generated by using the maximum-likelihood method with MEGA5 (www.megasoftware.net) after alignment was performed by using ClustalW (www.clustal.org).

Complete genome analysis showed that HAdV-65 was ≥5.0% distant from any other HAdV-D reference strains (online Appendix Figure 1, panel A, wwwnc.cdc.gov/EID/article/18/5/11-1584-FA1.htm). On the basis of hypervariable loop 1 and loop 2, which encode the neutralization epitope of HAdVs, this virus clustered with HAdV-10 (online Appendix Figure 1, panels B and C). However, HAdV-65 was closely related to HAdV-37 and HAdV-58 in the hypervariable loop 1 and the Arg-Gly-Asp (RGD) loop of the penton base gene, respectively (online Appendix Figure 1, panels D and E). Phylogenetic analysis also showed that this novel virus clustered with HAdV-9 on the basis of the fiber gene sequence (online Appendix Figure 1, panel F). Sequences of HAdV-65 and 3 other strains (DC 11, 253, and 303) isolated from infants in this

study had identical hexon, penton base, and fiber genes.

Potential recombination in HAdV-65 was investigated by using the SimPlot program (http://sray.med.som.jhmi.edu/RaySoft/simplot_old/Version1/SimPlot_Doc_v13.html). DNA sequence alignments were created by using DNASIS Pro (Hitachi Solutions, Tokyo, Japan). SimPlot analysis showed no potential recombination in the hexon and fiber genes (online Appendix Figure 2, panels A and C, wwwnc.cdc.gov/EID/article/18/5/11-1584-FA2.htm), and recombination between the hypervariable loop 1 and the RGD loop was predicted in the penton base gene (online Appendix Figure 2, panel B). HAdV-65 had nucleotide identities of 97.9% to HAdV-10 in the hexon gene, 92.3% and 96.7% to HAdV-37 and HAdV-58, respectively, in the penton base gene, and 98.2% to HAdV-9 in the fiber gene. The GenBank accession number for HAdV-65 is AP012285.

Conclusions

We report the complete genome of HAdV-65, a novel human adenovirus isolated from children with gastroenteritis. Recombination is an essential feature for viral evolution and immune escape. Recombination can be facilitated by antiviral immune pressure and co-infection with different HAdV strains of the same species (*9*). Recently, newly identified HAdVs appeared to originate by recombination among ≥2 viruses. HAdV-53 was reported as a novel recombinant HAdV with a close genetic relationship to loop 1 and loop 2 of HAdV-22, the penton base gene of HAdV-37, and the fiber gene of HAdV-8. HAdV-56 had a loop 1 and loop 2 highly similar to those of HAdV-15 (*10*,*11*). All other regions of the genome were genetically related to HAdV-9.

HAdV-58 was recently characterized as a novel HAdV with unique hexon and fiber genes of HAdV-25 and HAdV-29 (*12*). In the present study, we demonstrated that hexon and fiber coding regions of HAdV-65 were formed by recombination in regions around these genes but not by potential recombination within these genes. The potential recombination site within the penton base gene is located at the central position between hypervariable loop 1 and the RGD loop.

These findings suggest that the most conserved sequences around the hexon and fiber genes and in the penton base gene may play a major role in recombination. In addition, this recombination mechanism may be more efficient in enabling new processes of infection and immune escape for maintaining HAdVs than individual small mutations, such as insertions, substitutions, and deletions.

Most recombinant HAdVs have been found in AIDS patients. (*9*,*13*). The RGD loop of the penton base protein can be digested by trypsin secreted in the intestines, which results in inhibition of proliferation of HAdV except for HAdV-F types in the intestines (*14*). In this study, HAdV-65 was isolated from infants who had lower immunocompetence and secretion rates of digestive enzymes than adults. These results indicate that emergence of HAdV-65 might have been caused by long coexistence of multiple HAdV-D types and depending on a decrease in immunity, as observed in AIDS patients, and decreased digestive capacity in the intestines.

We detected this type of recombination not only in HAdV-65, but also in 3 other HAdV strains that had a genome sequence identical with that of HAdV-65 from children in Bangladesh during 2004–2005. This finding indicates that this virus might be a newly emerging HAdV, which might be a serious threat to public health.

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genomeA
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Genome Sequence of a Novel Virus of the Species Human Adenovirus D Associated with Acute Gastroenteritis

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A novel virus of the species human adenovirus D, HAdV-67 (P-New/H9/F25), was first isolated from diarrheal feces of six children in Dhaka City, Bangladesh. The genome of this novel virus may be composed of multiple recombinations among HAdV-9, HAdV-25, HAdV-26, HAdV-33, HAdV-46, and an unknown human adenovirus D which was an origin of HAdV-67.

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Human adenoviruses (HAdVs) infect humans of all age groups and are significant pathogens that cause a wide range of clinical diseases, including epidemic keratoconjunctivitis, acute respiratory illnesses, and acute gastroenteritis (1, 2, 3). Sixty-five types, including candidates from HAdV-55 to HAdV-65, have been identified and classified into seven species, A to G, each of which shows different organotropisms.

A novel virus of the species human adenovirus D was identified from diarrheal feces of six children in Bangladesh. This novel virus has been approved as a new type of HAdV-67 by the Human Adenovirus Working Group. For amplifying the genome fragments of HAdV-67, the previously published sequence of HAdV-9 was used to design primers. A complete genome of HAdV-67 was sequenced using primer walking. For the terminal genome sequence, the 5′ terminus of full-length DNA was phosphorylated with T4 polynucleotide kinase (Takara, Shiga, Japan). Afterward, a blunt-end EcoRI-NotI-BamHI adaptor (Takara, Japan) was ligated using the DNA Ligation Mighty Mix kit (Takara, Japan).

A complete genome of HAdV-67 was 35,075 bp in length and had an overall base composition of 22.47% (A), 20.48% (T), 28.56% (G), and 28.49% (C). Like those of other HAdV-Ds, the genome of HAdV-67 was predicted to encode 38 proteins in four early, two intermediate, and five late transcriptional regions. The inverted terminal repeat (ITR) of HAdV-67 was 123 bp in length and contained a conserved CATCATCAAT motif. Additionally, its transcription factor DNA binding sites, such as those for NFIII/OctI (ATGCAAAT) at nucleotide positions 42 to 49, SP1 (AGGG CGG) at nucleotide positions 64 to 70, and ATFs (TGACGT) at nucleotide positions 107 to 112, were present within the ITR. No nuclear factor I (NFI) binding sites, which exist in some HAdV-Ds (HAdV-9, HAdV-15, HAdV-19, HAdV-26, HAdV-29, HAdV-37, HAdV-53, HAdV-56, and HAdV-58), were identified in HAdV-67. In a phylogenetic analysis, HAdV-67 clustered with HAdV-9 (loops 1 and 2 of the hexon gene), HAdV-46 (HVL1 of the penton base gene), HAdV-33 (genes for a DNA binding protein and 100,000-molecular-weight protein [100K protein] in the L4 region), HAdV-26 (genes for CR1-α, gp19K, CR1-β, and CR1-γ in the E3 region), and HAdV-25 (fiber gene). Interestingly, HAdV-67 showed low similarities to other HAdV-Ds in the RGD loop of the penton base gene. These results suggested that the novel genome might be composed of multiple recombinations among HAdV-9, HAdV-25, HAdV-26, HAdV-33, HAdV-46, and an unknown human adenovirus D which was an origin of HAdV-67. The species HAdV-A and -F are primarily associated with acute gastroenteritis, whereas HAdV-Ds cause acute gastroenteritis in AIDS patients (4, 5, 6, 7, 8). We previously reported a novel HAdV-D (HAdV-65) isolated from diarrheal feces of infants without immunodeficiency (9). In this study, a novel HAdV-D, HAdV-67 (P-New/H9/F25), was found in acute gastroenteritic children who were previously healthy, indicating its potential threat associated with this illness. Moreover, this study demonstrates the importance of identifying recombinant regions within the genomes of HAdVs.

Nucleotide sequence accession number. The GenBank accession number for HAdV-67 (P-New/H9/F25) is AP012302.

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Identification of Contamination in the American Type Culture Collection Stock of Human Adenovirus Type 8 by Whole-Genome Sequencing

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Identification of Contamination in the American Type Culture Collection Stock of Human Adenovirus Type 8 by Whole-Genome Sequencing

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Human adenoviruses (HAdVs) infect billions of people worldwide, causing various diseases, such as conjunctivitis, respiratory disease, gastroenteritis, and hemorrhagic cystitis (1). HAdVs have been classified into six species and further classified into 51 types, available from the American Type Culture Collection (ATCC) as prototypes, based on serum neutralization and 16 additional type candidates (types 52 to 67) defined by the genome sequences.

Type 8 of HAdV-D (HAdV-8) is one of the major causative agents of epidemic keratoconjunctivitis (EKC) (2–9), and as such, we have been studying modes of the pathogenesis with genome sequencing and other means. During the course of our studies, by sequencing the genome of the HAdV-8 strain available from ATCC (lot number 59323195), extensive polymorphisms were identified over the genome, indicating that the HAdV-8 stock was contaminated.

Amplifying and sequencing the hexon protein-coding region, we found that the hexon sequences of the non-HAdV-8 viruses were identical to that of the HAdV-10 prototype (DDBJ accession number AB724351) that we had sequenced previously. We then proceeded to determine the whole-genome sequences of two types, i.e., HAdV-8 and -10, in ATCC HAdV-8 lot number 59323195. We first amplified genome regions with type-specific primers designed based on preexisting sequences available from our previous study (10) on HAdV-8 Trim (DDBJ accession number AB448767), HAdV-8b (DDBJ accession number AB448768), HAdV-8e (DDBJ accession number AB448769), and the HAdV-10 prototype and from other HAdV genomes available. We then sequenced, assembled, and analyzed the amplicons (see reference 10 for the methodology).

The genome of the ATCC HAdV-8 "Trim variant" (DDBJ accession number AB746853) is a new variant of HAdV-8 that is 34,978 bp in length and which is closest to the preexisting HAdV-8 Trim sequence, differing by seven nonsynonymous changes in five genes, five synonymous changes in four genes, and nine intergenic base substitutions, as well as three insertions and five deletions, including a single-nucleotide insertion (SNI) in CR1-α and an SNI and a single-nucleotide deletion in CR1-γ genes. The genome of the contaminant in the ATCC HAdV-8 stock (35,105 bp; DDBJ accession number AB746854) was almost identical to that of HAdV-10 (DDBJ accession number AB724351) except for a nonsynonymous change in the pTP gene and 12 base substitutions in the telomere regions. We also sequenced the genome of an HAdV-8 lot purchased from ATCC independently and propagated thereafter at the National Institute of Infectious Diseases of Japan (NIID) and found that the sequence (HAdV-10 NIID-1; DDBJ accession number AB695621) was entirely identical to that of the contaminant HAdV-10 (DDBJ accession number AB746854). In the phylogenetic tree of these newly sequenced

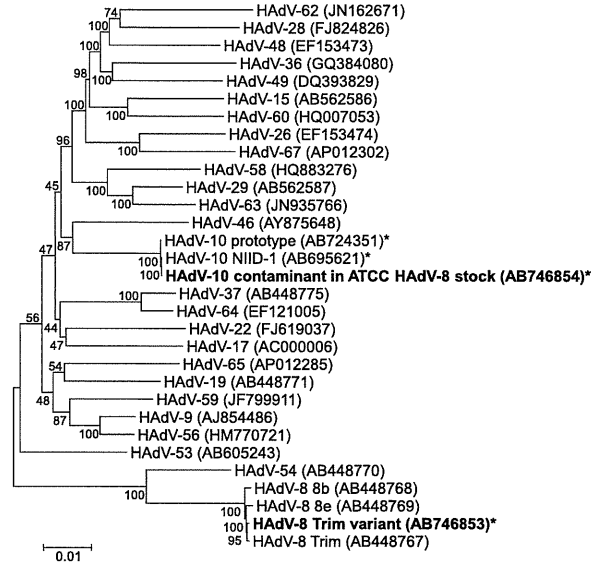

FIG 1 Molecular phylogenetic tree of the two genomes from the ATCC HAdV-8 stock (in bold) together with 29 complete genome sequences of HAdV-D viruses in the International Nucleotide Sequence Database (INSD). The multiple alignment of these sequences was constructed using MAFFT version 6 (11), the evolutionary distances were estimated using Kimura's two-parameter model (12) based on 33,884 gap-free sites in the alignment, and then an unrooted tree was constructed using the neighbor-joining method (13). The bootstrap probability calculated for 1,000 replicates is shown on each branch. The genomes that were newly sequenced in this study are indicated by an asterisk.

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genomes and those of 27 other HAdV types (Fig. 1), the genomes deposited under DDBJ accession numbers AB746853 and AB746854 were placed in the HAdV-8 and the HAdV-10 clades, respectively, with no ambiguity.

Here we report on the whole-genome sequences and phylogenetic analyses of the HAdV-8 and HAdV-10 reference virus stocks distributed by the ATCC and conclude that HAdV-8 lot number 59323195, provided by the ATCC, contained both HAdV-8 and HAdV-10 types. Due to HAdV-8 being one of the major causative agents of EKC, we would like to draw attention of the adenovirus research community to this contamination issue so as to prevent misinterpretation.

Nucleotide sequence accession numbers. The whole-genome sequences of the HAdV-8 Trim variant, the HAdV-10 contaminant in the ATCC HAdV-8 stock, HAdV-10 NIID-1, and the HAdV-10 prototype have been deposited at DDBJ under accession numbers AB746853, AB746854, AB695621, and AB724351, respectively.

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Laboratory and Epidemiology Communications

Isolation of an Intertypic Recombinant Human Adenovirus (Candidate Type 56) from the Pharyngeal Swab of a Patient with Pharyngoconjunctival Fever

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Human adenoviruses (HAdVs) are non-enveloped, double-stranded DNA viruses of the family *Adenoviridae*, genus *Mastadenovirus*. HAdVs have been categorized into seven species, A to G, on the basis of various biological and morphological criteria, nucleic acid characteristics, and homologies. The main tropism sites of HAdVs are determined by the species (Table 1).

HAdV-15/29/H9 reported by Kaneko et al. (1) is a novel intertypic recombinant type of adenovirus. HAdV candidate type 56 (HAdV-56) (2) has the same sequence as HAdV-15/29/H9.

HAdV-15/29/H9 (HAdV-56) caused epidemic keratoconjunctivitis (EKC) throughout Japan (1) and other countries (2). In this short report, we present the first case of pharyngoconjunctival fever (PCF) caused by

Table 1. Species of human adenoviruses and their main tropism

Species	Adenovirus type	Main tropism site
A	12, 18, **31**	Gut
B1	**3**, 7, 16, 21, 50	Respiratory tract and/or eye
B2	**11**, 14, 34, 35, 55*	Urogenital system, respiratory tract (severe infection among immunocompromised patient)
C	**1**, **2**, **5**, **6**	Respiratory tract (ARD among children) and gut
D	**8**–10, 13, 15, 17, **19**, 20, 22–30, 32, 33, 36, 37–39, 42–49, 51, **53**, **54**, **56***	Eye (epidemic keratoconjunctivitis)
E	**4**	Respiratory tract
F	**40**, **41**	Gut
G	52	Gut

* Human adenovirus types 1–56 are shown in this table.
Those most commonly associated with particular syndromes are in bold type.
The asterisk indicates the candidate type as of May 2012.

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Table 2. Complete nucleotide sequences of hexon-, fiber-, and penton base-coding regions of the HAdV in this study

Strain	Definition	Accession no.	bp	Reference	Hyogo6180 strain		
					Accession no. AB719408 2,856 bp	AB719409 1,089 bp	AB719410 1,560 bp
					Hexon-coding region	Fiber-coding region	Penton base-coding region
HAdV-15/29/H9 (isolate, 2307-S)	full genome	AB562588	35,067	1	100% identical (position, 17,727-20,582)	100% identical (position, 30,896-31,984)	100% identical (position, 13,486-15,045)
HAdV-56 (candidate)	full genome	HM770721	35,066	2	100% identical (position, 17,726-20,581)	100% identical (position, 30,895-31,983)	100% identical (position, 13,485-15,044)

HAdV-56.

On May 12, 2011, an immunocompetent 11-year-old boy from Himeji city, Hyogo Prefecture, developed a fever of 38.0°C. The following day, when his fever increased to 39.0°C, he visited Okafuji Pediatric Clinic in Himeji. He presented with symptoms of upper respiratory tract infection, such as red pharynx, as well as excessive redness of the right eye. Based on his clinical presentation, the patient was diagnosed with PCF but not with EKC. A bedside immunochromatographic test (Check Ad; Alfresa Pharma Corp., Osaka, Japan) conducted using a pharyngeal swab confirmed the presence of HAdV. Another pharyngeal swab was collected for type identification, as a part of the protocol for infectious agent surveillance in Hyogo Prefecture. Informed written consent for the laboratory test was obtained from the patient's guardian. The patient did not have any significant underlying disease.

The virus was isolated from the pharyngeal swab sample, as described previously (3). Briefly, the sample was inoculated into A549 and RD-18S cell lines. On the 7th day of the culture, cultured cells were passaged to a fresh culture of the same cell type. The typical cytopathic effect (CPE) of adenovirus appeared 6 days after passage into A549 cells. Nevertheless, no viral growth was observed in RD-18S cells. After culture isolation (strain: Hyogo6180), complete sequences of the hexon- (accession no. AB719408), fiber- (accession no. AB719409), and penton- (accession no. AB719410) coding regions were determined by the direct sequencing method, as described previously (1). All these sequences of the isolate were identical to those of HAdV-15/29/H9 (1) and HAdV-56 (2) (Table 2).

HAdV-56 had originally isolated in France in 2008 from the pulmonary biopsy of a 10-day-old neonate who had died because of a fatal respiratory infection, and from the conjunctival swabs of three healthcare workers who cared for the neonate and subsequently developed keratoconjunctivitis (2). An identical strain has been identified from cases of EKC throughout Japan since 2008. Genome sequence analysis revealed that the new strain in Japan is actually a recombinant virus carrying genetic material from different species D HAdVs, i.e., type 15 (and 29) in the hexon-conding region (hyper variable region), type 9 (and 26) in the penton-coding region, and type 9 in the fiber-coding region. Therefore, the new strain was designated as a novel intertypic recombinant, AdV-15/29/H9 (1). Robinson et al. (2) later performed a computational analysis of the genome sequence of the strain isolated in France from both the neonate and a healthcare worker, and found extensive recombination between HAdV-9, -15, -26, -29, and/or another adenovirus. They assigned the strain as a novel type, HAdV-56.

The present study is the first to indicate HAdV-56 as a causative agent of PCF. PCF is a highly contagious disease that affects young children more frequently than adults, resulting in outbreaks among institutionalized children. Worldwide data show that HAdV-3, HAdV-7 (species B), HAdV-1, HAdV-2, HAdV-5, HAdV-6 (species C), and HAdV-4 (species E) are the most frequent causative agents of PCF (4,5). In PCF, the virus infects epithelial cells of the respiratory tract and may or may not cause conjunctiva. The isolation of HAdV-56 from a patient with PCF signifies that specimens from respiratory tract infection cases should be examined for the presence of HAdV, including type 56. To identify HAdV-56, adenoviral fiber- and penton base-coding regions should be sequenced in addition to the hexon-coding region.

Since 2011, HAdV-56 has been included in reports to the National Epidemiological Surveillance of Infectious Disease (NESID) system in Japan. A total of 30 isolates were reported until April 2012, and 27 (90%) of these isolates came from the eye swabs of EKC patients. Following our isolation of HAdV-56 from PCF, another PCF case (detection from pharyngeal swab) and a "fever of unknown origin" (detection from feces) case associated with HAdV56 were reported (detailed data were not available). The tissue tropism of HAdV-56 has not yet been sufficiently resolved. This case underscores the necessity for continuous surveillance in order to understand the epidemiological nature of HAdV-56.

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Conflict of interest None to declare.

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