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Outbreak of Swine-Origin Influenza A (H1N1); Experience of a Regional Center in Seoul during a Month, August-September 2009

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Background: The aim of this study is to clarify the epidemiology of swine-origin influenza A (H1N1) virus 2009 (S-OIV) during the first month of outbreak at one of influenza clinic in Seoul, Korea.
Methods: We documented the epidemiologic and clinical features of S-OIV-confirmed cases who visited a university hospital in Northeastern Seoul between August 21 and September 20, 2009. Nasopharyngeal swab of patients with acute febrile respiratory illnesses were evaluated with rapid influenza antigen tests and multiplex RT-PCR for S-OIV and seasonal influenza A.
Results: A total of 5,322 patients with acute febrile respiratory illnesses were identified at our influenza clinic for the study period. S-OIV was confirmed in 309 patients by RT-PCR. The patients ranged from 2 months to 61 years of age and 189 patients (61.2%) were teenagers. Eighty-one patients had known contact with S-OIV-confirmed patients in schools (N=61), households (N=15), and healthcare facilities (N=3). Frequent symptoms were fever (94.5%), cough (73.1%), sore throat (52.1%), and rhinorrhea (50.5%). Gastrointestinal symptoms were also present in 10 patients (4.9%). Ten patients (4.9%) required hospitalizations. Seventy patients (22.7%) could not take oseltamivir at the first visits, however, all of them recovered without complication. Rapid antigen tests showed the sensitivity of 44.4% (130/294). Patients with positive antigen tests, compared with negative antigen tests, showed higher frequencies of rhinorrhea (60.8% vs 43.3%, *P*=0.004) and stuffy nose (33.8% vs 20.1%, *P*=0.012).
Conclusion: S-OIV infections spread predominately in school-aged children during the early accelerating phase of the outbreak. Rapid influenza antigen tests were correlated with nasal discharge and obstruction.
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Key Words: Influenza A virus, Swine-origin influenza, H1N1

INTRODUCTION

The novel swine-origin influenza A (H1N1) virus (S-OIV) has spread rapidly across the globe since the first documented case of human infection occurred in the United States and Mexico on 17 April 2009[1]. The first Korean case of S-OIV was identified in a person returning from Mexico on 2 May 2009. Most of the Korean S-OIV cases identified in May and June were persons returning from foreign countries or those who had contact with S-OIV confirmed patients. The epidemic accelerated rapidly when new school semester started in August. The Korean Ministry for Health, Welfare and Family Affairs designated 455 hospitals and pharmacies nationwide as centers for influenza tests and treatment. One of these centers, as of 21 August 2009, is our institution, a tertiary-care hospital located in Nowongu district, Northeastern Seoul. The population of this district (615,000) is the second largest of the 25 districts in Seoul. Here we report the epidemiologic, clinical, and laboratory features of 309 patients with S-OIV infection cared for at our influenza clinic during the first month.

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MATERIALS AND METHODS

1. Patients

We organized an influenza clinic on August, 21, 2009 for the care of the patients with acute febrile respiratory illnesses (sudden onset of fever ($\geq$37.8°C) with any signs or symptoms of acute

respiratory infection such as cough, sore throat, rhinorrhea and stuffy nose)[2]. Patient's demographic details such as name, sex, age, contact with S-OIV patients, recent travel history to foreign country, and clinical symptoms, were also surveyed. Nasopharyngeal swab specimens were sent to the hospital laboratory for a rapid influenza antigen test. Patients with influenza A antigen positive results were regarded as 'S-OIV suspected'. They were educated as to the need for in-house isolation and some were advised of confirmatory polymerase chain reaction (PCR) tests. Patients with negative results on influenza A antigen tests received an explanation concerning the possibility of false negative results and were strongly urged to agree to confirmatory PCR tests. Patients judged to be at high-risk included pregnant women, children ≤59 months-of-age, adults ≥65 years-of-age, and persons with chronic underlying disorders including pulmonary, cardiovascular, renal, hepatic diseases, cancers, diabetes, and immunocompromised conditions. High-risk patients, S-OIV suspected patients, and patients presenting with symptoms compatible with influenza according to the attending clinician were given oseltamivir as a nationally-mandated control measure.

2. Methods

Each nasopharyngeal swab specimen was dissolved in dedicated lysis buffer and analyzed using a SD Bioline Influenza A/B assay (Standard Diagnostics, Inc., Seoul, Korea). This immunochromatographic assay consists of a lateral-flow strip containing monoclonal antibodies against influenza A and B antigens. Another swab specimen was delivered to the molecular laboratory for confirmatory multiplex reverse transcription (RT)-PCR tests with Seeplex Flu A ACE subtyping (Seegene, Seoul, Korea). This test includes five pairs of dual-priming oligonucleotide primers; influenza A matrix protein 1 gene, hemagglutinin genes of S-OIV, seasonal simian-origin influenza A H1N1/H3N2, and avian influenza H5N1[3]. PCR products were detected using the ScreenTape auto-capillary electrophoresis system (Lab901, Loanhead, UK). 'S-OIV confirmed patient' was defined as positive results for influenza A and S-OIV, and negative result for seasonal H1N1, H3N2, and avian influenza H5N1 on multiplex RT-PCR. We reviewed the medical records including the survey and the laboratory results of the patients who visited influenza clinic from 21 August to 20 September 2009. Data were analyzed by Chi square tests. *P* values less than 0.05 were regarded as statistically significant.

RESULTS

1. Patients

A total of 5,322 patients with acute febrile respiratory illnesses visited our influenza clinic during the study period. For the first week of the clinic's operation, the number of visits and S-OIV confirmed patients did not seem to be increasing. However, these numbers abruptly increased at the end of August (Fig. 1). Of these patients, 5,293 (99.5%) underwent rapid influenza A antigen tests; 308 patients (5.8%) had positive results. Among the patients with influenza A antigen positive results, 126 patients had the confirmatory PCR tests and all of them showed positive S-OIV results on multiplex RT-PCR. In total, PCR tests were performed on 2,083 patients and detected S-OIV in 309 patients (14.8%). The number of S-OIV suspected or confirmed patients was 491. Oseltamivir was prescribed for 1,658 patients during the study period. Among the patients who received oseltamivir, 1,218 patients (73.5%) were negative for both of influenza A antigen and PCR tests.

2. S-OIV confirmed cases

Among the 309 patients with confirmed S-OIV infection, 199 patients (64.4%) were male (Table 1). Their median age was 15 years (range: 2 months to 61 years) and 189 patients (61.2%) were teenagers. The mean (±SD) time between the onset of illness and visit was 1.54±1.27 days (range: 0～10 days).

Eighty-five patients (27.5%) had a history of close contact with S-OIV confirmed patients including contact in schools (N=61, 19.7%), households (N=15, 4.9%), hospitals (N=3, 1.0%), and workplace (N=2, 0.9%). Three patients who were proved to be ac-

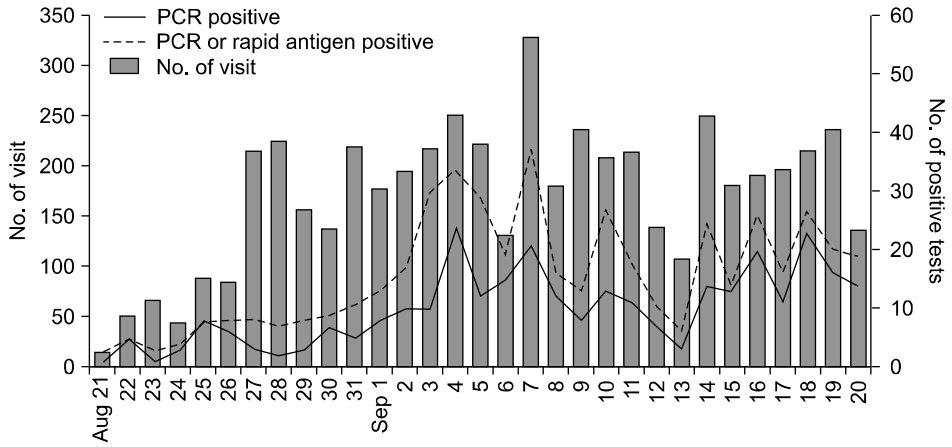

Fig. 1. Daily numbers of patients who visited the influenza clinic due to acute febrile respiratory illnessess (N=5,322), patients with PCR-confirmed S-OIV (N=309), and patients with PCR or rapid influenza A antigen positive results (N=491), August to September 2009. Abrupt increase of the visits to the influenza clinic was observed at the end of August, and it followed by the increase of S-OIV patients.

quired in hospital were two nurses and one trainee working in the department of emergency medicine. Two hundred eighteen cases (71.4%) were regarded as sporadic (no contact with a confirmed case within the 7 days before onset of illness). Recent travel history to foreign countries was confirmed in only six patients; two to North America and four to Asian countries.

Fever was the most common symptom, being present in 292 patients (94.5%); 211 patients exhibited fever (≥37.8°C) at the clinic and 81 patients had a history of fever and taking antipyretics before the visit. Other common symptoms included cough (N=226, 73.1%), sore throat (N=161, 52.1%), rhinorrhea (N=156, 50.5%), stuffy nose (N=79, 25.6%), headache (N=22, 7.1%), myalgia (N=10, 3.4%), and gastrointestinal symptoms (N=10, 3.4%). Ten patients (3.2%) required hospitalization and seven of them had clinical and radiologic evidence of pneumonia at presentation. No patients required care in the intensive care unit or experienced respiratory failure. All of the hospitalized patients recovered from the fever on day 2～3 of hospitalization and discharged 3～7 days later.

Table 1. Demographic and clinical features of 309 patients with confirmed swine-origin influenza A (H1N1) virus

Characteristics	Value
	No./total No. (%)
Male	199/309 (64.4%)
Age	
Median	15 years
Range	3 months to 61 years
Age group	No./total No. (%)
0～4 years	17/309 (5.5)
5～9 years	51/309 (16.5)
10～14 years	57/309 (18.4)
15～19 years	132/309 (42.7)
20～29 years	34/309 (11.0)
30～39 years	9/309 (2.9)
40～49 years	1/309 (0.3)
≥50 years	8/309 (2.6)
Underlying isorder	No./total No. (%)
Absent	287/296 (97.0)
Congestive heart failure	4/296 (1.3)
Asthma	3/296 (1.0)
Chronic renal failure	2/296 (0.6)
Intervals from onset of symptom to visit	No./available No. (%)
≤24 hours	44/281 (15.7)
24～48 hours	119/281 (42.3)
48～72 hours	70/281 (22.7)
72～96 hours	33/281 (10.7)
≥96 hours	15/281 (5.3)
Sources of S-OIV infection	No./total No. (%)
School	61/304 (20.1)
Household	15/304 (4.9)
Healthcare	3/304 (1.0)
Workplace	2/304 (0.7)
Outside Korea	5/304 (1.6)
Sporadic	218/304 (70.9)
Clinical symptoms	No./total No. (%)
Fever	292/295 (97.3)
Cough	226/295 (76.6)
Sore throat	161/295 (54.6)
Rhinorrhea	156/295 (52.9)
Stuffy nose	79/295 (26.8)
Headache	22/295 (7.5)
Myalgia	10/295 (3.4)
Diarrhea	6/295 (2.0)
Nausea	4/295 (1.4)
Abdominal pain	3/295 (1.0)

All patients with influenza A antigen positive results (except 1 patient who refused the medication) took oseltimivir at their first visit. Among the 309 S-OIV confirmed patients, 239 patients (77.3%) received oseltamivir at the first visit, 20 patients (6.5%) did at the second visit after the notification of positive PCR results. Fifty patients (16.2%) had no oseltamivir because their symptoms improved and the time passed the oseltamivir-effective period at their second visit.

3. Rapid influenza antigen test

Among the 309 confirmed cases, 294 patients received rapid influenza antigen tests at our clinic and 15 patients at primary-care hospitals. Of the 294 rapid antigen tests conducted at our institution, 130 were positive for influenza A antigen (44.4% of sensitivity). All of the rapid influenza positive cases were confirmed as S-OIV infection by PCR tests (100% of specificity). None of them were seasonal influenza A or false positive. The patients positive for influenza A antigens, compared with those negative for antigens, showed higher frequencies of rhinorrhea (60.8% vs 43.3%, P=0.004) and stuffy nose (33.8% vs 20.1%, P=0.012). For the intervals from onset to antigen tests, the mean times (days) were not significantly different between antigen-positive and -negative groups (1.8±1.4 days vs 1.4±days) (Table 2). However, the antigen positive rates was significantly higher in patients who had tests after 24 hours (11/44, 25.0%) compared to those within 24 hours (116/234, 49.6%, P=0.005) (Table 3). Similar findings were observed between antigen tests after 48 hours (63/115, 54.8%) and within 48 hours (64/148, 39.3%, P=0.015). Other symptoms and patients' age/sex were not significantly different in two groups (Table 2, 3).

DISCUSSION

The present study was undertaken to describe the epidemio-

Table 2. Frequencies of each clinical symptom according to rapid influenza A antigen test results in 204 patients with confirmed swine-origin influenza A (H1N1) patients

	Antigen (+) patients (N=130)	Antigen (−) patients (N=164)	P value
Fever, N (%)	128 (96.9%)	154 (93.9%)	0.260
Cough, N (%)	93 (71.5%)	123 (75.0%)	0.588
Sore throat, N (%)	71 (54.6%)	84 (51.2%)	0.644
Rhinorrhea, N (%)	79 (60.8%)	71 (43.3%)	0.004
Stuffy nose, N (%)	44 (33.8%)	33 (20.1%)	0.012

Table 3. Positive rates of rapid influenza A antigen test according to age and symptom interval in 204 patients with confirmed swine-origin influenza A (H1N1) patients

	No. of patients (N=294)	No. of antigen (+) patients (N=130)	% of antigen (+) patients
Age			
0～4 years	17	9	52.9%
5～9 years	48	19	39.6%
10～19 years	185	81	43.8%
≥20 years	44	21	47.7%
Interval from onset			
≤24 hours	44	11	25.0%
24～48 hours	119	53	44.5%
48～72 hours	69	40	58.0%
≥72 hours	46	23	51.1%

logic, clinical, and laboratory features of S-OIV infection confirmed in a tertiary-care hospital in Seoul, Korea during the accelerating phase of H1N1 outbreak of 2009. As of 20 September 2009, 15,160 Koreans had been diagnosed with S-OIV, according to the Korea Centers for Disease Control and Prevention[4]. Among them, 21% of patients resided in Seoul and 9,235 (60.9%) of the affected were male. The age distribution of the nation-wide data revealed 8,042 patients in 10～19-years-of age (53%), 3,187 patients in 20～29-years-of-age (21%), and 2,320 patients younger than 10-years-of-age (15.3%). Our study showed a similar proportion of male (64.4%, *P*=0.234), but a larger proportions of teens (61.1% in our institution vs 53% in nation-wide, *P*=0.006) and children <10-years-of-age (21.0% in our institution vs 15.3% nation-wide, *P*=0.002). The present age distribution was probably due to favored school-based transmission and population characteristics of local community. In another study about visitors to flu clinic to December 2009, higher number of visits lasted longer in children than in adults in our institution compared to other institutions[5]. The nation-wide data showed increase of incidence up to late October. However, the introduction of vaccination at late October with priority to health-care workers and school-aged children slowed down the spread of influenza, especially the school-based outbreaks[6].

Most of the S-OIV infected patients do not require hospitalization and progress to pneumonia[7]. In our series, fever was a major symptom and most of the patients had one or more acute respiratory symptoms. While diarrhea, nausea, and vomiting were frequent symptoms among U.K. and U.S. patients (about 25%) in previous reports[1,7], ≤5% of patients in our series presented these symptoms. However, Noh et al reported the lower proportion of fever (67.3%) and higher proportions of headache (57.6%), myalgia (65.4%), nausea/vomiting (27.4%), diarrhea (9.3%) in a tertiary-care hospital in Seoul from 2 May 2009 to 31 March 2010[8]. They selected 10% of patients aged more than 15 years randomly. More data needed to be collected through the studies in larger numbers of patients including pediatric patients.

An important observation is that the rapid influenza antigen test used as an initial diagnostic tool showed negative results in 55.6% of our S-OIV-confirmed patients. As in previous reports, the rapid influenza antigen test did not detect half of the S-OIV in our patients[9-13]. However, the 100% specificity in our series and the rarity of seasonal flu during this period allowed the presumptive diagnosis of S-OIV infection based on the positive influenza A antigen results. Early diagnosis of S-OIV by rapid tests made the consequent infection control measures and treatment possible. Nasal symptoms such as a runny or stuffy nose increased the chance of influenza antigen detection by the rapid immunochromatographic assay. In our study, the patients with rhinorrhea showed significantly high antigen positive rates (79/150, 52.7%) than those without rhinorrhea (47/131, 35.9%) (*P*=0.011). The patients with stuffy noses also showed more antigen positive results (44/77, 57.1% vs 82/204, 40.2%, *P*=0.023). This finding suggests that copious secretion is advantageous for the antigen detection. Some investigations reported that more secretions in children increased rates of antigen detection than in adults [12,14]. They also reported that sampling at the early phase of illness improved antigen detection rates[12,14]. In a study in adults, the overall sensitivity of a kind of rapid influenza antigen test kit was 74%, ranging from 86% on day 1 of illness to 50% on the second day and 0% on the fourth day[15]. In our study, the antigen tests within 24～48 hours presented lower sensitivity compared with tests after 24～48 hours. Kim et al reported the higher sensitivity in 24～72 hours than within 24 hours, although it was not statistically significant[16]. It was consistent with our findings and suggested increase of virion in nasal secretions in the 2nd or 3rd days compared with the first day of illnesses. However, Heo et al reported the opposite findings, higher positive rate within 48 hours (81.8%) than after 48 hours (18.2%)[17]. However, the differences by intervals might have been masked because about 95% of our patients visited the clinic within 72 hours. More studies with larger population of broad age distributions and longer intervals need to be performed.

During the study period, no seasonal flu, H1N1, and H3N2 subtypes were detected in our influenza clinic. However, a H3N2 subtype was detected with the rapid influenza A antigen test and multiplex RT-PCR in a patient who visited our influenza clinic in the last week of September. The H3N2 subtype is susceptible to oseltamivir and is associated with higher mortality[18]. However, in many counties including Korea, seasonal influenza A H1N1 subtype is the dominant circulating strain and is virtually 100% oseltamivir-resistant[19]. Surveillance for the emergence of seasonal influenza and identification of its subtype is an important issue, considering these different antiviral susceptibilities. In this regard, multiplex RT-PCR had advantage for the simultaneous detection of pandemic S-OIV and seasonal H1N1 or H3N2 influenza.

Oseltamivir is effective in the treatment of S-OIV when given early in the course of infection (e.g. within 48～72 h after onset of illness)[18]. In our study, 70 S-OIV confirmed patients (22.7%) did not receive oseltamivir at the first visit because they presented

with atypical or mild symptoms, displayed negative influenza A antigen results, had no risk factors, or presented late in the course of their illness. However, all of them were improved without complications. Among the patients who empirically received oseltamivir, 73.5% had no evidence of influenza A virus by laboratory tests. Neuraminidase inhibitors, oseltamivir and zanamivir are less prone to selecting for resistant influenza compared to the amantanes[20]. However, the recent emergence of oseltamivir-resistant S-OIV in patients treated with this drug[21] is a matter of great concern. In fact, a case of oseltamivir-resistant S-OIV infection was reported in a patient who were initially started with oseltamivir for laboratory-confirmed influenza, in Korea[22]. The ongoing global virologic surveillance and monitoring of antiviral resistance are important in influenza pandemics under prophylactic treatment guidelines with oseltamivir[23].

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=국문초록=

신종인플루엔자 A (H1N1)의 유행; 2009년 8월-9월 서울 시내 한 신종인플루엔자 거점병원에서 한 달간의 경험

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배경: 2009년 유행한 swine-origin influenza A (H1N1) virus (S-OIV)에 대해 서울의 한 지정병원에서 인플루엔자 진료소를 운영한 첫 한 달간 관찰한 임상적 양상을 살펴보고자 함이다.

방법: 2009년 8월 21일부터 9월 20일 한달간 서울 동북부에 위치한 한 대학병원의 발열진료소를 내원한 환자 중 S-OIV로 확진된 환자들의 역학적, 임상적, 검사실적 특성을 기술하였다. 독감유사증상으로 내원한 환자들의 비인두 도말 검체를 채취하여 S-OIV 및 계절성 influenza A (H1N1 and H3N2)에 대한 다중 RT-PCR을 실시하였고 일부 환자에서는 influenza A/B 항원에 대한 신속 항원 검사를 실시하였다.

결과: 연구 기간 동안 총 5,322명의 환자가 독감 유사 증상으로 발열진료소를 내원하였다. 이중다중 RT-PCR을 통해 S-OIV로 확진된 환자는 309명이었다. 환자들은 2개월부터 61세의 연령 분포를 보였고, 189명(61.2%)이 10대였다. 81명의 환자가 S-OIV 확진 환자와의 접촉력을 갖고 있었고, 접촉 경로는 학교(N=61), 가족구성원(N=15), 의료기관(N=3), 직장(N=2)이었다. 흔한 증상은 발열(94.5%), 기침(73.1%), 인후통(52.1%), 비루(50.5%)이었다. 위장관 증상을 호소한 환자는 10명(4.9%)이었다. 입원 치료를 필요로 한 환자는 10명(4.9%)이었다. 70명(22.7%)의 환자가 첫번째 내원 당시 oseltamivir를 투약받지 못하였으나 이들 모두 합병증 없이 회복되었다. RT-PCR에 비교하여 신속항원검사는 44.4% (130/294)의 민감도를 보였다. 항원 양성인 환자들은 항원 음성군에 비해 비루(60.8% vs 43.3%, P=0.004)와 코막힘(33.8% vs 20.1%, P=0.012) 증상의 빈도가 더 높았다.

결론: S-OIV 감염 유행의 초기 증가 시기에 주로 학생 연령군에서의 발병 빈도가 높았다. 신속항원검사는 코의 분비물 및 막힘 증상과 관련이 있었다. [대한임상미생물학회지 2010;13:103-108]

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