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Ⅳ．研究成果の刊行物・別刷

Transmission of *Bordetella holmesii* during Pertussis Outbreak, Japan

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We describe the epidemiology of a pertussis outbreak in Japan in 2010–2011 and *Bordetella holmesii* transmission. Six patients were infected; 4 patients were students and a teacher at the same junior high school. Epidemiologic links were found between 5 patients. *B. holmesii* may have been transmitted from person to person.

Bordetella holmesii, a small gram-negative coccoid bacillus that was first reported in 1995 (*1*), was originally identified as a member of the Centers for Disease Control and Prevention nonoxidizer group 2. The organism is associated with bacteremia, endocarditis, and pneumonia, usually in patients with underlying disorders such as asplenia or sickle cell anemia, and has been isolated from blood and sputum samples (*1–5*). *B. holmesii* may also be responsible for causing symptoms similar to those of pertussis (whooping cough) in otherwise healthy patients (*6*). Large surveillance studies in the United States and Canada have shown that the organism was detected in nasopharyngeal swab (NPS) specimens of patients with pertussis-like symptoms (*7,8*). Although humans may be infected with *B. holmesii*, transmission of *B. holmesii* between humans has not yet been fully elucidated.

Pertussis is a highly contagious disease caused by the bacterium *B. pertussis*. The organism is known to be transmitted from person to person by airborne droplets (*9*). During a recent pertussis outbreak, we conducted a laboratory-based active surveillance study and detected 76 suspected cases of pertussis. Among these cases, we identified not only *B. pertussis* infection but also *B. holmesii* infection. The purpose of this study was to describe the epidemiology of the pertussis outbreak and to determine the epidemiologic relatedness of *B. holmesii* transmission.

The Study

During 2010–2011, a pertussis outbreak occurred in a suburban town (town A) in Nobeoka City in Miyazaki Prefecture, Japan. Town A has a population of 4,227 persons and an elementary school and junior high school. The first pertussis case (in a 17-year-old boy) was reported in September 2010. From that time, we conducted a laboratory-based active surveillance study in the area until April 2011, in cooperation with clinics, hospitals, and local health departments. Pertussis-suspected cases were defined as an illness with cough lasting for ≥2 weeks, and pertussis-confirmed cases were defined as the presence of 1 of the following laboratory findings: a culture-positive result for *Bordetella* species from NPS specimens, or a positive result for molecular testing for *Bordetella* species.

For molecular testing, we conducted conventional PCR specific for insertion sequence IS*481*, which is known to detect *B. pertussis* and *B. holmesii*, and *B. pertussis*–specific loop-mediated isothermal amplification assays (*10–12*). To further confirm *B. holmesii* infection, *B. holmesii*–specific real-time PCR specific for the *recA* gene was also performed as described (*8*). For confirmed cases of *B. holmesii* infection, we collected the general information for patients (clinical symptoms, treatment, contact information, and outcome) by face-to-face interview or questionnaire.

During the pertussis outbreak, we identified 76 suspected pertussis cases. Among these suspected cases, 35 cases were confirmed by laboratory testing and involved persons 2–63 years of age (median age 13 years); 1 case occurred in a 2-year-old child, 14 in 6- to 12-year-old children, 14 in 13–15-year-old children, and 6 in persons >16 years of age. Despite pertussis vaccination rates in childhood of 82.3%–92.6%, most (80%) patients were students 6–12 and 13–15 years of age. Among the 35 confirmed case-patients, 29 and 6 patients showed *B. pertussis* and *B. holmesii* infection, respectively. Confirmed cases of *B. holmesii* infection were observed within the last half of the epidemic curve, i.e., weeks 1–12 of 2011 (Figure 1). There were no cases of co-infection with *B. pertussis* and *B. holmesii*.

All NPS specimens from patients with *B. holmesii* infection showed a negative result for the *B. pertussis* loop-mediated isothermal amplification, but showed positive results in the IS*481* PCR and *B. holmesii recA* real-time PCR (Table 1). *B. holmesii*-like organisms were obtained

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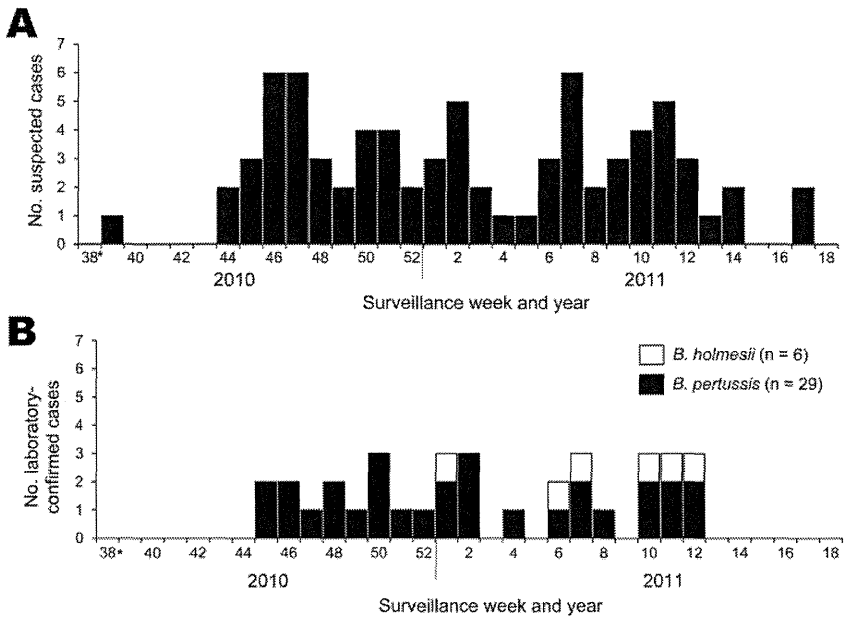

Figure 1. Epidemic curve of a pertussis outbreak, September 2010–April 2011, Japan. A) Suspected cases of pertussis. B) Laboratory-confirmed cases of *Bordetella pertussis* and *B. holmesii* infection. *As of September 20–26, 2010.

from 5 patients, and these were identified as *B. holmesii* by *recA* gene sequencing. Patient 6 had been treated with antimicrobial drugs before the culture test, which probably resulted in a culture-negative test result in this patient. Real-time PCR confirmed that patient 6 had a low *B. holmesii* DNA load (threshold cycle 36.6) in her NPS specimen. All patients experienced paroxysms of coughing without posttussive vomiting, especially at night, and 3 also experienced a whoop (Table 2). Five of 6 patients had a persistent cough lasting >2 weeks. No patients experienced any complications, and they were treated mainly with azithromycin, resulting in complete recovery. None of the patients were immunocompromised.

Our surveillance study showed epidemiologic linkage between 5 patients (patients 2–6), but not for patient 1 (Figure 2). Patient 1, a student in high school A, had the first case of *B. holmesii* infection (probable index case). He lived in a dormitory outside Nobeoka City. However, his family home was in town A in Nobeoka City, and he returned to his home at the end of 2010 and remained there in 2011. Patients 2, 3, and 4 were students at the same junior high school (B) in town A and were close friends. Patient 5 was a teacher at junior high school B and was in charge of patient 4. Patient 6 was a medical practitioner at clinic C, which is 1 of 2 clinics in town A. Patients 1–5 visited clinic C in early January, late February, late February, early March, and late March, 2011, respectively. The duration of illness in patient 1 did not overlap with that of the other patients, whereas that of patients 2–6 clearly overlapped.

Conclusions

In the past 16 years, ≈70 *B. holmesii* clinical strains have been isolated from human patients in several countries, mainly the United States. All reported cases of *B. holmesii* infection have been sporadic occurrences. Thus, the reservoir of *B. holmesii* is currently unknown. Moreover, whether *B. holmesii* is transmitted between humans is not known. In this report, we have demonstrated that 5 patients infected with *B. holmesii* showed epidemiologic linkage. In particular, the fact that 4 of these patients attended the same junior high school suggests that *B. holmesii* may be transmitted from person to person.

Table 1. Characteristics of *Bordetella holmesii* infection in 6 patients during pertussis outbreak, Japan, September 2010–April 2011*

Patient no.	Age, y/sex	Duration of cough, d†	*B. holmesii* test results	
			recA real-time PCR (C_t)‡	Culture§
1	17/M	5	+ (28.7)	+
2	15/F	4	+ (23.4)	+
3	15/F	>14	+ (21.6)	+
4	14/F	8	+ (25.1)	+
5	40/M	8	+ (27.0)	+
6	45/F	15	+ (36.6)	–

*All patients had negative results for *B. pertussis* loop-mediated isothermal amplification and positive results for IS*481* PCR. C_t, cycle threshold; –, negative; +, positive.
†At time of specimen collection.
‡Detection limit was a C_t value of 38.7, corresponding to 100 fg of DNA of *B. holmesii* ATCC51541.
§All strains were isolated from nasopharyngeal swab specimens.

Table 2. Clinical and epidemiologic characteristics for 6 *Bordetella holmesii*–infected patients during pertussis outbreak, Japan, September 2010–April 2011*

Patient no.	Whoop	Duration of cough	Treatment	DTP vaccine status, no. doses	Medical history	Epidemiologic findings
1	+	10 d	AZM	4	Asthma	Student at high school A. His 14-year-old sister, who was given a diagnosis of pertussis, was a student at junior high school B.
2	+	28 d	AZM	4	–	Student at junior high school B. Her 18-year-old brother had similar symptoms, but laboratory test results were negative.
3	–	>4 wk	AZM	4	–	Student at junior high school B. Her close friends began coughing after her disease onset.
4	+	15 d	AZM	4	Chlamydial pneumonia	Student at junior high school B. Her 11-year-old sister was given a diagnosis of pertussis before her disease onset.
5	–	28 d	AZM	UNK	Allergic rhinitis	Teacher at junior high school B in charge of patient 4.
6	–	23 d	AZM, CFPN-PI, GRNX	UNK	Rheumatoid arthritis	Medical staff at clinic C, which was visited by patients 1–5.

*All patients had a paroxysmal cough and coughed at night; none had posttussive vomiting. DTP, diphtheria-tetanus-pertussis; +, positive; AZM, azithromycin; –, negative; UNK, unknown; CFPN-PI, cefcapene pivoxil; GRNX, garenoxacin.

A previous report suggested that *B. holmesii* and *B. pertussis* may co-circulate in young adults (*7*). However, the relationship between pertussis epidemics and *B. holmesii* infection is not fully understood. Our active surveillance study showed that *B. holmesii* infection spread concurrently with the *B. pertussis* epidemic, but that there was no co-infection of *B. holmesii* and *B. pertussis*. Our observations demonstrate that accurate diagnosis is needed to discriminate between *B. holmesii* and *B. pertussis* infections during a pertussis outbreak because symptoms associated with these 2 diseases are similar.

In 2012, a patient with *B. holmesii* infectious pericarditis was reported in Japan (*13*). This is probably the first case report of *B. holmesii* infection in Asia. Previous surveillance studies conducted in the United States and Canada have shown low rates (0.1%–0.3%) of *B. holmesii* infection in patients with cough (*7,8*). However, in a recent study, *B. holmesii* DNA was detected in 20% of NPS specimens collected from patients in France who had been given a diagnosis of *B. pertussis* infection (*14*). These surveillance data indicate that *B. holmesii* infection is present in adolescents and adults, and that the organism is associated with pertussis-like symptoms. However, other causes of viral or bacterial respiratory infection cannot be excluded. Because of lack of specific diagnostic tools to detect bordetellae, *B. holmesii* infection may have been underestimated. Accurate diagnosis and further studies are required to fully elucidate the nature of *B. holmesii* infection.

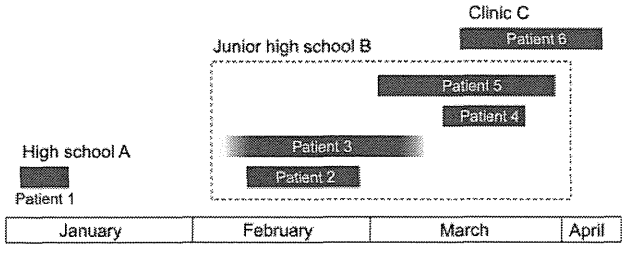

Figure 2. Epidemiologic linkage in 6 patients infected with *Bordetella holmesii* during pertussis outbreak, Japan, 2011. Duration of illness for each patient is shown as a gray box. Patient 3 provided unreliable information about the date of onset and recovery, but the patient's cough lasted for ≥1 month. Epidemiologic linkage was observed between 5 patients (patients 2–6), but not for patient 1.

Acknowledgments

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Dr Kamiya is a pediatrician and medical officer at the National Institute of Infectious Diseases in Tokyo, Japan. His research interests focus on surveillance and control of vaccine-preventable diseases.

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etymologia

Rabies

[ra′bēz]

From the Latin *rabere* (to rage), which may have roots in the Sanskrit *rabhas* (to do violence). Acute progressive fatal encephalomyelitis caused by neurotropic viruses in the genus Lyssavirus—from the Greek *lyssa* (frenzy or madness). In Greek mythology, Lyssa was the goddess of rage, fury, and rabies, known for driving mad the dogs of the hunter Acteon and causing them to kill their master.

Democritus (460–370) described rabies, and Hippocrates is believed to refer to the disease when he said that "persons in a frenzy drink very little, are disturbed and frightened, tremble at the least noise, or are seized with convulsions." According to Aristotle, "Dogs suffer from the madness. This causes them to become irritable and all animals they bite to become diseased." The disease in humans was characterized by hydrophobia, in which the sick person was simultaneously tormented with thirst and fear of water. The Roman writer Cardanus described the saliva from a rabid dog as a *virus*, the Latin word for poison.

Canine rabies has been eliminated in the continental United States. However, dog bites remain a concern for travelers to areas where the disease is enzootic.

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Laboratory of Epidemiology and Communications

Molecular Analysis of Genome of the Pandemic Influenza A(H1N1) 2009 Virus Associated with Fatal Infections in Gunma, Tochigi, Yamagata, and Yamaguchi Prefectures in Japan during the First Pandemic Wave

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In May 2009, a novel swine-origin pandemic influenza A(H1N1) 2009 virus [A(H1N1)pdm09] was first identified in Osaka and Hyogo prefectures of Japan, which then spread to other prefectures in the next several weeks (1). During the summer, pandemic influenza activity remained low; however, it subsequently increased and reached a peak in November 2009 (2). In most cases, infection by A(H1N1)pdm09 caused mild disease, though there have been sporadic cases with severe or fatal outcomes (2). One hundred and ninety-eight fatal cases were reported during the first pandemic wave in Japan (2). Several countries report that an amino acid substitution of aspartic acid by glycine at position 222 (D222G) in hemagglutinin (HA) is associated with disease severity; however, many A(H1N1)pdm09 isolates without this mutation have been identified in severe and fatal cases (3–5). To further explore the molecular determinants of A(H1N1)pdm09 that are associated with severity, we performed whole genome analysis on virus isolates obtained from the fatal cases identified in Gunma, Tochigi, Yamagata, and Yamaguchi prefectures between May 2009 and March 2010.

Nasal swabs were collected from hospitalized influenza patients and sent to the district's prefectural public health institute for diagnosis and viral strain surveillance. Clinical specimens were first inoculated onto Madin-Darby canine kidney (MDCK) cells for virus isolation. Viral RNA was then extracted from the virus culture supernatant using QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, Calif., USA) according to the manufacturer's instructions. Reverse transcription-polymerase chain reaction was carried out using the One Step RNA PCR Kit (AMV) (TaKaRa Bio Inc., Otsu, Japan) and virus-specific primers established by the National Institute of Technology and Evaluation and the National Institute of Infectious Diseases, Japan (6). Amplicons were purified using ExoSAP-IT (USB Corp., Cleveland, Ohio, USA) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA). The sequence data were assembled and analyzed using the Sequencher for Macintosh V4.9 software (Hitachi Software Engineering Co., Tokyo, Japan) and GENETYX-Mac Ver. 13.0.17 (Genetyx Corp., Tokyo, Japan).

In Gunma, Tochigi, Yamagata, and Yamaguchi prefectures, a total of 12 fatal cases were reported to the Ministry of Health, Labour and Welfare of Japan during the first pandemic wave. A(H1N1)pdm09 isolates were successfully obtained from 9 fatal cases; the patients were aged between 4 and 85 years (Table 1). Of these patients, 2 developed pneumonia (Patients 6 and 9) and 1 patient developed multiple organ failure (Patient 3). Patient 8 suffered a comorbidity, namely a subarachnoid hemorrhage. Moreover, 5 patients had underlying diseases: 3 had chronic obstructive pulmonary disease, 1 had multiple myeloma and diabetes mellitus, and 1 had lung cancer. Of the 7 patients taking medication, 4 were taking oseltamivir and 3 zanamivir. In this study, no pandemic influenza-related deaths were reported in pregnant women. For comparison, we obtained 6 isolates from influenza patients presented with mild conditions (Patients 10–15).

Nucleotide sequencing of entire viral genome segments (i.e., polymerase basic 2 [PB2], polymerase basic 1 [PB1], polymerase acidic [PA], HA, nucleoprotein [NP], neuraminidase [NA], matrix [M] protein, and nonstructural [NS] protein genes) was conducted for all 15 viral isolates. The amino acid sequences of viral proteins were deduced from the nucleotide sequences. Table 2 shows the amino acid differences between the virus isolates analyzed in this study and A(H1N1)pdm09 isolates (as consensus sequences) collected worldwide between April 2009 and March 2010. The consensus

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Table 1. Fatal and mild cases of A(H1N1)pdm09 infection and virus isolates in this study

Patient	Sex/age (yr)	Clinical feature	Severity	Medication	Underlying disease	Sampling date	Virus isolate	GenBank accession no.
1	M/85	Influenza	Fatal	Oseltamivir	None	Nov. 25, 2009	A/Gunma/287/2009	AB704443-AB704450
2	M/60	Influenza	Fatal	Zanamivir	Multiple myeloma, diabetes mellitus	Dec. 15, 2009	A/Gunma/293/2009	AB704451-AB704458
3	M/83	Multiple organ failure	Fatal	Oseltamivir	COPD	Oct. 29, 2009	A/Tochigi/350/2009	AB704459-AB704466
4	F/8	Influenza	Fatal	Zanamivir	None	Nov. 24, 2009	A/Tochigi/445/2009	AB704467-AB704474
5	M/56	Influenza	Fatal	None	None	Jan. 13, 2010	A/Tochigi/2/2010	AB704475-AB704482
6	F/62	Pneumonia	Fatal	Oseltamivir	COPD	Nov. 9, 2009	A/Yamagata/473/2009	AB704491-AB704498
7	F/13	Influenza	Fatal	Zanamivir	COPD	Nov. 21, 2009	A/Yamaguchi/217/2009	AB704507-AB704514
8	M/4	Subarachnoid hemorrhage	Fatal	None	None	Dec. 2, 2009	A/Yamaguchi/247/2009	AB704515-AB704522
9	M/60	Pneumonia	Fatal	Oseltamivir	Lung cancer	Dec. 5, 2009	A/Yamaguchi/248/2009	AB704523-AB704530
10	M/10	Influenza	Mild	None	None	Nov. 19, 2009	A/Gunma/262/2009	AB704419-AB704426
11	M/9	Influenza	Mild	Zanamivir	None	Nov. 16, 2009	A/Gunma/263/2009	AB704427-AB704434
12	M/4	Influenza	Mild	Oseltamivir	None	Nov. 25, 2009	A/Gunma/267/2009	AB704435-AB704442
13	M/56	Influenza	Mild	Unknown	None	Jan. 12, 2010	A/Tochigi/10/2010	AB704483-AB704490
14	F/50	Influenza	Mild	None	None	Nov. 27, 2009	A/Yamagata/674/2009	AB704499-AB704506
15	M/7	Influenza	Mild	Oseltamivir	None	Dec. 18, 2009	A/Yamaguchi/273/2009	AB704531-AB704538

COPD, chronic obstructive pulmonary disease.

sequences of each segment consist of 2728 (PB2), 2312 (PB1), 2366 (PA), 4733 (HA), 2440 (NP), 4401 (NA), 3449 (MP), and 2376 (NS) nucleotide sequences, which were downloaded from the Global Initiative on Sharing All Influenza Data EpiFlu database (http://platform.gisaid.org/epi3/frontend#34c9cf). Amino acid substitutions, namely S203T in HA, V100I in NP, V106I and N248D in NA, and I123V in NS1, which are known as specific markers for cluster 2, were present in all the analyzed sequences, indicating that these virus isolates belonged to the cluster containing a large majority of circulating A(H1N1)pdm09 strains in Japan, during the peak phase of the pandemic (7). There was no reassortment with other seasonal (either H1N1 or H3N2), swine, or avian influenza A viruses. A total of 39 unique amino acid differences were found in 9 isolates obtained from fatal cases: 5 in PB2, 4 in PB1, 9 in PA, 6 in HA, 2 in NP, 6 in NA, 3 in M2, 2 in NS1, and 2 in nuclear export protein (NEP). Of these differences, only V19I in HA was common to 2 of the isolates (i.e., A/Yamaguchi/217/2009 and A/Yamaguchi/248/2009). A marker for oseltamivir resistance, H275Y in NA, was identified in A/Yamaguchi/248/2009 that was derived from an oseltamivir-treated patient. Frequently observed changes in the fatal cases, defined as more than 3 out of 9 isolates, were commonly observed in mild cases (i.e., T257A, I435V, and N537S in PB1; S69L, D274N, and E374K in HA; and G41E in NA), suggesting that these amino acid substitutions are unlikely to be associated with the level of severity.

The amino acid location at position 222 in the receptor binding site of HA predicts that alterations to this position would influence the binding specificity of viruses. Previous studies have reported that a D222G substitution confers enhanced binding to α2,3-linked (avian-like) rather than α2,6-linked (human-like) sialic acids, suggesting an augmented ability to bind to lung cells in the lower respiratory tract in humans and cause an exacerbation of the disease (3). No D222G substitution in the HA was observed in any of the isolates analyzed in this study. Alternatively, a D222E substitution was found in 1 of the isolates, A/Yamaguchi/247/2009, which was derived from a fatal infection. This substitution, however, seems to be unrelated to the disease severity of the A(H1N1)pdm09 as previously reported (4,8). Another V132E amino acid mutation in the receptor binding site was found in 2 isolates, a fatal case-strain (i.e., A/Tochigi/2/2010) and a mild case-strain (i.e., A/Yamaguchi/273/2009); however, the precise impact of this mutation is unclear. A mixed population of viruses possessing 163K/E in an antigenic site was found in the isolate A/Yamaguchi/217/2009, which was also derived from a fatal infection. However, the antigenicity of this isolate was similar to that of the vaccine strain, A/California/07/2009 (data not shown).

Virus isolates derived from patients with fatal infection manifested sporadic amino acid changes in the PB2 and PA proteins more frequently than those derived from patients with mild infections (Table 2). Notably, 6 of the 9 isolates from fatal cases had 1 or 2 amino acid substitutions in the PA, e.g., E2K, A70V, P325L, V387I, S405A, V432F, L589I, S594G, and A598P. The RNA-dependent RNA polymerase of influenza viruses is a complex of 3 viral proteins, PB1, PB2, and PA, and

Table 2. Amino acid differences in the viral proteins of A(H1N1)pdm09 isolates obtained from fatal and mild cases

Virus isolate[1)]	Viral protein[2)]																																	
	PB2									PB1										PA														
	121	251	368	495	588	616	660	673	682	36	76	94	257	383	393	435	537	609	652	2	70	224	325	387	405	432	578	589	594	598				
Consensus	K	R	R	V	T	I	K	G	G	T	D	F	T	E	R	I	N	V	A	E	A	S	P	V	S	V	G	L	S	A				
A/Gunma/287/2009					I				R			L														A			I						
A/Gunma/293/2009							R	R	R				A																	P					
A/Tochigi/350/2009																V	S																	
A/Tochigi/445/2009																V	S																	
A/Tochigi/2/2010							R						A							K					F									
A/Yamagata/473/2009			K													V	S																	
A/Yamaguchi/217/2009				I																			L											
A/Yamaguchi/247/2009	R													D					V					I										
A/Yamaguchi/248/2009		K											A			V		A			V							G						
A/Gunma/262/2009							R																											
A/Gunma/263/2009																V	S																	
A/Gunma/267/2009								R	R		N				T	V	S																	
A/Tochigi/10/2010							R			M																								
A/Yamagata/674/2009							R																											
A/Yamaguchi/273/2009						V	R						A														S							
A/California/07/2009																						*P*												

Virus isolate	Viral protein																								
	HA																NP								
	19	69	83	132	163	171	197	203	222	258	274	297	304	321	339	374	393	31	100	119	297	363			
Consensus	V	S	S	V	K	K	A	T	D	E	D	P	P	V	G	E	V	R	I	V	Y	V			
A/Gunma/287/2009													S		R						H	I			
A/Gunma/293/2009																K									
A/Tochigi/350/2009		L									N					K									
A/Tochigi/445/2009		L									N					K									
A/Tochigi/2/2010				E												K									
A/Yamagata/473/2009		L									N					K									
A/Yamaguchi/217/2009	I				K/E																				
A/Yamaguchi/247/2009									E			S					I								
A/Yamaguchi/248/2009	I																								
A/Gunma/262/2009						R	T													I					
A/Gunma/263/2009		L									N					K									
A/Gunma/267/2009		L								V	N					K									
A/Tochigi/10/2010						R	T								R										
A/Yamagata/674/2009						R	T											K							
A/Yamaguchi/273/2009				E												K									
A/California/07/2009			*P*					*S*						*I*					*V*						

Table 2. Continued

Virus isolate	Viral protein																														
	NA																M1	M2				NS1							NEP		
	41	62	76	82	106	110	140	221	248	275	351	381	382	416	465		64	39	50	51	63	2	64	93	100	112	122	123		2	89	105
Consensus	G	V	A	S	I	S	L	N	D	H	F	T	G	D	F		F	I	C	I	P	D	I	M	D	I	A	V		D	A	L
A/Gunma/287/2009						C												V	Y		A		T								
A/Gunma/293/2009																								I							
A/Tochigi/350/2009	E																														
A/Tochigi/445/2009	E							S																							
A/Tochigi/2/2010																								I							
A/Yamagata/473/2009	E																														I
A/Yamaguchi/217/2009																														V	
A/Yamaguchi/247/2009												N			L												T				
A/Yamaguchi/248/2009				P						Y																					
A/Gunma/262/2009		I					I						E	N			Y														
A/Gunma/263/2009	E																														
A/Gunma/267/2009	E																														
A/Tochigi/10/2010													E	N			Y					G			N				G		
A/Yamagata/674/2009													E	N			Y			T						M					
A/Yamaguchi/273/2009			T																					I							
A/California/07/2009					*V*				*N*		*Y*																	*I*				

[1]: Virus isolates obtained from fatal cases and their amino acid substitutions are indicated with a bold font. The vaccine strain for the 2009–2010 influenza season and its amino acid substitutions are indicated in an italicized font.

[2]: PB2, polymerase basic protein 2; PB1, polymerase basic protein 1; PA, polymerase acidic protein; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M1, matrix protein 1; M2, matrix protein 2; NS1, nonstructural protein 1; NEP, nuclear export protein. Fatal case-specific substitutions are indicated with opened boxes. Positions of antigenic and receptor binding sites in the HA are indicated with gray and dotted backgrounds, respectively.

plays a key role in viral growth within the mammalian host cells. Recent studies demonstrated that mutations within PB2 or PA lead to increased pathogenicity of the A(H1N1)pdm09 in mice (9-11). A limitation of this study is the relatively small size of the analyzed data set. In addition, the pathogenetic role of the observed viral mutations remains to be elucidated.

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Conflict of interest None to declare.

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Original Article

The post-infection outcomes of influenza and acute respiratory infection in patients above 50 years of age in Japan: an observational study

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Objectives Influenza can be a serious illness, especially for older people, and reducing the impact of influenza in elderly is important. The objective of this study was to estimate the prevalence and postinfection outcomes of influenza among the over-50 population in Japan.

Design An observational study was designed to ascertain the proportion of influenza cases in a population aged ≥50 years with acute respiratory infection (ARI) and to determine the postinfection outcomes of their illness during the 2008–09 influenza season in Japan. Respiratory specimens obtained from a total of 401 patients were tested by PCR for influenza viruses, respiratory syncytial virus (RSV) and human metapneumovirus (hMPV). The effectiveness of the seasonal trivalent influenza vaccine was estimated by a test-negative case control analysis.

Setting Seventeen outpatient clinics located in four separate areas of Japan.

Sample Respiratory swab specimens from the ARI patients aged ≥50 years.

Main outcome measures Laboratory confirmed influenza in patients presenting with ARI.

Results In all, 89 (22.2%) of the patients were positive for one of the tested viruses; 70 (78.7%) with influenza, 17 (19.1%) with RSV, and 2 (2.2%) with hMPV. Cough (95.7% vs 73.4%), loss of appetite (67.1% vs 35.5%), absence from work (50.0% vs 23.0%), impact on daily activity (90.0% vs 62.5%), and caregiver absence from work (5.7% vs 0.6%) were observed higher in influenza patients. The duration of feeling weakness (6.3 ± 5.4 vs 3.6 ± 1.9 days) and average days of reduced activity (5.2 vs 3.6 days) were longer for influenza patients. Vaccine effectiveness was estimated to be 32.1% (95% CI: −14.9, 59.9%).

Conclusions Influenza was the dominant ARI-causing virus and the clinical and socio-economic outcomes imposed on patients over 50 years of age was high for influenza.

Keywords Acute respiratory infection, human metapneumovirus, influenza, influenza-like illness, post-infection outcomes, respiratory syncytial virus, test-negative case–control, vaccine effectiveness.

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Introduction

Seasonal influenza is an important cause of morbidity and mortality.[1] Influenza viruses have the potential to cause not only epidemics but also occasional pandemics. While influenza viruses remain of high scientific interest, studies based on clinical features without laboratory confirmation of diagnoses may lack reliability because of the similarity and overlap of influenza symptoms with those of other etiological agents that can give rise to acute respiratory infections (ARIs).

Older people are at greater risk from complications arising from influenza than younger adults.[2] In the United States, the elderly account for a significant number of influenza-associated hospitalizations.[3] In Japan, a study of excess mortality associated with influenza epidemics across all ages during the period 1987–2005 estimated that 85–90% occurred among persons aged 65 and above.[4] With declining birth and death rates, Japan already has one of the highest rates of aging globally,[5] and it is projected that by 2050 over 30% of the Japanese population aged 60 or older will be 80 or above.[6] Recently, treatment

for influenza with neuraminidase inhibitors has become popular in Japan, and they are prescribed very often even for mild cases of influenza. However, there have been no studies addressing the post-infection outcomes of laboratory-confirmed seasonal influenza on those aged 50 and above under such special circumstance. We therefore conducted an observational study using a population of patients with ARI in this age group from a primary care setting. The primary objective was to determine the proportion of ARI cases that were affected by influenza among the study population during the 2008–2009 influenza season. Secondary objectives were to ascertain both the clinical and socioeconomic post-infection outcomes imposed on the subjects during their influenza episode. We also carried out a test-negative case–control analysis to assess the effectiveness of the trivalent influenza vaccine (TIV) in preventing influenza in this age group.

Methods

The observational study was conducted in compliance with Good Clinical Practice (GCP) guidelines and the Japanese Ministry of Health, Labor and Welfare's (MHLW) ethical guidelines for epidemiological research. A central Institutional Review Board (IRB) provided ethics review and approval. Seventeen outpatient clinics located in four separate areas of Japan, Fukuoka, Ishikawa, Gifu and Tokushima prefectures participated in the study. The recruitment period was from November 2008 to May 2009. Patients aged 50 years or over presenting with ARI were enrolled following informed consent. The inclusion criteria were a temperature of 37·5°C or more and/or feverishness at least one of the following respiratory symptoms: coryza and/or nasal congestion, cough, and sore throat (criteria adapted from the ARI subsection of 'influenza case definitions' specified by the European Centre for Disease Prevention and Control[7]).

Medical histories were collected to obtain baseline data on demographics, underlying medical conditions (pneumonia, chronic obstructive lung disease, asthma, immunocompromised, diabetes, dialysis, arteriosclerosis, coronary artery disease, cardiac failure, cerebrovascular disease, regular smoking), and whether the patient had received a TIV vaccination prior to the 2008–2009 influenza season. Clinical symptoms and medical histories were recorded by the physician in clinical interview and through the subjects' medical record. Participants were requested to keep a daily record of body temperature, clinical symptoms, medication taken and information on the socioeconomic outcomes of their ARI episode, such as reduced activity and workdays lost by themselves or their care givers. Follow-up contact was made by telephone 12–21 days after the initial visit, when participants were asked to complete a questionnaire while referring to their diary as a memory aid. Interviewers were blinded to the laboratory test results of the subjects. All subjects participating in the study were accounted for and followed up.

Two respiratory swab specimens were taken, one for rapid testing as part of the clinical diagnosis and the other for subsequent laboratory testing. Specimens for laboratory testing were collected and sent to the central laboratory at Hara-doi Hospital by designated courier. All clinical specimens were labeled, handled, analyzed, and stored in accordance with GCP and JNIPH guidelines and standard operating procedures (SOPs). Agreement between rapid diagnosis kit and laboratory confirmation was analyzed to assess any misclassification of disease.

Clinical samples were tested for influenza, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) by reverse transcription polymerase chain reaction (RT-PCR). The PCR mixture comprised 7·5 μl nuclease-free water, 6·2 μl GoTaq® Green Master Mix, 2X (Promega KK, Promega Corp., Madison, WI, USA), 0·15 μl forward primer, 0·15 μl reverse primer, and 1·0 μl template. Detection and subtyping of influenza type A (H1N1 and H3N2) and type B viruses were carried out as described by Stockton *et al.*[8] Amplification conditions consisted of initial denaturation at 94°C for 10 seconds followed by 94°C for 5 seconds, 53°C for 20 seconds, and 72°C for 20 seconds applied for 32 cycles in the first round and 28 cycles in the second round, and final extension at 72°C for 10 seconds.

Infections by RSV were detected as described by Falsey *et al.*[9] Amplification conditions consisted of initial denaturation at 95°C for 10 seconds followed by 95°C for 5 seconds, 42°C for 20 seconds, and 72°C for 20 seconds applied for 32 cycles in the first round and 28 cycles in the second round, and final extension at 72°C for 10 seconds. Infections by hMPV were detected as described by Peret *et al.*[10] Amplification conditions consisted of initial denaturation at 94°C for 10 seconds followed by 94°C for 5 seconds, 55°C for 20 seconds, and 72°C for 20 seconds applied for 40 cycles in the first round and 40 cycles in the second round, and final extension at 72°C for 10 seconds.

The statistical software used were sas 8.2 (or later versions) and STATA/SE 10. Standard parametric techniques employed for statistical analysis (including comparison of baseline characteristics) were χ^2, Fisher's exact and Student's *t*-tests. With the power of the study set at 80% and significance level at 5%, the minimum sample size was estimated to be 500.

To evaluate crude estimates of the effectiveness of the seasonal vaccine in preventing influenza, we conducted a test-negative case–control analysis using the data obtained for the study population of patients with ARI who were infected with one of the three influenza viruses contained in the seasonal TIV (A/H1N1, A/H3N2, and B). The

controls were patients with ARI who tested influenza negative. Participants were considered vaccinated if they had received the TIV at least 14 days before presenting with ARI.

Results

A total of 401 patients were enrolled into the study, 233 (58·1%) women and 168 (41·9%) men. A comparison of detailed baseline characteristics of the study population grouped by influenza diagnosis revealed that baseline characteristics of the influenza and non-influenza groups were similar in terms of age, gender, and vaccination status (Table 1). The two groups were also similar with respect to the presence of an underlying medical condition (45·7% and 43·2%, respectively). The medical conditions did not differ significantly between the two groups (data not shown).

In all, 89 (22·2%) of the 401 study participants were found to be infected with one of the tested viruses: 70 (78·7%) with influenza, 17 (19·1%) with RSV, and 2 (2·2%) with hMPV. According to the assessment of possible misclassification of disease between the rapid diagnosis kit and laboratory confirmation, specificity, sensitivity, positive predictive value, and negative predictive value were 93·0%, 78·6%, 70·5%, and 95·3%, respectively. Only laboratory-confirmed influenza was categorized as influenza positive for further analyses. Among the 70 patients positive for influenza, H1N1, H3N2, and B were detected in 33 (47·1%), 33 (47·1%), and 4 (5·7%) cases, respectively. Influenza was diagnosed in 70 (17·5%) of the 401 participants, of whom 28 (40·0%) had received the seasonal vaccination, while 42 (60·0%) had not. Among the 70 patients positive for influenza, neuraminidase inhibitors were prescribed at the initial visit to 50 (71·4%), of whom 23 (46·0%) had been vaccinated, but 27 (54·0%) had not. Among the 331 influenza-negative patients, 164 (49·5%) had been vaccinated and 167 (50·5%) had not (Table 1).

Table 1. Characteristics of Japanese patients with ARI in 2008–2009 by influenza infection status

Characteristics	Influenza (+)* (n = 70)	Influenza (–)** (n = 331)	Difference (P-value)
Age			
Average	63·1	65·0	0·330
Range	50–84	50–95	
50–64 years	43 (61·4%)	171 (51·7%)	
65–74 years	18 (25·7%)	107 (32·3%)	
≥75 years	9 (12·9%)	53 (16·0%)	
Gender			
Males	29 (41·4%)	139 (42·0%)	0·931
Females	41 (58·6%)	192 (58·0%)	
Underlying medical condition			
Yes	32 (45·7%)	143 (43·2%)	0·700
No	38 (54·3%)	188 (56·8%)	
Vaccinated for influenza 2008–2009 season			
Vaccinated	28 (40·0%)	164 (49·5%)	0·146
Unvaccinated	42 (60·0%)	167 (50·5%)	

ARI, acute respiratory infection.
*Influenza positive.
**Influenza negative.

Significant differences were observed between influenza and non-influenza cases with regard to both clinical symptoms and socioeconomic post-infection outcomes (Table 2). The prevalence of cough, headache, loss of appetite, and both the feeling and duration of weakness were significantly higher among influenza-positive patients: Cough was observed in 95·7% and 73·4% ($P < 0·01$), headache in 64·3% and 49·5% ($P = 0·03$), loss of appetite in 67·1% and 35·5% ($P < 0·01$), feeling of weakness in 32·9% and 20·5% ($P = 0·03$), of influenza positive and negative, respectively. The duration of feeling weakness was 6·3 ± 5·4 days for positive and 3·6 ± 1·9 days ($P < 0·01$) for negative participants. Although there was no significant difference, it is particularly worth noting that the total duration of illness was more than 2 weeks in influenza-positive patients probably due to the vulnerability of the study population of this age. In terms of the socioeconomic outcomes of their ARI episode, absence from work, impact on daily activity, and caregiver absence from work were all reported to be significantly higher for those with influenza: Absence from work were observed in 50·0% and 23·0% ($P < 0·01$), impact on daily activity in 90·0% and 62·5% ($P < 0·01$), caregiver absence from work in 5·7% and 0·6% ($P = 0·010$) of influenza positive and negative, respectively (Table 2). Average days of absence was 3·1 days and 2·2 days ($P = 0·026$), and days of reduced activity was 5·2 days and 3·6 days ($P < 0·001$) for influenza-positive and influenza-negative participants. When the clinical symptoms and socioeconomic outcomes of only the influenza-positive cases were compared between those vaccinated or unvaccinated in the 2008–2009 season, there were no significant differences for any of the clinical or socioeconomic parameters (Table 3).

The test-negative case–control analysis of vaccine effectiveness indicated that the 2008–2009 seasonal influenza vaccination was 32·1% (−14·9, 59·9%) effective in preventing influenza in the study population overall. We were unable to determine age-specific vaccine effectiveness because of the small number of influenza cases in each age group.

Discussion

This is the first study in Japan to quantify the post-infection outcomes of seasonal influenza confirmed by labora-

Table 2. Clinical symptoms and socioeconomic outcomes in 401 patients with ARI in Japan 2008–2009 by laboratory confirmation

	Influenza (+) (n = 70)*	Influenza (–) (n = 331)**	OR (95%CI)	P-value
Clinical symptoms				
Coryza and/or nasal congestion	53 (75·7%)	235 (71·0%)	1·27 (0·70–2·31)	0·43
Cough	67 (95·7%)	243 (73·4%)	8·09 (2·48–26·37)	<0·01
Headache	45 (64·3%)	164 (49·5%)	1·83 (1·07–3·13)	0·03
Loss of appetite	47 (67·1%)	117 (35·5%)	3·74 (2·16–6·46)	<0·01
Myalgia	42 (60·0%)	160 (48·3%)	1·60 (0·95–2·71)	0·08
Sore throat	50 (32·9%)	256 (77·3%)	0·73 (0·41–1·31)	0·29
Feeling of weakness	23 (32·9%)	68 (20·5%)	1·89 (1·08–3·33)	0·03
Duration of feeling of weakness (days)	6·3 ± 5·4	3·6 ± 1·9	2·09 (1·14–3·83)	<0·01
Total duration of illness (days)	19·0 ± 3·4	18·4 ± 5·7	1·41 (0·64–3·12)	0·39
Socioeconomic outcomes				
Absence from work	35 (50·0%)	76 (23·0%)	3·36 (1·97–5·72)	<0·01
Days absent (average)	3·1	2·2	1·62 (1·04–2·54)	0·026
Impact on daily activity	63 (90·0%)	207 (62·5%)	5·93 (2·39–12·14)	<0·01
Days of reduced activity	4	3	–	–
Days of reduced activity	5·2	3·6	1·51 (1·18–1·93)	0·001
Caregiver absence from work	4 (5·7%)	2 (0·6%)	9·97 (1·79–55·55)	0·010
Days absent (average)	3·5	2	3·15 (0·16–63·12)	0·492

ARI, acute respiratory infection.
*Influenza positive.
**Influenza negative.

tory testing of clinical respiratory specimens in a cohort of patients with ARI aged 50 and above. Because the symptoms of influenza are similar to those arising from other viral respiratory pathogens, diagnostic respiratory samples obtained from each participant were laboratory-confirmed using RT-PCR, which is highly sensitive and specific for detecting influenza viruses, RSV, and hMPV.[8,9] The latter two commonly cocirculate with influenza in winter months and give rise to similar symptoms, which can be severe in the elderly.[2,9]

The results showed that in the study population of 401 patients with ARI, 70 (17·5%) were influenza positive. Among the respiratory viruses tested for and identified, influenza was dominant (almost 80·0%), which agrees with previous reports relating to the elderly.[2,11] In our study, influenza viruses were four times more prevalent than RSV and 35 times more common than hMPV. The features may vary by studied season or year. Because the study was conducted in the flu season, a possible over-representation of influenza prevalence cannot be eliminated. There is a lack of reliable comparative data in Japan on the prevalence of laboratory-confirmed influenza among ARI cases, but studies conducted elsewhere[12,13] support our finding that influenza is the predominant ARI-causing viral pathogen among the viruses tested for.

Our study demonstrated that influenza-positive patients suffered more severe outcomes in terms of clinical symptoms than patients with ARI who were influenza negative including those who may have been infected with other viruses and those with no pathogen detected. In particular, the prevalence of cough, headache, loss of appetite, feeling of weakness, and duration of weakness was all significantly greater for those with laboratory-confirmed influenza, even though anti-influenza drug was prescribed to 50 of the 70 influenza-positive cases (71·4%). Previous studies have also reported that elderly influenza patients suffered from longer-lasting coughs[14] and that weakness was a common symptom of influenza.[15] The socioeconomic outcomes on influenza-positive patients was also significantly greater than for the non-influenza patients with ARI in terms of impact on daily activity and the absence from work by both the patient and caregiver. It is particularly noteworthy that half of the influenza patients reported being absent from work (with a median duration of 3 days), whereas less than a quarter of the non-influenza ARI patients reported work days lost (median duration 2 days). In Japan, the majority of elderly people continue to do some form of work. Sometimes full-time worker, but part-time jobs and volunteering are regarded as work. The number of days of absenteeism of other persons who provided patient care during the follow-up period was analyzed because it is quite common for people around patients to care for the patient because of the limited availability of home nursing service in Japan. Patient absenteeism and the need for care-

Table 3. Clinical symptoms and socioeconomic outcomes of influenza-positive vaccinated and unvaccinated patients in Japan 2008–2009

	Vaccinated (*n* = 28)	Unvaccinated (*n* = 42)	OR (95% CI)	*P*-value
Clinical symptoms				
Coryza and/or nasal congestion	23 (82·1%)	30 (71·4%)	1·84 (0·57–5·96)	0·31
Cough	26 (92·9%)	41 (97·6%)	0·32 (0·03–3·68)	0·56
Headache	17 (60·7%)	28 (66·7%)	0·77 (0·29–2·09)	0·61
Loss of appetite	19 (67·9%)	28 (66·7%)	1·06 (0·38–2·93)	0·92
Myalgia	15 (53·6%)	27 (64·3%)	0·64 (0·24–1·70)	0·37
Sore throat	18 (64·3%)	32 (76·2%)	0·56 (0·20–1·61)	0·28
Feeling of weakness	10 (35·7%)	13 (31·0%)	1·24 (0·45–3·41)	0·68
Duration of feeling of weakness (days)	8·1 ± 6·7	5·2 ± 4·4	1·62 (0·69–3·82)	0·27
Total duration of illness (days)	18·6 ± 3·2	19·3 ± 3·6	0·32 (0·02–4·60)	0·41
Socioeconomic outcomes				
Absence from work	14 (50·0%)	21 (50·0%)	1·00 (0·38–2·60)	1·000
Days absent (average)	3·3	2·9	1·49 (0·45–4·94)	0·52
Impact on daily activity	24 (85·7%)	39 (92·9%)	0·46 (0·09–2·24)	0·43
Days of reduced activity (average)	5·5	4·9	1·15 (0·69–1·94)	0·59
Caregiver absence from work	2 (7·1%)	2 (4·8%)	1·54 (0·20–11·61)	1·00
Days absent (average)	1·5	5·5	–	0·13

givers to also take time off work appears to have also been substantially higher in the influenza-positive group. The burden of reduced daily activity would be reflected in diminished performance – with its consequent economic impact and loss of productivity, as well as intruding on the quality of life of both patients and their care givers.

Comparison of the clinical symptoms and socioeconomic criteria between vaccinated and unvaccinated influenza-positive participants revealed no significant differences. In addition, the proportion of vaccinated and unvaccinated patients was similar among those who had antiviral prescriptions (23/50 versus 27/50, respectively). Among this group of above 50 years of age, the seasonal vaccine appeared to have only a limited mitigating effect regarding clinical symptoms and reducing the socioeconomic outcomes of influenza.

To assess the effectiveness of the 2008–2009 TIV seasonal vaccine, we employed a test-negative case–control analysis in which the control group consisted of all the ARI participants who tested negative for influenza. Such a design is relatively easy to implement, controls better for bias related to healthcare service utilization than the traditional case–control method and has been shown to provide accurate estimates of vaccine effectiveness.[16] The findings on the effectiveness of TIV in preventing influenza in this age group were inconclusive [32·1% (−14·9, 59·9%)]. However, this study was not primarily designed to study vaccine effectiveness, and these results should be interpreted with caution given the limited power and potential bias including lack of control for confounding. Nevertheless, the degree of effectiveness is broadly comparable with the results of large-scale cohort studies carried out annually by the Japan Physicians Association, which indicate that classical influenza vaccination of the over 65s has generally provided only moderate benefit to this age group.[17] In the United States, the Centers for Disease Control estimates that vaccination of the elderly can be 30–70% effective.[18] Although this refers to the prevention of hospitalization rather than the disease itself, our figure of 32·1% lies at the lower end of this range. Vaccine effectiveness in any given year is influenced by how well the strains used in the seasonal vaccine antigenically match those circulating in the community.[19] In 2008–2009, the vaccine and circulating A/H1N1 subtype matched (A/Brisbane/59/2007); the A/H3N2 subtype matched in the first part of the season (A/Uruguay/716/2007), but there was a mismatch from March when A/Perth/16/2009 began circulating. In the case of type B, there was also a mismatch between the vaccine strain and the dominant circulating strain.[20]

Seasonal influenza vaccination is recommended for the elderly in Japan[21] and is available at a subsidized cost,[22] and the benefits are mostly seen in factors such as reduced hospitalization, fewer complications, and lower mortality.[2,23,24] However, the evidence for seasonal vaccination in reducing the incidence of influenza in this age group remains weak,[25] indicating a need for improved vaccines.

The study had some limitations. First, although the participants were living in the community, they were recruited solely from among clinic attendees and may therefore not be representative of the Japanese over-50

population as a whole in terms of their general health. In fact, baseline data showed that nearly half of the subjects (43·6%) had an underlying medical condition, even though more than half were aged under 65. Second, our estimate of TIV effectiveness based on a test-negative case–control analysis needs to be treated with some caution because of possible overestimation[26] and lack of adjustment for confounders and limited sample size. Nevertheless, baseline comparison of the influenza-positive and influenza-negative groups showed that overall the two groups were very similar. Furthermore, information/recall bias was minimized by ensuring that participants were able to keep track of the study variables by means of the prospective diary they were provided.

In summary, these results indicate that influenza is an important cause of ARI leading to higher socioeconomic outcomes and more severe symptoms than other viral ARI. Although clinical effectiveness of neuraminidase inhibitors has been reported, burden of influenza is significant. Current TIV vaccines may not offer an effective prevention against influenza in the elderly population. Continuing research and development toward improving influenza vaccines may play a vital part in reducing the clinical and socioeconomic outcomes of the influenza illness for a growing older population.

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Conflicts of interest

HI has received funding from Daiichi Sankyo, Toyama Chemical, GSK, Taisho Toyama Pharmaceutical Company, Chugai Pharmaceutical Company for his scientific researches, expert testimony, lectures and travel for academic presentation. Authors YT and MR are employed by GlaxoSmithKline. YT owns stock in GSK.

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Original Article

Increased symptom severity but unchanged neuraminidase inhibitor effectiveness for A(H1N1)pdm09 in the 2010–2011 season: comparison with the previous season and with seasonal A(H3N2) and B

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Background No studies of the clinical symptoms before starting therapy or of the effectiveness of neuraminidase inhibitors (NAIs) have been carried out of the 2009–2010 and 2010–2011 seasons that compare A(H1N1)pdm09 or the three circulating types of influenza virus.

Methods The clinical symptoms and duration of fever (body temperature ≥37·5°C) after the first dose of an NAI (oseltamivir, zanamivir, laninamivir) were analyzed. PCR was carried out for 365 patients with A(H1N1)pdm09 in the 2009–2010 season and for 388 patients with one of the three types of influenza circulating in the 2010–2011 season. IC_{50} for the three NAIs was also analyzed in 51 patients in the 2010–2011 season.

Results The peak body temperature was significantly higher in 2010–2011 than in 2009–2010 for patients under 20 years with A(H1N1)pdm09, and in the 2010–2011 season for children 15 years or younger with A(H1N1)pdm09 than for those with other virus types. The percentage of A(H1N1)pdm09 patients with loss of appetite or fatigue was significantly higher in 2010–2011 than in the previous season. The duration of fever was not affected by the kind of NAI or by age in multiple regression analysis. The percentage of patients afebrile at 48 hours after the first dose of NAI was significantly higher for A(H1N1)pdm09 than for A(H3N2) (laninamivir) or B (oseltamivir and laninamivir).

Conclusion Although the clinical symptoms of A(H1N1)pdm09 were slightly more severe in the 2010–2011 season, the effectiveness of the NAIs remained high in comparison with 2009–2010 and with other types of seasonal influenza.

Keywords A(H1N1)pdm09, clinical symptom, IC_{50}, laninamivir, neuraminidase inhibitor.

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Introduction

Influenza A(H1N1)pdm09 was highly prevalent in the 2009–2010 season, with few cases of A(H3N2) or B reported.[1,2] However, all three subtypes (types) spread widely and almost simultaneously in the 2010–2011 winter season.[1,3,4] Little study has been carried out of the differences in the clinical symptoms or the effectiveness of neuraminidase inhibitors (NAIs) between these two seasons for A(H1N1)pdm09 viruses or among the three influenza subtypes. A(H1N1)pdm09 was reported mainly in the autumn (mostly September–December) of the 2009–2010 season, but prevailed in winter (mostly January–March) in the 2010–2011 season, which is similar to the usual influenza season in Japan.[1] Therefore, we thought it would be interesting to determine how the clinical features of A(H1N1)pdm09 might have differed between these two seasons.

We have reported the usefulness of neuraminidase inhibitors (NAIs) almost annually[5–11] and have shown reduced effectiveness of oseltamivir in the 2008–2009 season, when the oseltamivir-resistant (H275Y NA mutation) A(H1N1) viruses were highly prevalent, compared with the previous