軸とするカプランマイヤー曲線を作成し、ログランク検定（log-rank test）を用いて2つの曲線の差を検定する。

Cox比例ハザード・モデルを用いて、抗菌薬服用開始日から解熱（37.5度未満）までに要した時間に影響を与える因子を解析する。

（倫理面への配慮）

本研究のすべての担当者は、「ヘルシンキ宣言（2008年10月修正）」および「臨床研究に関する倫理指針（平成20年7月31日改正、以下臨床研究倫理指針）」を遵守して実施する。本研究は、北海道大学病院自主臨床研究審査委員会の審査を経て、北海道大学病院により承認されている（臨床研究番号：自012-0174）。

C．研究結果

1．解析の対象となった肺炎マイコプラズマ肺炎患者

2012年12月より2015年8月までに合計905名の患者から検体を採取し、139名（15.0%）の検体より肺炎マイコプラズマが検出された。139名中78名（56.1%）から採取された検体からML感受性肺炎マイコプラズマが検出され、残りの61名（43.9%）から採取された検体からML耐性肺炎マイコプラズマが検出された。ML耐性株は全て23SリボゾームRNA上にA2063G変異を有していた。

肺炎マイコプラズマによる肺炎患者139名のうち、診療情報が揃っている92名（ML感受性群50名、ML耐性群42名）に加えて、2013年7月から2014年1月までに釧路市内の病院で血清学的に肺炎マイコプラズマによる肺炎と診断された17症例をML耐性群として解析した。この時期に釧路で検出された肺炎マイコプラズマ株は全例ML耐性であることに加えて、17症例の有熱期間はML耐性群の有熱期間と区別できないことの2つの理由からである。

2．分離された肺炎マイコプラズマ株のML感受性

A2063G変異を有さない肺炎マイコプラズマ株のマクロライド系抗菌薬（EM, CAM, AZM等）のMIC90値は低値であったが、A2063G変異を有する肺炎マイコプラズマ株のマクロライド系抗菌薬のMIC90値はいずれも（A2063G変異を有さない肺炎マイコプラズマ株と比べて）10^5倍以上高値であった（表1）。MINOとTFLXについてはA2063G変異がある株とA2063G変異がない株との間にMIC90値の差異はなかった（表1）。

3．肺炎マイコプラズマ患者の背景および抗菌薬開始から解熱までの日数

解析の対象となった109名（ML感受性群50名、ML耐性群59名）の患者背景を表2に記載した。年齢、性別、発熱から抗菌薬開始までの日数、選択された抗菌薬に差異はなかった。

抗菌薬開始日から解熱までの日数に関与する各種因子をCox回帰分析による多変量解析にて解析したところ、①入院の有無、②最初に選択した抗菌薬、③ML耐性遺伝子が影響を与えることが判明した（表3）。外来で治療を継続した患者は、経過中に入院した患者に比べると解熱する確率が2.08倍高い。AZM群に比べてMINO群では解熱する確率が2.83倍高く、CAM群に比べてMINO群では解熱する確率が3.87倍高く、TFLX群に比べてAZM群では解熱する確率が2.24倍高く、TFLX群に比べてMINO群では解熱する確率が6.34倍高い。ML耐性に比べてML感受性では解熱する確率が2.43倍高かった（表3）。抗菌薬開始から解熱するまでの時間は、最初に選択する抗菌薬によって大きく異なる（図1）。

ML感受性群（50症例）に限定したサブ解析を行うと、①入院の有無、②発症から抗菌薬服用までの日数が抗菌薬開始日から解熱までの日数に影響を及ぼすが、最初に選択した抗菌薬は影響を与えなかった（表4）。ML耐性群（59症例）に限定したサブ解析を行うと、①入院の有無、②最初に選択した抗菌薬が抗菌薬開始日から解熱までの日数に影響を与えた（表5）。AZM群に比べてMINO群では解熱する確率が37.2倍高く、CAM群に比べてMINO群では解熱する確率が56倍高く、TFLX群に比べてMINO群では解熱する確率が77倍高かった（表5）。

ML感受性群とML耐性群における、抗菌薬開始から解熱までの日数を抗菌薬別に解析したところ、ML耐性群にML系抗菌薬（CAM, AZM）を使用した場合、ML感受性群に比べて発熱が3日

間程度長引くことが判明した（図1, 2）。MINOとTFLXを使用した場合、ML感受性群とML耐性群には有熱期間の有意差はなかった（図2）。

抗菌薬開始から解熱するまでの日数を、ML感受性あるいは耐性株に分けて検討したところ、抗菌薬による治療開始後2日以内に解熱する症例の78%はML感受性群であり、発熱が3日以上持続する症例の85%はML耐性群であった（図3）。

D. 考察

1. ML感受性およびML耐性肺炎マイコプラズマによる肺炎に有効な抗菌薬

ML感受性肺炎マイコプラズマ感染症にAZM, CAM, MINO, TFLXを使用した場合の抗菌薬開始から解熱までの日数を比較すると、全体としては4種類の抗菌薬間に有意差を認めるものの（図1）、2種類の薬剤を個々に比較した場合には有意な違いはなかった（表4）。

一方、ML耐性肺炎マイコプラズマ感染症にAZM, CAM, MINO, TFLXを使用した場合の抗菌薬開始から解熱までの日数を比較した場合には、MINO使用群で抗菌薬開始日から解熱までの日数が有意に短かった（表5、図1）。その他の3剤（AZM, CAM, TFLX）使用群については、抗菌薬開始日から解熱までの日数に有意差はなかった。

ML耐性肺炎マイコプラズマ感染症に抗菌薬を使用した場合の抗菌薬開始日から解熱までの日数の違いを各抗菌薬のMIC値の違いのみで説明することはできない。MINOとTFLXのMIC90値はML感受性肺炎マイコプラズマとML耐性肺炎マイコプラズマで差異がないが（表1）、ML耐性肺炎マイコプラズマに対する臨床効果は両薬剤で明らかに異なる（表5）。MINOに比べてTFLXの半減期が長いこと、あるいは肺組織における移行率に違いがあるため等の理由が考えられる。

2. ML耐性肺炎マイコプラズマによる肺炎に対する治療

肺炎マイコプラズマによる肺炎患者を目の前にしたとき、その肺炎マイコプラズマがML感受性であるかML耐性であるかを区別することは困難である。ML耐性肺炎マイコプラズマの場合、MINOのみが有効であるが、歯牙着色などの問題があり8歳以上の患者にのみ使用可能である。肺炎マイコプラズマによる肺炎をCAMやAZMなどのマクロライド系抗菌薬で治療開始した場合、治療開始後2～3日以内に解熱しない場合、8歳以上の患者であればMINOの使用も選択肢となり得る。8歳未満の患者の場合には、もし細菌感染症が否定されれば、ステロイド剤使用も検討されて良いかもしれない。

肺炎マイコプラズマのML耐性率には大きな地域差が存在することがわかっている（Jpn J Infect Dis. 2015 Jul 10）。日本国内の肺炎マイコプラズマのML耐性率は50%程度とする報告が多いが、個々の医療機関にとって「受診する患者の半数がML耐性」を意味する訳ではない。肺炎マイコプラズマのML耐性率に関する地域毎のサーベイランスが有用と考えられる。

E. 結論

ML感受性肺炎マイコプラズマ感染症にAZM, CAM, MINO, TFLXを使用した場合の抗菌薬開始から解熱までの日数を比較すると、全体としては4種類の抗菌薬間には有意差を認めるものの、2種類の薬剤を個々に比較した場合には有意な違いはなかった。一方、ML耐性肺炎マイコプラズマ感染症にAZM, CAM, MINO, TFLXを使用した場合の抗菌薬開始から解熱までの日数を比較すると、MINO使用群で抗菌薬開始日から解熱までの日数が有意に短かったが、その他の3剤（AZM, CAM, TFLX）については、抗菌薬開始日から解熱までの日数に有意差はなかった。

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(順不同)

F. 研究業績

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G. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

表1．分離された肺炎マイコプラズマのMIC90

抗菌薬	MS *M. pneumoniae* (n=23)	MR *M. pneumoniae* (n=27)
Erythromycin	0.0039	>256
Clarithromycin	0.001	256
Azithromycin	<0.000125	64
Clindamycin	0.5	128
Levofloxacin	0.5	0.5
Ciprofloxacin	1	1
Tosufloxacin	0.5	0.25
Minocycline	1	1

表2．患者背景

		ML感受性	ML耐性	*P* value
患者数		50	59	
平均年齢(歳) ± 標準偏差 (範囲)		9.2 ± 3.3 (2 - 15)	9.0 ± 3.2 (3 - 17)	0.7745
男性/女性		29/21	33/26	0.8280
抗菌薬開始前の平均日数 ±標準偏差 (範囲)		3.6 ± 2.1 (0 - 8)	3.1± 1.8 (0 - 6)	0.2056
最初に選択した抗菌薬	AZM	11 (22.0%)	18 (30.5%)	0.2217
	CAM	26 (52.0%)	29 (49.2%)	
	MINO	9 (18.0%)	4 (6.8%)	
	TFLX	4 (4.0%)	8 (13.5%)	

AZM, アジスロマイシン; CAM, クラリスロマイシン;
MINO, ミノサイクリン; TFLX, トスフロキサシン

表3．治療開始から解熱までの日数に影響を与える因子

独立因子	基準	ハザード比 (95% CI)	*P*
年齢	1歳	1.00 (0.93-1.07)	0.9896
性別	女性	0.80 (0.53-1.21)	0.2810
経過中に入院したか否か	経過中に入院	2.08 (1.28-3.40)	0.0031*
発症から受診までの日数	1日	1.12 (0.99-1.27)	0.0621
最初に選択した抗菌薬			
AZM	CAM	1.37 (0.82-2.24)	0.2279
MINO	AZM	2.83 (1.17-6.74)	0.0215*
MINO	CAM	3.87 (1.64-8.84)	0.0024*
AZM	TFLX	2.24 (1.10-4.88)	0.0256*
CAM	TFLX	1.64 (0.84-3.50)	0.1554
MINO	TFLX	6.34 (2.30-17.84)	0.0004*
経過中に抗菌薬の変更があったか否か	変更なし	0.71 (0.40-1.22)	0.2273
ML耐性かML感受性か	ML耐性	2.43 (1.56-3.80)	<0.0001*

表4．治療開始から解熱までの日数に影響を与える因子（ML感受性50例）

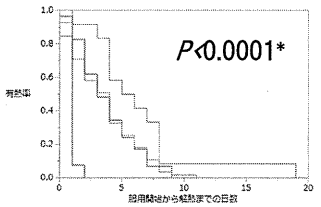
独立因子	基準	ハザード比 (95% CI)	P
年齢	1歳	1.00 (0.90-1.11)	0.9496
性別	女性	0.59 (0.31-1.13)	0.1075
経過中に入院したか否か	経過中に入院	2.58 (1.15-6.00)	0.0240*
発症から受診までの日数	1日	1.26 (1.03-1.54)	0.0256*
最初に選択した抗菌薬			
AZM	CAM	1.34 (0.58-2.91)	0.4785
MINO	AZM	1.42 (0.45-4.85)	0.5529
MINO	CAM	1.91 (0.65-5.55)	0.2378
AZM	TFLX	3.07 (0.94-12.09)	0.0639
CAM	TFLX	2.29 (0.81-8.35)	0.1269
MINO	TFLX	4.37 (0.99-22.51)	0.0511
経過中に抗菌薬の変更があったか否か	変更なし	0.58 (0.18-1.58)	0.3269

表5．治療開始から解熱までの日数に影響を与える因子（ML耐性59例）

独立因子	基準	ハザード比 (95% CI)	P
年齢	1歳	1.00 (0.91-1.10)	0.9467
性別	女性	0.88 (0.48-1.62)	0.6772
経過中に入院したか否か	経過中に入院	2.02 (1.02-3.93)	0.0404*
発症から受診までの日数	1日	1.09 (0.91-1.31)	0.3452
最初に選択した抗菌薬			
AZM	CAM	1.51 (0.78-2.87)	0.2228
MINO	AZM	37.20 (5.02-758.89)	0.0003*
MINO	CAM	56.01 (7.74-1129.74)	<0.0001*
AZM	TFLX	2.07 (0.79-5.97)	0.1410
CAM	TFLX	1.38 (0.55-3.87)	0.5060
MINO	TFLX	77.08 (9.47-1655.99)	<0.0001*
経過中に抗菌薬の変更があったか否か	変更なし	0.89 (0.45-1.71)	0.7350

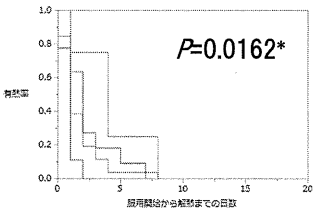
図１．最初に選択した抗菌薬と服用開始から解熱までの日数

ALL



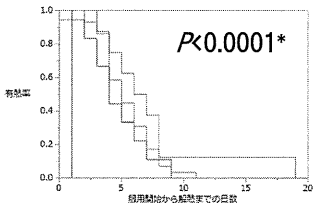
	抗菌薬	n	平均	SE
—	AZM	29	3.8	0.5
—	CAM	55	3.7	0.4
—	MINO	13	0.9	0.1
—	TFLX	12	6.4	1.3
	合計	109	3.7	0.3

ML感受性



	抗菌薬	n	平均	SE
—	AZM	11	2.5	0.6
—	CAM	26	1.7	0.3
—	MINO	9	0.9	0.2
—	TFLX	4	4.3	1.4
	合計	50	1.9	0.3

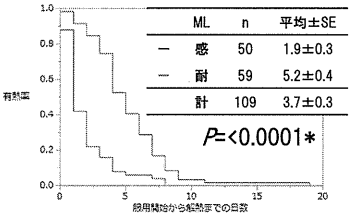
ML耐性



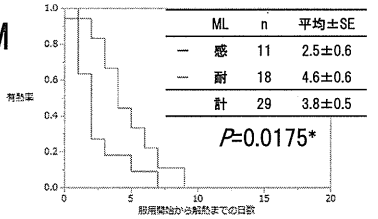
	抗菌薬	n	平均	SE
—	AZM	18	4.6	0.6
—	CAM	29	5.5	0.4
—	MINO	4	1.0	0.0
—	TFLX	8	7.5	1.8
	合計	59	5.2	0.4

図２．ML感受性/耐性の違いと服用開始から解熱までの日数

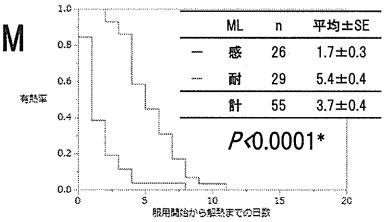
ALL



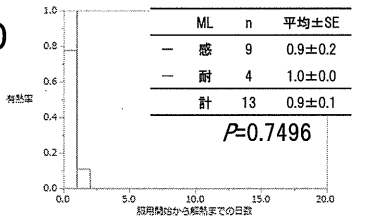
AZM



CAM



MINO



TFLX

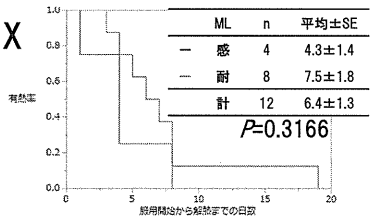
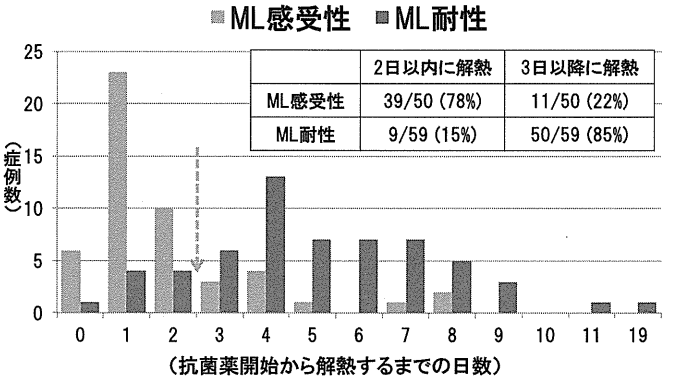


図３．抗菌薬開始から解熱するまでの日数



	2日以内に解熱	3日以降に解熱
ML感受性	39/50 (78%)	11/50 (22%)
ML耐性	9/59 (15%)	50/59 (85%)

Ⅲ. 研究成果の刊行に関する一覧表

平成27年度研究成果の刊行に関する一覧表

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kanayama A, Yahata Y, Arima Y, Takahashi T, Saitoh T, Kanou K, Kawabata K, Sunagawa T, Matsui T, Oishi K.	Enterohemorrhagic *Escherichia coli* outbreaks related to childcare facilities in Japan, 2010-2013.	BMC Infect Dis.	15(1)	539	2015
Murakami Y, Hashimoto S, Kawado M, Ohta A, Taniguchi K, Sunagawa T, Matsui T, Nagai M.	Estimated Number of Patients with Influenza A(H1)pdm09, or Other Viral Types, from 2010 to 2014 in Japan.	PLOS ONE	11(1)	e0146520	2016
Obuchi M, Yagi S, Oguri A, Takizawa T, Kimura H, Sata T.	Outbreak of Human Bocavirus 1 Infection in Young Children in Toyama, Japan.	Japanese Journal of Infectious Diseases	68(3)	259-261	2015
田中敏博	小児における伝染性紅斑の概要と地域における状況	IASR	37(1)	3-4	2016
Ishiguro N, Koseki N, Kaiho M, Kikuta H, Togashi T, Oba K, Morita K, Nagano N, Nakanishi M, Hazama K, Watanabe T, Sasaki S, Horino A, Kenri T, Ariga T; Hokkaido Pediatric Respiratory Infection Study Group.	Regional differences in rates of Macrolide-Resistant *Mycoplasma pneumoniae* in Hokkaido, Japan.	Japanese Journal of Infectious Diseases	Epub ahead of print		2015
Ishiguro N, Koseki N, Kaiho M, Kikuta H, Togashi T, Watanabe T, Ariga T; Hokkaido Pediatric Respiratory Infection Study Group.	Sensitivity and Specificity of a Loop-Mediated Isothermal Amplification Assay for the Detection of Mycoplasma Pneumonia from Nasopharyngeal Swab Samples Compared with those of Real-time PCR.	Clinical Laboratory	61(5-6)	603-606	2015
石黒信久	マイコプラズマ感染症－再流行に備えて～薬剤耐性の現状と抗菌薬治療～	小児科	56(6)	785-792	2015

Ⅳ. 研究成果の刊行物・別刷

Kanayama *et al. BMC Infectious Diseases* (2015) 15:539
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BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Enterohemorrhagic *Escherichia coli* outbreaks related to childcare facilities in Japan, 2010–2013

Atsuhiro Kanayama[1,2], Yuichiro Yahata[3*], Yuzo Arima[3], Takuri Takahashi[3], Takehito Saitoh[3], Kazuhiko Kanou[3], Kunio Kawabata[1], Tomimasa Sunagawa[3], Tamano Matsui[3] and Kazunori Oishi[3]

Abstract

Background: Enterohemorrhagic *Escherichia coli* (EHEC) is an important cause of gastroenteritis in Japan. Although non-O157 EHEC infections have been increasingly reported worldwide, their impact on children has not been well described.

Methods: We collected national surveillance data of EHEC infections reported between 2010 and 2013 in Japan and characterized outbreaks that occurred in childcare facilities. Per Japanese outbreak investigation protocol, faecal samples from contacts of EHEC cases were collected regardless of symptomatic status. Cases and outbreaks were described by demographics, dates of diagnosis and onset, clinical manifestations, laboratory data, and relation to specific outbreaks in childcare facilities.

Results: During 2010–2013, a total of 68 EHEC outbreaks comprised of 1035 cases were related to childcare facilities. Among the 66 outbreaks caused by a single serogroup, 29 were serogroup O26 and 22 were O157; 35 outbreaks were caused by *stx1*-producing strains. Since 2010, the number of reported outbreaks steadily increased, with a rise in cases and outbreaks caused by *stx1*-producing O26. Of 7069 EHEC cases reported nationally in 2010–2011, the majority were caused by O157 ($n = 4938$), relative to O26 ($n = 1353$) and O111 ($n = 195$). However, relative to 69 cases of O157 (2 %) associated with childcare facility EHEC outbreaks, there were 131 (10 %) such cases of O26, and this trend intensified in 2012–2013 (O157, 3 %; O26, 24 %; O111, 48 %). Among family members of childcare facility cases, the proportion of cases that were symptomatic declined with age; 10/16 cases (63 %) aged 6 years or younger, 16/53 cases (30 %) 6–19 years old, 23/120 cases (19 %) 20–49 years old and 2/28 cases (7 %) 50 years or older were symptomatic. Thirty one of the 68 outbreaks (46 %) were classified as foodborne-related.

Conclusions: Childcare facility EHEC outbreaks due to non-O157 serogroups, particularly O26 and O111, increased during 2010–2013. These facilities should pay extra attention to health conditions in children. As older family members of childcare facility cases appear to be less symptomatic, they should be vigilant about hand-washing to prevent further transmission.

Keyword: Enterohemorrhagic *Escherichia coli*, Non-O157, Childcare facility, Outbreak, Surveillance

* Correspondence: yahata@nih.go.jp
[3]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan
Full list of author information is available at the end of the article

Background

Enterohemorrhagic *Escherichia coli* (EHEC) is known to cause diarrhea and hemorrhagic colitis in humans [1]. Vulnerable populations of EHEC infection include small children and the elderly. Severe cases are reported more often in children than in adults (under 65 years of age), and may include life-threatening hemolytic uremic syndrome (HUS) [2]. EHEC cases in outbreaks have predominantly been attributed to EHEC O157:H7; however, recent studies have reported an increased in the number of cases affected by non-O157 serogroups, including O26, O103, O111, and O145 [3]. Non-O157 *E. coli* strains have been isolated from patients in the USA [4], Europe [5, 6], Australia [7], New Zealand [8], and Japan [9]. Notably, non-O157 *E. coli* tended to affect younger children more often than O157 in the USA [4].

EHEC outbreaks in childcare facilities have been reported from several countries [10–19]. Higher rates of secondary transmission have been detected in EHEC O157 outbreaks among people with a median age of less than 6 years compared with those with a median age of 6–59 years [12]. Although information regarding non-O157 outbreaks in childcare settings have been limited, non-O157 infections seem to occur more frequently than O157 infections in childcare facilities [13–19]. However, the reasons remain unknown and there is no consensus regarding how these outbreaks should be controlled.

EHEC is a notifiable disease in Japan and approximately 4000 EHEC cases are reported annually in the country, including outbreaks associated with childcare facilities [16–19]. After being notified of a case by a physician, Local Public Health Centers (LPHCs) conduct active case-finding among contacts of case patients as described previously [20]. LPHC staff interview contacts about their health status, collect their faecal specimens (regardless of symptomatic status), and monitor their health status. Such protocol is designed to allow for rapid and timely reporting of outbreaks, including those associated with childcare facilities, through the National Epidemiological Surveillance of Infectious Diseases (NESID) system from each LPHC. In August 2013, an increase in the reported number of EHEC outbreaks in childcare facility settings was noted, especially after 2012. Although more than 2.2 million children attending childcare facilities are at potential risk of EHEC infection [21], the overall picture on outbreaks in these settings has yet to be determined.

We describe a recent increase in the number of reported EHEC outbreaks related to childcare facilities in Japan based on analysis of national surveillance data. We discuss the outbreaks in terms of number of outbreaks and cases, proportion of symptomatic cases among cases detected through active investigations, characteristics of isolated serogroups and *stx* types, and mode of transmission.

Ethics

This study was conducted by the Act on Prevention of Infections and Medical Care for Patients with Infections in Japan. Thus, this study did not require approval from an ethics committee.

Methods

Data collection

EHEC infection is a notifiable disease in Japan, requiring an immediate report after diagnosis per "Act on Prevention of Infections and Medical Care for Patients with Infections". Local governments collect and submit case-based data via NESID to the Ministry of Health, Labour and Welfare (MHLW). For the present study, the following data were extracted from NESID: dates of notification, age, sex, occupation, clinical manifestations, serogroup and *stx* type from isolated EHEC strains, suspected route of infection (foodborne, direct contact, both foodborne and direct contact, or unspecified, as indicated by the reporting physicians and LPHC), and relationship to a specific outbreak in a childcare facility.

A symptomatic case was defined as EHEC infection confirmed by culture and serological testing for anti-Shiga toxin (*stx*) antibody or Polymerase Chain Reaction (PCR), after the development of at least one gastrointestinal illness, such as watery diarrhea, abdominal pain or cramps, bloody diarrhea, or vomiting. The presence of Shiga toxin in stool samples was confirmed by enzyme immunoassay or PCR. Standardized methods for laboratory tests are recommended to LPHCs under the guidance of the National Institute of Infectious Diseases, based on guidelines from World Health Organization for *Escherichia coli* and *Klebsiella* [22]. An asymptomatic case was defined as an EHEC infection confirmed by the laboratory tests described above for symptomatic cases, detected by active case finding during outbreak investigation or from routine screenings required in designated occupations (e.g. food handler, nursery school employee).

Childcare facility-related EHEC outbreaks

Per Japanese outbreak investigation protocol, after being notified of a case by a physician, LPHCs conduct active case-finding among contacts of case patients. When symptomatic EHEC cases are detected at a childcare facility, asymptomatic children at the same facility are screened by the laboratory tests described above. For our study, an outbreak at a childcare facility was defined as a cluster of at least two cases aged less than 7 years, attending the same childcare facility, affected by the same EHEC serogroup and *stx* type, reported from the same LPHC, and occurring within 3 weeks of diagnosis

(approximately double the 10 days-incubation period). Outbreak-related cases were defined as cluster cases described above and cases with the same serogroup and *stx* as the cluster cases, and sharing the same information as cluster cases for any of the following: belonged to the same family, living in the same household, or epidemiologically linked to the same childcare facility or cluster. The suspected route of infection was indicated by the reporting physicians and LPHCs and categorized as either foodborne, direct contact, or unspecified.

All descriptive statistical analyses were based solely on the extracted NESID surveillance data. Based on the extracted data, cases reported during the period when an increase in EHEC outbreaks in childcare facilities was observed (2012–2013) were compared to an immediately prior period of the same length (2010–2011).

Results

Reported number of EHEC cases and childcare facility outbreaks

Approximately 4000 EHEC cases were reported annually, peaking consistently at around epidemiological week 30 during 2010–2013 (Fig. 1). We identified 1035 outbreak-related cases among the 68 childcare facility outbreaks that occurred during this period. The number of outbreaks doubled during 2010–2013 and outbreak-related cases increased steadily over this period (Table 1). The increase in number of cases per outbreak in 2011–2013 was largely due to an increase in asymptomatic cases. However, the median number of symptomatic cases per outbreak remained the same (4, range: 1–15 for 2010–2011 and 1–47 for 2012–2013); moreover, in 2012–2013 there were 3 large scale outbreaks with more than 15 symptomatic cases compared to none in 2010–2011. Among the 68 outbreaks, 65 (96 %) outbreaks occurred between epidemiological weeks 22 and 50.

Majority of EHEC cases in 2010–2013 were caused by six serogroups (O26, O157, O111, O103, O121 and O145). While O157 was responsible for the majority of EHEC cases with a single serogroup and *stx1* and/or *stx2* type identified (4938/7069), O26 was the major serogroup among childcare-facility outbreaks (131/218), with 1 % of O157 cases vs. 10 % of O26 cases being outbreak-related (Table 2). This contrast between O157 and O26 became greater in 2012–2013 (2 % and 24 %, respectively). There were no childcare facility outbreaks associated with O111 in 2010–2011, but 173 O111 outbreak-related cases were reported in 2012–2013. Moreover, the proportion of O111 outbreak-related cases reached 48 % in 2012–2013.

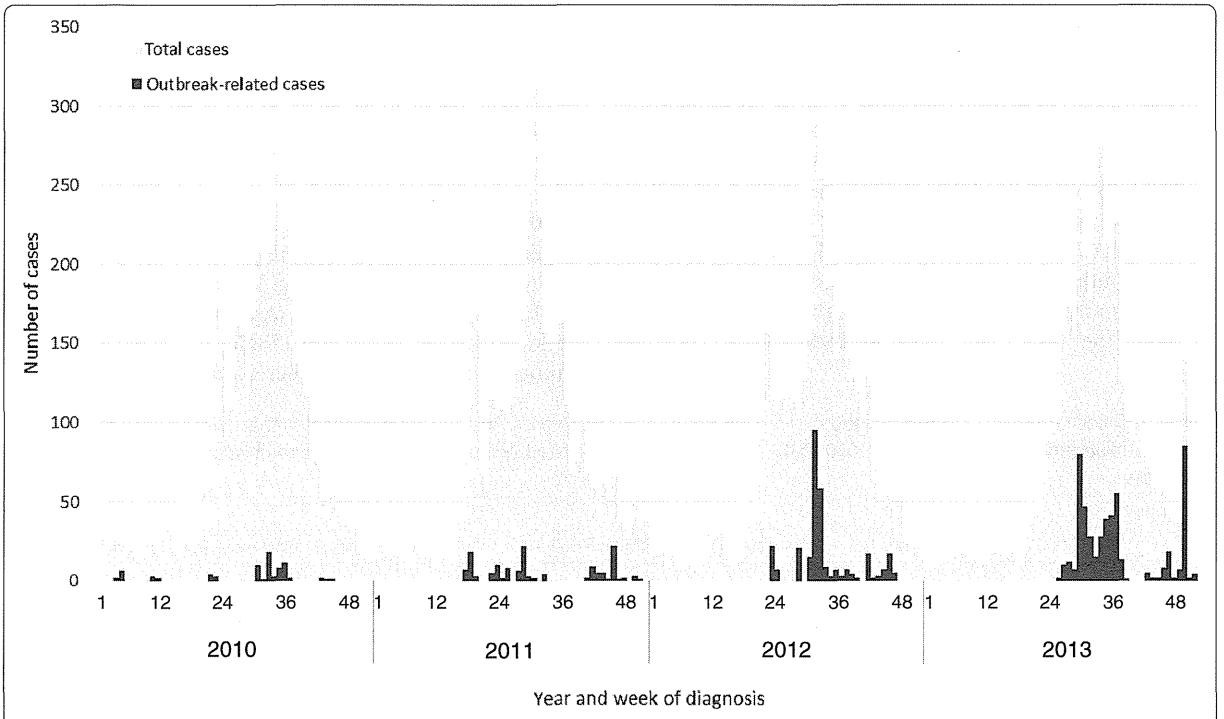

Fig. 1 Epidemiological curve of EHEC cases in childcare facility-related outbreaks in Japan, 2010–2013. EHEC cases in childcare facility-related outbreaks are shown by week of diagnosis during 2010–2013 in black (indicated as Outbreak-related cases); total EHEC cases for the same period are shown in grey (indicated as Total cases)

Table 1 Childcare facility-related EHEC outbreaks, 2010–2013, Japan. All outbreaks listed below in the table refer to childcare facility-related outbreaks

	Total	2010	2011	2012	2013
Total no. reported cases	15,887	4134	3940	3768	4045
No outbreaks	68	9	12	15	32
No. outbreak-related cases	1035	77	141	304	513
No. cases per outbreak	15.2	8.6	11.8	20.3	16.0
No. asymptomatic cases per outbreak	9.4	3.7	6.8	14.8	9.6
No. Hemolytic Uremic Syndrome cases in outbreaks	4	0	1	2	1

Characteristics of cases related to childcare facility outbreaks

Among a total of 1035 outbreak-related cases, 484 (47 %) were male and the median age was 4 years (range: 0–88 years) (Table 3). A total of 761 (74 %) cases were cluster cases, i.e. pediatric cases who were attending the childcare facilities that had an outbreak. Of 761 cases, 329 (43 %) were symptomatic. The majority of the remaining outbreak-related cases were family members, and the majority of family members were aged 20–49 years (120 cases). In 2010–2011, older age groups were less likely to be symptomatic than other family members. For example, 60 % of siblings of cluster cases less than 7 years old and with no evidence of going to the same childcare facility were symptomatic, while all 16 family members over 50 years old were asymptomatic. This trend continued in 2012–2013, when this youngest age group remained more likely to be symptomatic (64 %) than cases 7–19 and 20–49 years of age (23 % and 10 %, respectively). During both 2010–2011 and 2012–2013, among family member cases, age was inversely associated with being symptomatic, with only 7 % of those 50 years and older being symptomatic during 2010–2013 (Table 3). Four HUS cases were reported during 2010–2013; all four were less than 7 years old and affected by O157 that had both *stx1* and *stx2* and recorded in three outbreaks in 2013. Based on the available information among symptomatic cases, the longest interval between the onsets of cases in these outbreaks was 18 days.

Serogroups and stx types of the isolated EHEC strains in the childcare facility outbreaks

Among outbreaks caused by a single serogroup ($n = 66$; excluding two O26/O103 co-infection outbreaks), the *stx1*-producing strain was the most predominant (35/66, 53 %) (Table 4); 19/66 (29 %) outbreaks were associated with both the *stx1*- and *stx2*-producing strains and 12/66 (18 %) outbreaks were associated with the *stx2*-producing strain. Considering both Serogroups and *stx* types, O26 *stx1* accounted for the largest proportion (26/66, 39 %), followed by O157 *stx1* and *stx2* (14/66, 21 %) and O157 *stx2* (7/66, 11 %). During 2012–2013, there was a greater increase in both the number of cases and outbreaks associated with O26 relative to those associated with O157, particularly for *stx1*-producing O26 (Table 4).

Mode of transmission

Among the 68 outbreaks, suspected transmission routes were direct contact (24 outbreaks, 35 %), both foodborne and direct contact (24 outbreaks, 35 %), foodborne (7 outbreaks, 10 %), and unspecified (13 outbreaks, 19 %).

Discussion

In this study, we identified 68 EHEC outbreaks associated with childcare facilities in Japan from 2010 to 2013. Notably, the proportion of cases associated with outbreaks varied by serogroup (Table 2). In particular, the number of such outbreaks increased in 2012–2013. O26

Table 2 Childcare facility outbreak-related cases, total number of EHEC cases, and proportion of cases that were childcare facility outbreak-related cases, by serogroup, 2010–2013, Japan. All outbreaks listed below in the table refer to childcare facility-related outbreaks

Serogroup	2010–2011			2012–2013		
	Outbreak cases (n)	Total cases (n)	Outbreak/Total (%)	Outbreak cases (n)	Total cases (n)	Outbreak/Total (%)
O157	69	4938	1 %	100	4075	2 %
O26	131	1353	10 %	417	1707	24 %
O111	0	195	0 %	173	364	48 %
O103, O121 or O145 [a]	18	583	3 %	64	684	9 %
Total [b]	218	7069	3 %	754	6830	11 %

[a] excludes cases related to two O26/O103 co-infection outbreaks
[b] excludes cases with serogroups other than O157, O26, O111, O103, O121, or O145, co-infections of more than one serogroup and infections with unknown serogroup or stx type (1005 cases in 2010–2011 and 983 cases in 2012–2013)

Table 3 Demographic characteristics of childcare facility EHEC outbreak-related cases, 2010–2013, Japan. All outbreaks listed below in the table refer to childcare facility-related outbreaks

	Total				2010–2011				2012–2013			
	Symptomatic (n)	Asymptomatic (n)	Total (n)	Symptomatic (%)	Symptomatic (n)	Asymptomatic (n)	Total (n)	Symptomatic (%)	Symptomatic (n)	Asymptomatic (n)	Total (n)	Symptomatic (%)
Male	197	287	484	(41 %)	54	46	100	(54 %)	143	241	384	(37 %)
Female	196	355	551	(36 %)	50	68	118	(42 %)	146	287	433	(34 %)
Age in years												
<7, childcare facility	329	432	761	(43 %)	75	66	141	(53 %)	254	366	620	(41 %)
<7, family	10	6	16	(63 %)	3	2	5	(60 %)	7	4	11	(64 %)
7–19, family	16	37	53	(30 %)	6	4	10	(60 %)	10	33	43	(23 %)
20–49, family	23	97	120	(19 %)	15	22	37	(41 %)	8	75	83	(10 %)
≧50, family	2	26	28	(7 %)	0	8	8	(0 %)	2	18	20	(10 %)
Others [a]	13	44	57	(23 %)	5	12	17	(29 %)	8	32	40	(20 %)

[a] Others include those who were not or not known to be family members of the child attending the childcare facility

Table 4 Number of childcare facility EHEC outbreak-related cases by serogroups and *stx* types, 2010–2013, Japan. All outbreaks listed below in the table refer to childcare facility-related outbreaks

Serogroup	*stx* type	Total		2010–2011		2012–2013		Ratio of 2012–2013 values to 2010–2011 values	
		Case	Outbreak	Case	Outbreak	Case	Outbreak	Case	Outbreak
O157	*stx*1	11	1	11	1	0	0	0	0
	*stx*2 or *stx*1/2	158	21	58	9	100	12	1.7	1.3
	Total	169	22	69	10	100	12	1.4	1.4
O26	*stx*1	478	26	86	7	392	19	4.6	2.7
	*stx*2 or *stx*1/2	70	3	45	2	25	1	0.6	0.5
	Total	548	29	131	9	417	20	3.2	2.2
O111	*stx*1	44	3	0	0	44	3	-	-
	*stx*2 or *stx*1/2	129	3	0	0	129	3	-	-
	Total	173	6	0	0	173	6	-	-
O103, O121 or O145 [a]	*stx*1	48	5	11	1	37	4	3.4	4
	*stx*2 or *stx*1/2	34	4	7	1	27	3	3.9	3
	Total	82	9	18	2	64	7	3.6	3.5
Total	*stx1*	581	35	108	9	473	26	4.4	2.9
	*stx*2 or *stx*1/2	391	31	110	12	281	10	2.6	0.8
	Total	972	66	218	21	754	45	3.5	2.1

[a] excludes cases related to two O26/O103 co-infection outbreaks

stx1 cases comprised the majority of cases related to such outbreaks (39 % of outbreaks) during the period and contributed to much of the increase. O111 cases were also associated with childcare facility outbreaks and nearly half of all reported O111 cases were related to such outbreaks that occurred during 2012–2013 (Table 2). O157, however, appeared to be much less associated with such outbreaks. Fortunately, *stx1*-producing O26 is known to be less severe than O157 [2]. In fact, all four reported HUS cases during the study period were among O157 infections and much of the increase in outbreak-related cases was associated with an increase in asymptomatic cases. Family members, particularly older persons, of cases that were attending childcare facilities were less likely to be symptomatic. To our knowledge, this is the first study to show profiles of childcare facility-related EHEC outbreaks in Japan.

These results suggest that certain non-O157 strains, such as O111 and O26, may be more likely to occur in childcare facility settings and could indicate transmission concerns in these settings, particularly given the presence of asymptomatic cases. In fact, direct contact was the suspected route of transmission of EHEC infections in 48 of the 68 outbreaks (direct contact in 24 outbreaks and both foodborne and direct contact in 24 outbreaks), relative to foodborne transmission suspected in 31 outbreaks (foodborne in 7 outbreaks and both foodborne and direct contact in 24 outbreaks). Majority of adult family member cases were asymptomatic, particularly those over 50 years of age. Taken together, we speculate that a sizable proportion of adolescent and adults were unaware of their EHEC infection, and could transmit the infection to other family members, including their children, before control measures could be undertaken. Thus, adolescents and adults in close contact with young children should be vigilant about hand-washing even if they do not notice any signs or symptoms of gastroenteritis themselves. In particular, as O26, the most frequent serogroup detected among childcare facilities, tends to be more asymptomatic than O157, asymptomatic cases may contribute to further spread, resulting in larger outbreaks.

As EHEC shedding is known to be relatively long lasting, earlier detection of events and rapid outbreak investigations are important in minimizing the number of infected cases and reducing potential further spread. While only few studies have assessed such information, long duration of shedding has been documented for several EHEC serotypes. The duration of shedding ranged from 15 to 46 days in an EHEC O26 outbreak in the USA [15]. Shedding periods of up to 71 days were found in an EHEC O145 outbreak in a Norwegian childcare center [23]. Furthermore, a sensitive detection method used in a German outbreak showed that shedding lasted up to 62 days in those with diarrhea or hemorrhagic colitis [24]. Moreover, some outbreaks in Japan were difficult to control quickly, taking a mean of 46.5 days (range: 32–63) after the onset of index cases, partly due to secondary infections [25], emphasizing the need for early detection to prevent transmission. Family members

are also affected by childcare facility-related cases, and while the direction of transmission between family members and cases in childcare facilities is unknown, proper hand washing and hygiene practice are important in households.

While the O157 serogroup is known for its severity, the United States Department of Agriculture pointed out that awareness of non-O157 serogroups as a food safety concern has increased in the USA and added six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) to the list of pathogens that should be considered to be of public health importance [26]. Indeed, while O26 serogroup is known to be relatively less severe, concerns regarding O26 are starting to emerge. For example, a novel type of O26 clone harboring the *stx2* gene was found in Germany, and has been spreading across Europe, the USA, and South America [5, 27–30]. These studies suggested that *stx2*-producing O26 might be a more virulent type, and could pose a threat to children in childcare facilities in Japan and in other countries. The *stx2*-producing strain of O157 can also result in severe outcomes, including HUS [2]. The second major serogroup in our study was *stx2*-producing EHEC O157, and indeed, all four HUS cases detected in our study were affected by *stx1* and *stx2*-producing O157.

A key limitation in our study is understanding the reason why childcare facility-related non-O157 outbreaks have increased, which remains unclear from national surveillance-based analysis. As the increase was associated with increase in asymptomatic cases, possibility of enhanced outbreak investigations at child care facilities exist. However, there was no change in policy for diagnostic practices or surveillance criteria. Moreover, while the median number of symptomatic cases per outbreak remained the same, there was an increase in large scale outbreaks in 2012–2013. In addition, while specific alerts regarding childcare facility-related EHEC outbreaks were distributed from MHLW to LPHCs in late August 2013, this notification was based on increased EHEC cases from NESID data; thus, it cannot explain the increase in the number of outbreaks in 2012 or the increase during the period up to August in 2013, when the number of outbreaks had already reached almost twice as many as that in 2012.

Conclusions

In Japan, the reported frequency of EHEC outbreaks and cases related to childcare facilities, especially those with non-O157 strains, have been increasing. Once infections with non-O157 serogroups in childcare facilities are recognized, family members should be vigilant about hand washing to prevent further transmission. Early detection and response is required to minimize the extent of outbreaks, and further investigation into the potential reasons of the increase in such outbreaks is warranted.

Abbreviations

EHEC: Enterohemorrhagic *Escherichia coli*; HUS: Hemolytic uremic syndrome; *stx*: Shiga toxin; NESID: National Epidemiological Surveillance of Infectious Diseases; MHLW: Ministry of Health Labour and Welfare.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AK designed the study and conducted all descriptive and statistical analyses of the data and drafted the manuscript. YY and YA helped with the design, analyses, and interpretation and revised the manuscript. TT, TS, KK, KK, TS, TM and KO contributed to the revision. All authors read and approved the final manuscript.

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Author details

[1]Field Epidemiology Training Program, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. [2]Department of Global Infectious Diseases and Tropical Medicine, National Defense Medical College, Saitama, Japan. [3]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan.

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RESEARCH ARTICLE

Estimated Number of Patients with Influenza A(H1)pdm09, or Other Viral Types, from 2010 to 2014 in Japan

Yoshitaka Murakami[1]*, Shuji Hashimoto[2], Miyuki Kawado[2], Akiko Ohta[3], Kiyosu Taniguchi[4], Tomimasa Sunagawa[5], Tamano Matsui[5], Masaki Nagai[3]

1 Department of Medical Statistics, Toho University, Tokyo, Japan, **2** Department of Hygiene, Fujita Health University School of Medicine, Aichi, Japan, **3** Department of Public Health, Saitama Medical University Faculty of Medicine, Saitama, Japan, **4** Department of Pediatrics, National Mie Hospital, Mie, Japan, **5** Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan

* yoshitaka.murakami@med.toho-u.ac.jp

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Abstract

Infectious disease surveillance systems provide information crucial for protecting populations from influenza epidemics. However, few have reported the nationwide number of patients with influenza-like illness (ILI), detailing virological type. Using data from the infectious disease surveillance system in Japan, we estimated the weekly number of ILI cases by virological type, including pandemic influenza (A(H1)pdm09) and seasonal-type influenza (A(H3) and B) over a four-year period (week 36 of 2010 to week 18 of 2014). We used the reported number of influenza cases from nationwide sentinel surveillance and the proportions of virological types from infectious agents surveillance and estimated the number of cases and their 95% confidence intervals. For the 2010/11 season, influenza type A(H1) pdm09 was dominant: 6.48 million (6.33–6.63), followed by types A(H3): 4.05 million (3.90–4.21) and B: 2.84 million (2.71–2.97). In the 2011/12 season, seasonal influenza type A(H3) was dominant: 10.89 million (10.64–11.14), followed by type B: 5.54 million (5.32–5.75). In conclusion, close monitoring of the estimated number of ILI cases by virological type not only highlights the huge impact of previous influenza epidemics in Japan, it may also aid the prediction of future outbreaks, allowing for implementation of control and prevention measures.

Introduction

Infectious disease surveillance systems provide both quantitative and qualitative information crucial for protecting populations from disastrous epidemics [1–6]. Infectious disease surveillance in Japan, initiated in 1981, has evolved into a comprehensive system of infectious disease control, particularly since the revision of the Infectious Disease Control Law in 1999 [6]. Current surveillance system in Japan, called National Epidemiological Surveillance for Infectious Diseases (NESID), which includes mandatory reporting system for national notifiable diseases

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and sentinel surveillance system for ubiquitous infectious diseases. National notifiable disease must require having a laboratory confirmation. Sentinel surveillance is based on disease symptom and a confirmed case distribution was reported by infectious agents surveillance, which is 10% samples of the sentinel surveillance. Specimen were randomly selected from these cases and then tested and reported by the Local Public Health Institutes. These relationships were shown in the flow diagram (S1 Appendix). Compared with other surveillance systems around the world, the Japanese system is based on a number of sentinel institutions throughout the country. In the case of influenza-like illness (ILI), almost 5000 sentinel institutions were sampled from across Japan that were representative of geographical regions (public center areas and prefectures), medical institutions (clinics/hospitals and medical departments) and population densities [7]. This carefully designed surveillance system enabled us to estimate the number of ILI patients nationwide and determine the 95% confidence intervals (95% CI) [8,9]. For example, incidence rate (per 1,000) of ILI patients in year 2005 epidemic season was estimated in 142.6 (95% CI: 135.6–149.6) [9]. This quantitative information provided important insight in to future influenza epidemics in Japan.

Identifying the virological types of influenza involved in outbreaks of infection provides essential information for the treatment and prevention of population-wide influenza epidemics. To examine the current influenza epidemic in Japan, infectious agents surveillance, which is laboratory based surveillance, investigated a small number of patients (300–600 patients in the epidemic season) and provided the distribution of virological types among patients every week. This information only reported a fraction of the virological types present among the population, but no further quantitative information was provided by the system. If the estimated number of specific virological types of influenza (i.e. pandemic influenza: A(H1)pdm09) were available, this information would help predict the threat of an influenza epidemic and therefore allow for the appropriate action to be taken.

This study estimated the number of cases of pandemic influenza (A(H1)pdm09) and seasonal-type influenza (A(H3) and B) infection from 2010 to 2014, using data provided from 5000 sentinel sites of the infectious disease surveillance system in Japan.

Methods

The infectious disease surveillance system in Japan

The infectious disease surveillance system in Japan comprises two types of surveillance: sentinel and infectious agents surveillance [7]. Sentinel surveillance, which covers 27 common infectious diseases including ILI, reports weekly numbers of patients throughout Japan. Sentinel medical institutions were selected by local government following the guidelines of the Ministry of Health, Labour and Welfare [10]. These guidelines were intended to ensure equal distribution of sentinel medical institutions throughout the nation. The number of sentinel medical institutions designated within each area and public health center is approximately proportional to its population size. For ILI, the total number of sentinel medical institutions in Japan is approximately 5000. ILI was confirmed by sentinel doctors (mostly using rapid diagnostic testing) of the sentinel medical institution and the number of ILI in each sentinels was reported every week using internet network system. In contrast, infectious agents surveillance is based on specimen samples, which was bought from selected sentinels (500 medical institutions). This laboratory-based surveillance confirmed the virus subtype by Local Public Health Institutes. After pathogens have been isolated from specimens, this information of local centers is sent to the central (Infectious Disease Surveillance Center) and is summarized in an infectious agents surveillance report. Together these surveillance systems provide weekly data on the frequency of infection with different virological types of influenza.

Estimation of the number of patients with specific virological influenza types

Methods for estimating weekly numbers of ILI patients have been described previously [8,9]. In brief, estimations are based on the assumption that all sentinel medical institutions in a public health center are randomly sampled and stratified by the characteristics of the medical institutions (i.e. department type, number of patients). We used a stratified random sampling technique to estimate the total number of ILI cases and their approximate 95% confidence intervals [9].

The weekly numbers of virological type-specific patients were estimated by multiplying the above-mentioned estimated numbers of ILI with the weekly fraction of each virological type identified from the laboratory confirmed cases. The formula of this estimation is shown in the Appendix. We estimated weekly numbers of influenza cases of virological types A(H1)pdm09, A(H3) and B, from week 36 (September 6) of 2010 to week 18 (May 10) of 2014. We also conducted the age-specific estimation by using same method mentioned above. These data were also categorised according to the age of patients: 0–4, 5–19, 20–59 and 60 years and over. All statistical analyses were performed using SAS, version 9.30 (SAS Institute Inc., Cary, NC, USA).

Results

Fig 1 and Table 1 show the weekly estimated number of virological type-specific influenza cases from week 36, year 2010 to week 18, year 2014, in Japan. In the 2010 epidemic season (from September 2010 to May 2011), influenza type A(H1)pdm09 was dominant [total estimated number: 6,480,000 (95% confidence interval (95% CI) 6,330,000–6,630,000]] and the

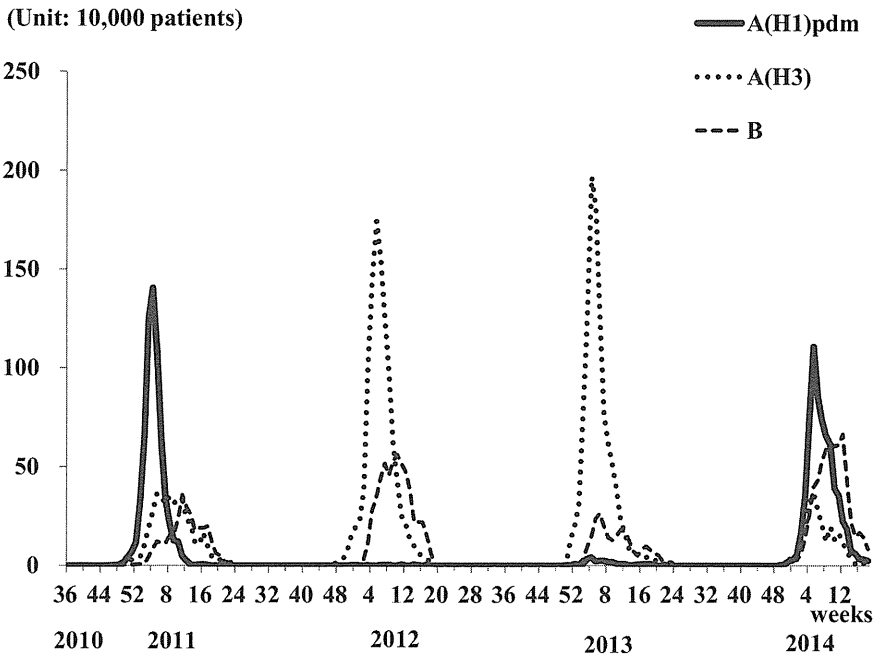

Fig 1. Estimated number of cases of influenza infection according to virological type from week 36, 2010 to week 18, 2014, Japan. Unit of the vertical line: 10,000 patients

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