A report on measles and rubella surveillance in Taiwan, 2005

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In 2005, forty (40) suspected cases of measles and 43 suspected cases of rubella were reported to CDC/Taiwan through the infectious diseases reporting system. Among them, 4 cases were reported as having both measles and rubella. Hence, a total of 79 suspected cases of measles/rubella were reported. A total of 66 cases were excluded and only 7 cases of measles and 6 cases of rubella were confirmed. Viral culture and serological examination showed that 2 cases of suspected measles and 1 suspected rubella were identified to be an enterovirus infection. In addition, adenovirus was isolated from a suspected measles case. Among the serologic tests, 4/79 were positive for parvo B19. Among the 19 cases under the age of 3, four were positive for HHV-6. Pathogens were identified in about 30% of reported cases.

Introduction

Measles is the next disease that will be eradicated globally after polio. Currently 4 of the 6 World Health Organization (WHO) regions have set a time

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table for its elimination: in Americas, by 2000; European, by 2010; Eastern Mediterranean, by 2010, and Western Pacific region, by 2012. Whereas in African and South-East Asia regions, the goal is to reduce the mortality rate of measles. Similarly, to reduce the burden of congenital rubella syndrome (CRS), Americas and Europe have also set the goal to eliminate rubella and reduce CRS. Since surveillance plays an important part to eradicate a disease, WHO has established the measles and rubella laboratory network (LabNet) to strengthen disease confirmation [1].

The "Monitoring the interruption of indigenous measles transmission" meeting held in Cape Town, South Africa in 2003 [2] suggested that the following five indicators to document progress toward elimination: 1. confirmed measles cases per million population per year, excluding imported cases should be less than one; 2. vaccination coverage for the first dose should reach 95% and 80% for the second; 3. at least 80% of administrative areas should have one suspected measles case reported per 100,000 population per year to demonstrate the monitor system is functional; 4. at least 80% of suspected cases should have serum specimens for IgM examination; 5. at least every transmission chain should have one specimen to confirm genotype of the virus. Our data in the recent three years showed that all indices have been satisfied except that the third index.

With the high vaccination coverage rates of measles vaccine and measles, rubella and mumps (MMR) vaccine, incidences of disease have decreased significantly and hence the positive prediction rates of clinical symptoms for measles and rubella have decreased accordingly. Therefore, suspected measles and rubella cases should be laboratory confirmed to understand the real incidence. We also attempt to test those cases which were excluded to have measles and rubella, in order to explore the etiology of fever and rashes.

Materials and Methods

Specimens

Blood, urine and throat swabs were gathered from reported cases of measles and rubella through the reporting system.

Virus culture and immunofluorescence examination

1. 100 μl of throat swab and urine specimens were inoculated onto B95a, Vero, A549 and RD cell lines and observe for the presence of cytopathic effect (CPE) everyday. If CPE occurred, supernatant were collected by centrifugation and re-suspended cell pellet in PBS and proceeded for immunofluorescence assay. Measles monoclonal antibody of the Measles IFA Kit (LIGHT DIAGNOSTIC Measles-IFA kit, Chemicon International,Inc.catalog-3187), rubella monoclonal antibody (Chemicon International,Inc.catalog-MAB925, 1:100 dilution), respiratory tract virus antibody (Chemicon International,Inc.catalog-5007), and enterovirus antibody (Chemicon International,Inc.catalog-3360) were used for examination. Specimens positive for respiratory tract virus and enterovirus were subtyped with other monoclonal antibodies. For enterovirus that can not be subtyped by immunofluorescence examination, molecular biology methods were used for identification of serotypes.

Serological testing

A. IgM and IgG antibodies for measles/rubella: Enzygnost Anti-Masern-Virus/IgM, Enzygnost Anti-Masern-Virus/IgG, Enzygnost Anti-Rubella-Virus/IgM, and Enzygnost Anti-Rubella-Virus/IgG (Dade Behring, Marburg, Germany) were used.

B. Parvovirus B19 IgM and IgG: Parvo B19 IgM EIA and parvo B19 IgG EIA kits (Biotrin) were used.

C. HHV-6 IgM and IgG: Human Herpesvirus –6 IgM ELISA/ Human Herpesvirus-6

IgG ELISA kits (Panbio) were used.

Molecular genotyping of measles virus

QIAGEN QIAamp Viral RNA Kit was used for RNA extraction, and OneStep RT-PCR kit was used for Reverse Transcription Polymerase Chain Reaction (RT-PCR) with the following primers: MV59 (5'-GAT ATG TGA CAT TGA TAC ATA TAT-3') and MV64 (5'-TAT AAC AAT GAT GGA GGG TAG-3'). The reaction parameters were: 50℃ for 30min, 95℃ for 15min, 35 cycles of 94℃ for 30sec, 51℃ for 30sec and 72℃ for 1 min, and finally 72℃ for 10 min. In addition, nested PCR was done with MBI FERMENTAS 2X PCR Master Mix with primer pairs MV60 (5'-GCT ATG CCA TGG GAG TAG GAG TGG-3') and MV63 (5'-GGC CTC TCG CAC CTA GTC TAG-3') and the following condition: 94℃ denature for 2min, 35 cycles of 94℃ for 30sec, 60℃ for 30sec and 72℃ for 1min, and finally 72℃ for 7min.

Agarose gel electrophoresis and phylogenetic analysis

Reaction products (around 580bp) were analyzed by 1.5% agarose gel. Amplified products were then purified for sequencing. After sequencing, 456bp of the sequence and reference strains for measles virus genotype from WHO were used for phylogenetic analysis with MEGA 3.1 software.

Results

In 2005, seven (7/40, 17.5%) measles cases were confirmed from the reporting system. Two viral strains were isolated (one from throat swab and one from urine). Six rubella cases (6/43, 14% of total reported cases) were confirmed. Rubella virus was isolated from the throat swab of a case. Among the reported measles cases, 14 cases (14/40, 35%) were below 3-year-old and 14 were more

than 18-year-old. Three cases were confirmed in each group. Twelve cases (30%) were reported from student of six to eighteen years old, and one was a confirmed case. The majority of reported rubella cases (23/40, 53% were older than eighteen years old. Five of them were confirmed cases (Fig. 1). Among the seven measles confirmed cases, 5 were positive for IgM. One of them was not positive for IgM, but had IgG converted from negative to positive. The other confirmed case was diagnosed by PCR of throat swab specimens from active surveillance cases from contract virology laboratory with symptoms including fever, rash and cough. Among the six rubella confirmed cases, all were positive for IgM, and three of them had IgG converted from negative to positive. Genotyping of viral strains from the confirmed measles cases showed that 3 strains were genotype H1, one was D5 and 2 were A (Fig. 2). No results were obtained from other three cases. The ages, dates of onset and specimen collection, types of specimens, vaccination, serological examination and genotyping of strains of the 13 measles/rubella confirmed cases are listed in Table 1.

In the 66 suspected cases that were excluded to have measles and rubella, three enteroviruses (2 Coxsackievirus B3, one CA 21) were cultured from RD cells. One adenovirus was isolated from A549. Serological examination of HHV-6 showed that among the 19 cases of reported measles/rubella cases younger than 3, four positive cases (three for HHV-6 IgM and one for HHV-6 IgG seroconversion) were identified. Among the all 79 suspected measles/rubella cases, four were postivie for IgM against parvo B19 (Table 2).

Antibodies against measles and rubella were also tested in reported cases. Among the 40 measles reported cases, after exclusion of the seven confirmed cases, 5 were negative for measles antibody. All of them were younger than 9

months old, the age to receive vaccination. The result of rubella antibody testing showed 11 cases were negative. Among them, 8 were younger than 15 months old, the age to receive MMR vaccination, one was a two years old case without scheduled vaccination, and two were adults with unknown vaccination history. Among the 43 rubella reported cases, five were negative for measles antibody; one was a 4 months old infant, one was a ten years old child with one dose of measles vaccine and two doses of MMR, and the other 3 were adults (two were born in 1980 and one was born in 1981). Among the 37 suspected cases excluding the 6 confirmed rubella cases, 3 were negative for rubella antibody, and all of them were younger than the age required for vaccination (Table 3).

The definitions for reporting measles cases are fever higher than 38.5℃, rash for more than 3 days with one of the following symptoms: cough, coryza, or conjunctivitis. The definitions for reporting rubella cases are mild fever (higher than 37.2℃) acute rash, and one of the following symptoms: arthritis, lymphadenopathy, or conjunctivitis. Accordingly, we compared confirmed and excluded cases of measles and rubella in term of fever, rash, cough, coryza and conjunctivitis for measles reported cases and fever, rash, arthritis, lymphadenopathy and conjunctivitis for rubella reported cases (Table 4). We found that there was no significant correlation between individual symptoms and whether a case is a confirmed case. The percentage of compatible symptoms was higher in confirmed cases than in excluded cases, but was not statistically significant by chi-square test.

Discussion

Measles is a highly contagious disease, but can be effectively prevented by

vaccination. Since the introduction of routine vaccination, the once prevalent childhood measles was rare now in Taiwan. The sporadic cases under 3 years old were cases not receive scheduled vaccine or younger than the age for vaccination. In the 3 confirmed cases younger than 3 years old, 2 were from the same family and did no receive scheduled vaccination. The cases were identified as a confirmed case in the reporting system. Traditional case investigation revealed that the younger brother of the case was also positive for measles virus as shown by viral culture from the throat swab. However, the younger brother of the confirmed case was initially diagnosed as common cold, with subsequent serological examination and viral culture showed measles virus infection. Based on this finding, we screened cases from influenza and enterovirus monitoring systemt from contract virology laboratory for cases having symptoms of fever, rash, cough or coryza, and gathered throat swabs for viral culture and PCR. In the 126 specimens, two were identified by PCR, and one of them was the brother of the family of the case suggesting that our current passive monitoring system is not sensitive enough. The numbers of suspected case reported each year were also lower than the recommendation from WHO, i.e., at least one suspected case of measles should be reported every one hundred thousand population every year in 80% of administrative areas [2]. Among adult measles cases, three confirmed cases were older than 18 years old (born in 1965, 1976 and 1986). One of the cases was a foreigner (Germany, born in 1965) and the other two cases were natives; one had a travel history to Japan one week before onset, and the other one had no travel history. The result of measles antibody test showed three antibody negative adults born in 1980 to 1981. In the 27 confirmed measles cases in 2002, nine were older than 18 (born in 1973 to

1984), suggesting that this population has more susceptible hosts, similar to the reports from Australia [3]. How to administer vaccine in adulthood is a practical difficulty by every country set to eliminate measles. Another issue worth of attention is secondary vaccine failure, identified in areas with sustain outbreak in highly vaccinated population [4]. One example is a confirmed measles case in 2005. The case was born in 1990. As to the vaccination history, records of measles and MMR vaccines had been lost except one MMR boostered in 2002. According to parents of the case, all vaccines had been administrated as scheduled. Serological examination did not show IgM positive, but measles IgG antibody showed seroconversion during the acute phase and recovery phase, and the symptoms were compatible with the definition for fever, rash and cough for reporting measles cases. Another case was born in 1994 with documented one shot of measles and two shots of MMR vaccination, but showed negative IgG for measles. In cases of measles among students in 2002, 8 cases were found to have one measles and one MMR vaccination (only two had detailed records). As to the timing of measles vaccine, different countries have different timing, such as 9, 12 or 15 months old. The concern is that administration too early will be interfered by maternal antibodies. Previous study also showed that vaccine-induced maternal measles antibody lasts shorter in infants than antibody generated by natural infection of measles [5].

Among confirmed rubella cases, all except one 16-year-old case were adults (born in 1954 to 1979). The teenager was from Belarus. Among the other five adult cases, two were foreign workers from Philippines and Indonesia and lived in Taiwan for two weeks before having the disease. Among the other three natives, one was in Philippines for 6 weeks and returned to Taiwan 10 days before onset

of the disease. The other two cases had no travel histories. The results of IgG antibody against rubella showed that 13/79 (16.5%) were negative for rubella antibody. Among them, 12 were younger than the age for MMR vaccination, and one was an adult born in 1966. For years, more than 95% of rubella cases were native adults. However, in 2005, half of the cases were foreigners, and the disease suggested to be imported as judged from the dates of onset and traveling history. The impact of foreign workers on eradication program of measles/rubella should be further evaluated.

As to the genotype of measles, among the 6 PCR positive cases, two were genotype A, three were H1, and one was D5. The two cases of genotype A had measles vaccine within one month, and hence were excluded as positive cases. Among the three cases of genotype H1, two were brothers and no infection source could be identified. The one case having genotype D5 was a foreigner, who went back to Germany two to three weeks before onset that occurred after coming back to Taiwan for one week. The case belongs to be an imported case. According to the Eurosurveillance the measles outbreak occurred in early 2005 in Hessen, Germany was genotype D4 and in Bavaria was D6 [6]. In early 2006 the measles outbreak occurred in Nordrhein Westfalen was genotype D6 [7]. Although we did not have detail traveling history data on this patient, our result indicates that the imported case (genotype D5) was possibly not related to any of the mentioned outbreaks in 2005 and 2006.

As to investigation of other pathogens, previous studies suggested that parvovirus, enterovirus and adenovirus could cause fever and rash, and HHV-6 could also cause infection in young children (8, 9). Hence, this study also investigate these four pathogens in cases excluded to have measles and rubella.

Erythema infectiosum, also called fifth disease, is caused by Parvovirus B19. The typical symptom is facial rash similar to slapped-cheek appearance. The rash is less around the nose and mouth. The rash will then spread to limbs and the trunk, and may be itching or stinging. The rash will have a reticular pattern after one week as the center fades. In the following weeks, the rash may recur after stimulation of temperature fluctuation or sunlight exposure. The route of transmission is by respiratory tract, but transmission by blood transfusion has been suspected in literature. Four cases were identified in 2005. Among them, one case was between 6 to 12 years old, one was between 12 and 18, and two were older than 18.

HHV-6 is related to roseola infantum, which occur most commonly in children younger than 3. Characteristic symptoms includes high fever, which may be up to 40℃, for 3 to 5 days, and rash that occurs after the fever subsides and starts from the trunk to limbs, neck, and face. The typical rash has diameter around 2 to 3 mm and fades upon light pressure. Results of the surveillance showed four positive cases among the 19 cases younger than 3. The ages were 4, 8, and 11 month-old, and 1year and 3 month-old.

Certain types of enterovirus and adenovirus may cause rash occasionally [8]. Was the enterovirus and adenovirus isolated from specimens related to the fever and rash? In one case in our surveillance enterovirus (CA21) was isolated and B19 IgM was also identified. Which one was the cause of the disease? Was it a concomitant infection? Without serum samples during the recovery phase, the cause could not be identified based on changes in antibodies. Enterovirus has also been isolated from cases without obvious changes in antibodies during the acute and recovery phases. Long-term surveillance is required to draw a

conclusion for diseases with measles-like rash.

Conclusion

Increasing herd immunity by a high vaccination rate can effectively prevent transmission of measles and rubella. The effectiveness of vaccination is clearly demonstrated by surveillance in 2005, since only sporadic confirmed cases of measles/rubella were identified. However, before global eradication can be achieved, importation from low vaccine coverage countries through traveling remains a potential threat to totally confine the disease. Strengthen the surveillance strategy on international travelers is an important aspect in public health network can never be underestimated.

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Table 1. Profiles of confirmed cases of measles/rubella in 2005

Measles Positive Cases									
Number	Age (year/month)	Onset dates of rash	Dates of specimens gathering	MV (date)	MMR (date)	Travel history (country)	Examination results	Types of specimens	Genotype
2005-09	14/3	2005/3/2	2005/3/4	yes	Yes (2002/4/12)	no	IgG conversion	whole blood, throat swab, urine	—
2005-10	1/2	2005/2/27	2005/3/3	no	no	no	Virus isolated	throat swab	H1
			2005/3/17				IgM positive	serum, urine	
2005-11	2/0	2005/3/8	2005/3/17	no	no	no	IgM positive	serum, urine	H1
2005-32	40/4	2005/11/12	2005/11/14	no	no	Yes (Germany)	IgM positive	serum, throat swab	D5
							Virus isolated	urine	
2005-M36	19/2	2005/12/5	2005/12/12	yes	yes	no	IgM positive	serum, throat swab, urine	
2005-39	0/9	—	2005/8/27	no	no	no	PCR positive	throat swab	H1
2005-R35	29/6	2005/11/7	2005/11/18	unknown	unknown	Yes (Japan)	IgM positive	serum	—
2005-07	0/9	2005/1/8	2005/1/27	Yes (2005/1/5)	no	no	Throat swab PCR positive	whole blood, throat swab, urine	A
			2005/2/23				IgG conversion	serum	
2005-13	0/10	2005/3/17	2005/3/28	Yes (2005/3/10)	no	no	Throat swab PCR positive	throat swab, urine	A
							IgM positive	whole blood	
Rubella positive cases									
2005-06	29/5	2005/4/21	2005/4/22	unknown	unknown	Yes (Philippines)	IgM positive	whole blood, throat swab, urine	—
2005-08	30/8	2005/5/11	2005/5/12	unknown	unknown	Yes (Philippines)	IgM positive	whole blood, throat swab, urine	—
2005-14	25/8	2005/6/18	2005/6/21	no	no	Yes (Indonesia)	IgM positive	serum, throat swab	—
2005-M25	15/11	2005/7/12	2005/7/15	unknown	unknown	Yes (Belarus)	throat swab virus isolated	throat swab, urine	—
							IgM positive	whole blood	—
2005-R36	51/6	2005/11/20	2005/12/5	unknown	unknown	no	IgM positive	whole blood, throat swab, urine	—
2006-01	45/5	—	2006/1/2	no	no	no	IgM positive	serum	—

Table 2. Pathogens identified in measles/rubella reported cases

Measles (40 cases)								Rubella (43 cases)						
Pathogens								Pathogens						
Age	cases	measles	Rubella	Adeno-virus	Entero-virus	B19	HHV-6	cases	measles	rubella	Adeno-virus	Entero-virus	B19	HHV-6
<3	14	3	0	1	0	0	3	6	0	0	0	0	0	1
3-6	0	0	0	0	0	0	–	2	0	0	0	0	0	–
6-12	6	1	0	0	1	0	–	5	0	0	0	0	1	–
12-18	6	0	1	0	0	0	–	7	0	1	0	1	1	–
>18	14	3	1	0	1	0	–	23	1	5	0	0	2	–
Total	40	7	2	1	2	0	3	43	1	6	0	1	4	1

Table 3. Negativity for antibody against measles/rubella among cases

	Measles (40 cases)			Rubella (43 cases)		
Age	Cases	Negative for measles antibody	Negative for rubella antibody	Cases	Negative for measles antibody	Negative for rubella antibody
<3	14	5	9	6	1	3
3-6	0	0	0	2	0	0
6-12	6	0	0	5	1	0
12-18	6	0	0	7	0	0
>18	14	0	1	23	3	0

Table 4. Statistics of clinical symptoms of measles/rubella

Measles (40cases)	Symptoms					
	Rash	Fever	Cough	Coryza	Conjunctivitis	Compatible with definition for report
Measles (7)	6 (85.7 %)	7 (100 %)	5 (71.4 %)	3 (42.9 %)	3 (42.9 %)	4
Negative (33)	32 (97.0 %)	26 (78.8%)	13 (39.4 %)	9 (28.1%)	6 (18.2 %)	11
Rubella (2)	2	2	0	0	0	0
Enterovirus (2)	2	2	1	0	1	1
Adenovirus (1)	1	1	1	1	0	1
HHV-6 (3)	3	2	1	2	0	1
Others (25)	25	21	10	6	5	8

Rubella (43)	Symptoms					
	Feverf	Rash	Conjunctivitis	Neck lymphadenopathy	Arthritis	Compatible with definition for report
Rubella (6)	4 (66.7 %)	4 (66.7 %)	3 (50 %)	3 (50 %)	1 (16.7 %)	2 (33.3 %)
Excluded cases (37)	25 (67.6%)	27 (73.0 %)	1 (2.7%)	8 (21.6 %)	2 (5.4 %)	6 (16.2 %)
Enterovirus (1)	1	1	0	0	0	0
B19(4)	2	4	0	1	0	0
HHV-6(1)	1	1	0	1	0	1
Others (31)	22	23	1	6	2	5

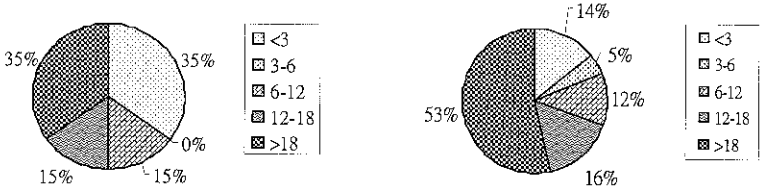

Figure 1. Distribution of ages of reported, cases of measles/rubella, 2005

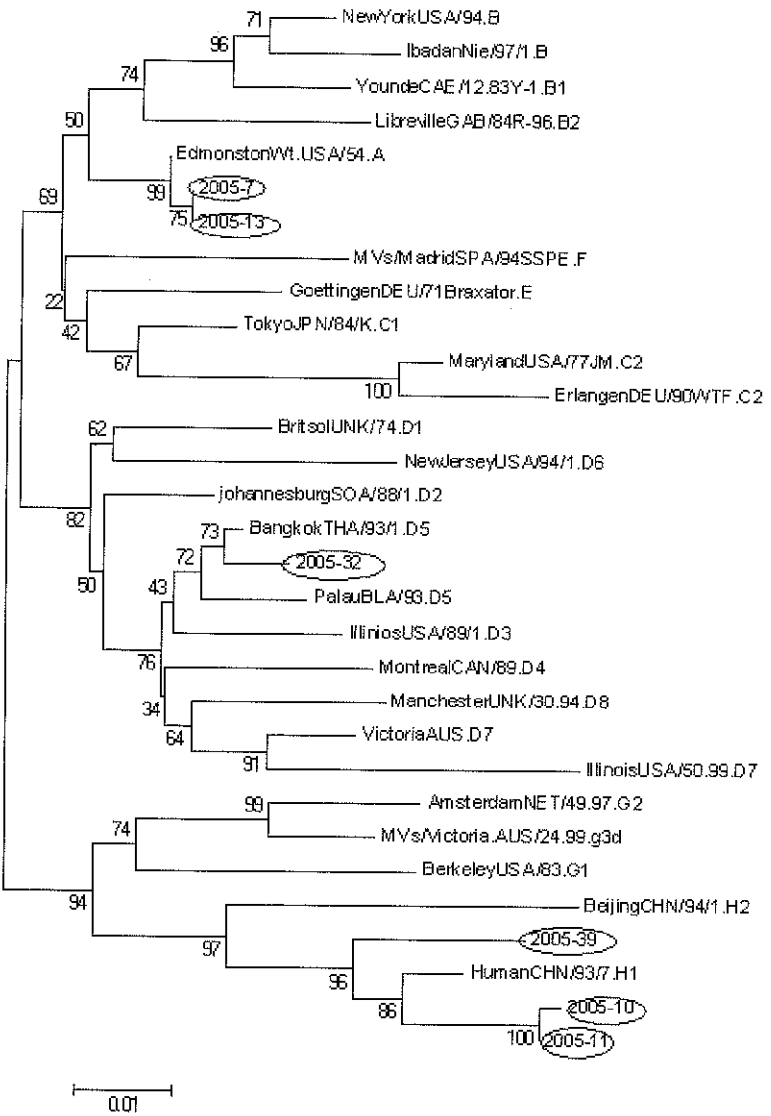

Figure 2. Phylogenic analysis of measles virus isolated in 2005. Molecular Evolutionary Genetic Analysis (MEGA) version 3.1 was used for analysis. Neighbor-joining with bootstrap for 1,000 times.