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Research Paper

Possible Spread of adenovirus type 3 from poultry to humans: indirect evidence from an outbreak in China

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Abstract

Objective: To explore the epidemiology and etiology for an outbreak of acute respiratory tract infection that occurred in one county of Jiangsu Province, China 2004. **Methods:** Only cases meeting the case definition were included in the study. We reviewed the medical records of the cases who were admitted to the local hospitals, interviewed cases by a standard questionnaire, and then described the epidemiologic features and analyzed risk factors by means of a case-control study. We collected pharyngeal swab specimens and sent them to different laboratories for isolation and culture. The laboratory used different detection methods such as DIF, PCR, electron microscope examination and microneutralization assay, to identify and then type the positive specimens. **Results:**A total of 871 cases were reported during the period from April 18 to July 4, 2004. The distribution of onset times presented two peaks, one in late May and another in middle June. The epidemic occurred mainly in the elementary and junior high schools in ten townships of one county, and the mean age of the cases was 12 years (range 7 months to 18 years). The course of the disease was acute, and was characterized by fever accompanied with sore throat and tonsillitis. The WBC count of cases was normal or elevated. The mean duration of illness was 5 days (range 2 to 12 days). No fatalities from illness were reported. A case-control study indicated that the possible risk factors were close contact with a case and/or poultry before onset and sharing of towels among members of the family. The typical CPE was observed through inoculating pharyngeal swab specimens into the HEP-2 cell cultures in different laboratories. An infection of adenovirus type 3 was verified by detecting positive specimens in different methods. **Conclusion:**This investigation demonstrated that the acute respiratory infection in cases was caused by adenovirus type 3. Cases occurred in over 70 schools in ten townships in 2004, and the route of transmission was possibly close contact with cases or droplet transmission.

Keywords: adenovirus; acute upper respiratory infection; outbreak

INTRODUCTION

Adenovirus infections in humans are ubiquitous. Adenovirus can cause coryza and pharyngitis in infants, upper respiratory, pharyngoconjunctival fever, diarrhea and haemorrhagic cystitis in children, acute respiratory disease and pneumonia in young adults, and epidemic keratoconjunctivitis in adults. Adenovirus is a non-enveloped icosahedral DNA virus. Over 100 serotypes have been discovered since the first successful adenovirus isolation in the 1950's. At least fifty-one serotypes belong to the human adenovirus group and can be divided into six different subgroups (A-F).

Various clinical syndromes may result from infection depending on the viral serotype causing disease and

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the immunologic status of the host.

We describe an outbreak of acute respiratory infection due to adenovirus type 3 that occurred in one county of Jiangsu Province, China, during the period from April 18th to July 4th 2004. Cases experienced fever, cough, sore throat (pharyngitis) and tonsillitis. Despite the mildness of the illness, it was characterized by high transmissibility and rapid spread.

MATERIALS AND METHODS

Patients and case definition

The clinical case definition:

Persons lived in the initial and neighborhood townships with illness onset after April 2004 and displaying the following symptoms: Fever(axillary temperature over 38℃), accompanied by manifestations of upper respiratory tract infection such as cough, headache, and sore throat, plus one or more of the followings:

a. pharyngitis, tonsillitis, or conjunctivitis;

b. normal or slightly increased WBC count;

c. thickening lung veins by chest X-ray.

Students who were reported as having a fever (temperature measured by local hospital doctors) were required to report to the local County CDC and then were determined whether meeting the clinical case definition by epidemiologists. The clinical records of some cases who were admitted to the local township health care hospitals were reviewed. Local public health personnel interviewed the cases with a standard questionnaire.

Specimens-swab and serum

Pharyngeal swab specimens from children and adolescent patients who were diagnosed with acute upper respiratory tract infections at Township A health care hospital during the outbreak from April through July 2005 were cultured for adenovirus. Swab specimens of healthy children in the Nanjing area, China were also included in the virological analysis were used as a control group during the same time period.

Acute and convalescent serum specimens collected from patients admitted to the local health care hospital were tested for adenovirus antibodies. Other serum specimens were collected randomly from healthy students as control serum from the same schools and same grade but in different classes where no cases were reported.

In addition, 57 specimens from healthy animals such as swine, sheep, poultry, duck and five flies were collected randomly to test for adenovirus.

Rapid Test-Viral DNA extraction and detection

DNA extraction was performed according to the directions of Kit(QIAGEN Company Cat. No. 51106) and TaqMan 2 × PCR Master Mix(LOT:F07868)

Nested polymerase chain reaction(PCR) was used to detect Adenovirus DNA[1]. After amplification two times, the length of the amplificatory objective fragment was 301 bp through 1% agarose electrophoresis to the amplification products, by using the PTC-200 PCR instrument.

Real-time PCR was also used to detect adenovirus DNA[2] using the RG-3000 fluorescence quantitation PCR instrument.

All the primers and probes needed in PCR detection were synthesized by Shanghai Bio-Engineer Biology Company.

Rapid Test-Anti-adenovirus IgM antibody detection

Enzyme-linked immunosorbent assay(ELISA) was used to detect IgM antibody in the patient's acute serums, using EUROIMMUN Company LOT: 030509 Kit.

The enzyme standard equipment/instrument from SUNRISE Company was used to measure the value of A450 with double waves(measurement wave 450 nm, reference wave 620nm). Test designed 200 RU/ml, 20 RU/ml, and 2 RU/ml three adjust standard samples and the A450 value of sample was among the allowance scope. A sample with A450 value equal to or greater than 20 RU/ml of standard sample A450 value was considered positive.

Viral culture and identification

Pharyngeal swab specimens were inoculated into human esophageal type 2(HEP-2) monolayer cell cultures(kindly provided by Prof. Wen-Bo Xu, Institute for Virosis Control and Prevention of National Center for Diseases Control and Prevention)[2]. These cell cultures were maintained in Eagle's minimum essential medium with 2% inactivated calf serum and 5% CO_2 at 37℃ for 14 days. The media were changed every 7 days. The cell cultures were observed for 3~4 weeks under the inverted microscope for confirmation of characteristic cytopathic effect(CPE) by indirect immunofluorescent antibody assays.

The cell cultures were examined for characteristic cytopathic effects(CPE) every day. After 10 to 15 days, the cell cultures with CPE continued to passage and the cell cultures without CPE stopped blind passage after 3 generations. Three continuous and stable generations with CPE was judged as adenoviral culture positive, and a blind passage for 3 generations without CPE or that cell cultures coud not passage continuously and stably was considered negative.

After a 10-fold dilution, the new isolate viral suspending fluids were inoculated into the HEP-2 cell cultures. Each dilution was divided and inoculated into

four tubes and cultured with 35℃and 5% CO_2. The $TCID_{50}$ of the isolate viral strains was calculated according to the highest dilution degree with CPE present.

The positive specimens were frozen and thawed repeatedly and then centrifugated for 30 minutes with 4 000 r/min. Supernatant samples from positive specimens with typical CPE, after negative staining, were observed and filmed under the H-600A type transmission electron microscope.

Serotyping of adenoviruses

Adenovirus serotypes were determined by use of neutralization assay with type-specific reference antiserum (anti-ADV 3 serum, provided by Prof. Jing-Ming Zhao, Capital Paediatric Institute, Beijing) and HEp-2 cells, and the neutralization antisera concentration was against 100-fold $TCID_{50}$.

In addition, Multi-primer real time PCR was also used to identify the serotype of isolated adenovirus with adenovirus type 3(Ad3) and adenovirus type 7(Ad7) as primers according to the methods used in reference [3].

Viral serological detection

A microneutralization assay was used to detect the titer of patients' paired (acute and convalescent period, interval of 2 to 3 weeks) serum with the identified adenoviral strains isolated from the patients admitted to the local health care hospital. A 4-fold or greater rise in titer between the acute and convalescent serum was considered diagnostic for a recent adenovirus infection.

Case-control study

To identify risk factors for illness, a case-control study was performed. Risk factors evaluated included contact with a case, contact with poultry, sharing towels in the family, egg and milk consumption, and hand-washing (frequent classified three grades as over 3 times per day, 1~2 times per day and never). Fifty cases and fifty-eight controls were randomly selected from two schools for the unmatched case-control study. A standardized questionnaire was administered to the cases and controls by face-to-face interview. Data obtained form the epidemiologic investigation and review of clinical records was entered into Microsoft Excel 2000, and SPSS 10.0 was used for statistical analysis.

Reference laboratory

Some of the pharyngeal swabs of the acute patients were sent to the laboratory of National Centers for Diseases Control and Prevention, Beijing and Key Laboratory of Public Health Safety, School of Public Health, Fudan University, Shanghai.

RESULTS

Epidemiology

As shown in ***Fig 1***, during April 18th to July 4th 2004, 871 cases met the case definition from one north central county of Jiangsu Province, China which holds 23 townships with a population of 1,162,000. Six hundred and thirty two of 871cases(72%) were admitted to the local health care hospital. The outbreak occurred in 75 of 168 schools(45%) in ten townships of the county. But there were 86.4% classes with case and the proportion of class with case over three attained 28.2% in township A. (Shown in ***Tab 1***) Initially, the outbreak affected only 19 junior high schools, elementary schools and kindergartens in two townships. The reported cases in the two townships were 351(40%) and 157(18%) respectively. (Shown in ***Tab 2***)

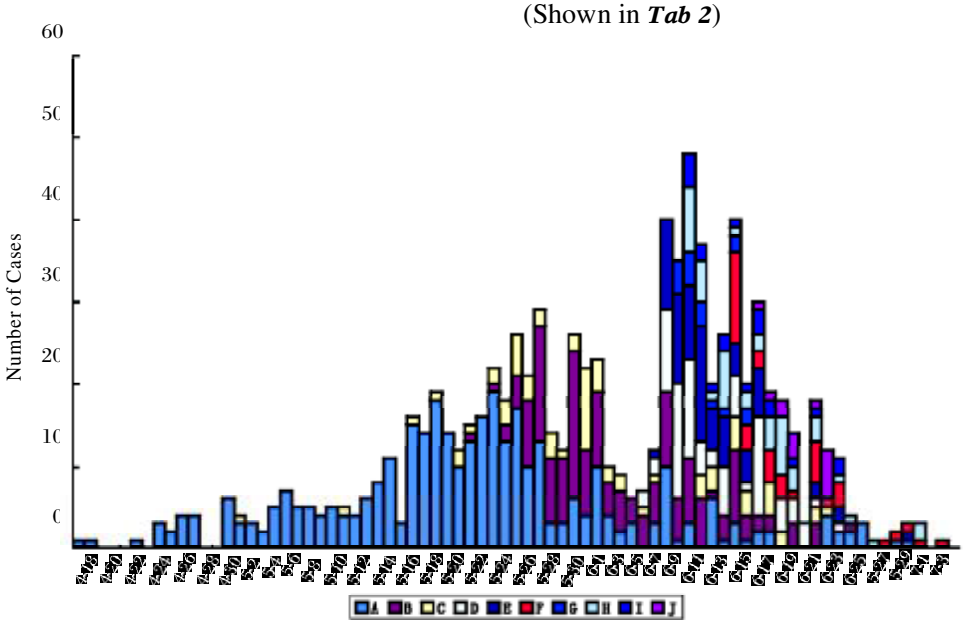

Fig 1 The epidemic curve from an outbreak of adenovirus type 3 in one county of Jiangsu Province, China 2004

Tab 1 **School and Class distribution of mild acute respiratory infection cases in China due to adenovirus type 3**

Township	Total Schools	Schools with cases	Total number of classes	Classes with cases	Classes with over 3 cases	Maximum number of cases per Class
A	30	24	103	89	34	24
B	16	8	102	44	8	6
C	11	8	72	19	4	14
D	27	7	207	31	1	3
E	12	8	161	18	2	2
F	18	4	74	8	0	2
G	13	5	84	13	0	2
H	22	7	123	14	2	4
I	19	4	122	4	1	3
Total	168	75	1048	240	52	24(Max.)

Tab 2 **Age and township distribution of mild acute respiratory infection cases in China due to adenovirus type 3**

Township	Age Group(year)					proportion(%)
	0~4	5~9	10~14	15~19	Total	
A	12	94	170	75	351	40.3
B	31	78	34	14	157	18.0
C	3	45	9	11	68	7.8
D	3	50	23	6	82	9.4
E	1	34	17	24	76	8.7
F	0	25	10	3	38	4.4
G	1	7	7	2	17	1.95
H	0	18	21	9	48	5.51
I	1	8	9	2	20	2.30
J	0	10	4	0	14	1.61
Total	52	369	304	146	871	
Proportion(%)	6.0	42.4	43.9	34.9		

The attack rate in school students in the 10 townships was 1.7%, but the attack rate in Township A was 5.9%, and Township B was 2.5%. For individual schools, the highest attack rate(25.6%) was one kindergarten in Township C, and the second highest attack rate(23.5%) was the Township A elementary school. For individual classes, the highest attack rate(56.9%) was class one, grade three in the Township A elementary school. From epidemiologic analysis of one class in the junior high school and three elementary schools in Township A, we found that the cases aggregated within classrooms, and the mean secondary attack rate in the classes ranged from 49.1% to 59.5%.

The index patient had onset of illness on April 18, 2004. Most of the case onsets were from mid May through middle June. The first peak occurred in late May, and the second one occurred in mid June. After June 24, the cases began to decrease, and the reported number was less than 5 cases per day in all ten townships. Epidemiological surveys showed that there was no significant association and assembly among the new cases.

Initially, the epidemic was limited to Township A, but Township B was implicated on May 29, and Township C on June 1, Township D on June 9, Township E and Township F on June 11, Township G and Township H on June 14, Township I on June 15, and Township J on June 16.

Most of the cases were students and children under the age of 18 years. The age of cases ranged from 7 months to 18 years, and most cases were between 10 and 14 years of age(43.9%)(Shown in ***Tab 2***). The median age of cases was 12 years. The outbreak happened primarily in the elementary and junior high school although some pre-school children were involved. No adult cases were reported. The male to female ratio of cases was 1.26 to 1, and the similar ratio of students in schools was 1.13 to 1 (RR=1.10, 95%CI: 0.94~1.37).

The average incubation period was 6 days(range 2 to 12 days) based on 56 cases who had a definite history of having contact with other patients before onset.

Clinical status

The clinical course in patients with acute respiratory infection characterized fever and cough as the first symptoms. The temperature fluctuated between 38.0℃ and 40.4℃, and the mean duration of fever was 5.3 ± 2.3 days(range 2 to 10 days). Most of the cases presented with a pattern of remittent fever or irregular fever and a few presented with a pattern of continued fever.

Of 379 cases with complete information, 379(100%) had fever, 346(91.4%) pharyngeal congestion, 224 (59.1%) swelling of tonsil, 182(48.0%) sore throat, and 119(31.4%) cough. Some of the cases had the symptoms of headache, dizziness, snuffle, rhinorrhea, diarrhea, vomiting, lymphadenitis below the jaw, and pharyngoconjunctival congestion. None of the cases had extrapulmonary manifestations such as toxemia, neck stiffness, rashes, arthralgia, or palpitation. 56.2% of cases had a WBC count of greater than 10×10^6/L. 55.1% of cases had a neutrophil count of greater than 70%. The thickening lung veins observed in X-rays occurred in 90.2% of cases. One percent of cases admitted to hospitals were diagnosed with pneumonia. Liver function tests, renal function tests, myocardial enzymes, and routine urine tests ranged within normal limits. No fatalities occurred. The mean duration of illness courses was 5.1 ± 2.0 days (range 2 to 10 days).

Case-control study

Significant factors for illness included contact with other cases, contact with poultry, and sharing towels among the members of a family. Significant risk factors which were protective against illness were frequent hand-washing, egg consumption and milk consumption. (shown in ***Tab 3***)

Stratification of data analysis was performed to evaluate confounding and interaction between the risk factors of contact with a case and contact with poultry. The results indicated that contact with poultry was still a significant risk factor despite removing the effect of contact with other cases. (Shown in ***Tab 4***)

Tab 3 **Epidemiologic Results of a case-control study**

Factors	Case	Control	OR	95%CI	P-Value
Contact with another cases	14/50(28%)	1/58(1.7%)	22.2	2.8–175.9	<0.001
Contact with Poultry	12/50(24%)	3/58(5.2%)	5.8	1.5–21.9	<0.006
Sharing Towels in the family	28/50(56%)	17/58(29%)	3.1	1.4–6.8	0.006
Egg consumption	25/50(50%)	45/58(77%)	0.3	0.1–0.7	0.004
Frequent hand washing	29/50(58%)	46/58(79%)	0.4	0.2–0.8	0.02
Milk consumption	10/50(20%)	22/58(38%)	0.4	0.2–1.0	0.057
Family poultry farm	21/50(42%)	20/58(35%)	1.4	0.6–3.0	0.435
Neighbor poultry farm	23/50(46%)	37/58(64%)	0.5	0.2–1.1	0.081
Family cattle farm	1/50(2%)	0/58(0%)	undefined		0.46

Tab 4 **Results of stratification analysis with contact with a case as the stratifying variable**

Contact with poultry	Contact with a case			No contact with a case		
	case	control	total	case	control	total
Yes	4	0	4	8	3	11
No	10	1	11	28	54	82
total	14	1	15	36	57	93
			$P = 0.73$	OR=5.14	95%CI:1.26–20.92	$P = 0.0136$

*OR_{Adjust}=5.44, 95%CI(Adjust):1.34–22.14, $P < 0.0116$

A retrospective cohort survey performed early in the outbreak in the junior high school of Township A indicated that students living in school had a higher risk than those who lived in their own home.(χ^2=21, $P <$ 0.001, RR=1.78, 95%CI:1.38~2.30)

Laboratory test

Adenovirus DNA detection

A total of 112 pharyngeal swab specimens were collected from acutely ill patients admitted to the local health care hospital.

Nested PCR was used to test 80 pharyngeal swab specimens collected from the patients and 20 specimens from controls. The positive rate was 60%(48/80) and 0%(0/20), respectively. The difference between the two groups was statistically significant($\chi^2 = 23.0769$, $P <$ 0.0001). Real time PCR was also used to test another 32 pharyngeal swab specimens from the acutely ill patients, and the positive rate was 62.5%(20/32). The detection rate between the two PCR methods to detect viral DNA displayed no significant statistical difference.

In addition, using the two same PCR test methods, other pathogens which can cause acute respiratory diseases(ARDs) such as influenza, parainfluenza, RSV, SARS,coronavirus, enteric virus, *Mycoplasma pneumoniae* had also been tested and been ruled out as the cause of illness.

Anti-adenovirus IgM antibody test

Twenty acute period patient sera and ten control sera were tested for anti-adenovirus IgM antibody. The positivity rate was 45%(9/20) and 10%(1/10), respectively. The detection rate of anti-adenovirus IgM antibody in

cases was higher than that in controls, but the results were not significant(Fisher exact *P*-value equal to 0.10).

Viral culture and identification

Eighteen viral strains were isolated from 35 pharyngeal swabs through inoculating specimens into Hep-2 cell cultures, and the isolation positivity rate was 51.4%. The $ICID_{50}$ of isolated viral strains was between10^{-3} and 10^{-4}. The typical CPE characters of adenovirus was observed under inverted microscope, where infected cells began swelling, became round, and developed into a grape cluster appearance in the end(***Fig 2, 3***)

The typical CPE, presented with a crystal lattice array of many adenovirus-like particles in the cell nucleus observed under negative staining electronic microscope. This CPE occurred in 13(65.0%) of 20 pharyngeal swabs by means of Hep-2 cell cultures in the laboratory of the School of Public Health, Fudan University, but no CPE occurred in the cultures of MDCK(35th generation), LLC-MK2, Vero E6 and MRC-5(39th generation). The presence of adenovirus was further confirmed by direct immunofluorescence(DIF) and polymerase chain reaction (PCR).

Morphology of virus

The diameter of the isolate virus under the electron microscope was about 80 nm with non-enveloped particles, which had an icosahedral cubic symmetric structure. The virus presented the typical morphology of adenovirus with a regular array of particles on the surface of triangle, two penton bases located on both sides and four hexon monomers located in the center of every line of the triangle(See ***Fig 2***).

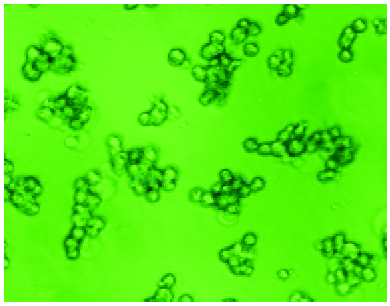

Fig 2 HEP-2 cells gathered and presented the cluster of grapes in the late phase of CPE(× 400)

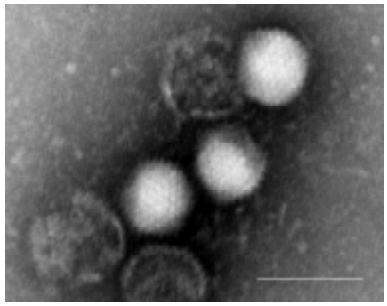

Fig 3 Electronic microscope photograph of isolate strains, where viral particles presented the typical adenovirus morphology(Bar=100 nm)

Multi-premier PCR was used to test 8 isolated viral strains using Ad3 and Ad7 primers, 4 viral strains were detected as adenovirus type 3 and no viral strains were detected as adenovirus type 7.

An infection caused by adenovirus type 3 was verified by entire gene sequence testing to 10 Nested PCR amplification products of positive specimens (from nine patients) in the laboratory of the National CDC of China.

Paired serum detection

Eighteen paired patient serums(acute and convalescent) were used to test neutralization titer with the isolate adenovirus type 3 viral strain simultaneously. Four-fold or a greater increase of neutralization titer in the acute period compared to the convalescent period occurred in 13 pairs, and the conversion rate was 73.2%.

Complement fixation tests were also used to detect the adenovirus neutralization titer of 10 paired patient sera. For three paired specimens there was a four-fold or more rise in adenovirus neutralization titer, for three paired specimens there was a two-fold rise, for three paired specimens the titer rise was less than 1:8, and for one paired specimens a four-fold decrease was found.

DISCUSSION

Adenovirus type 3(Ad3) and Ad7 have usually been associated with outbreaks of acute respiratory tract infections. The common types of adenovirus, which can lead to acute pharyngoconjunctival fever in children, have been type 3, 4, and 7[4]. Ad3 and Ad7 are responsible for most epidemics or outbreaks of LRTIs in children[5-7]. Three outbreaks caused by respiratory adenovirus infection were reported in Taiwan, China from 1999 through 2001. The epidemic viral strains isolated from the first outbreak that occurred from November 1999 through January 2000 was Ad3 and Ad7. The second outbreak that occurred from September to October 2000 and the third outbreak that occurred in September 2001 were primarily Ad4[8].

Outbreaks caused by adenovirus have been reported in China and other counties in recent years[10-12]. Three pharyngoconjunctival fever outbreaks caused by adenovirus type 3 were reported in China in 2005[8-10]. Research has shown that Ad3 is isolated more frequently

than other adenovirus serotypes in subgroup B. In Japan, isolation of Ad3 from infants and children with acute gastroenteritis increased rapidly from 4.0% in 1998 to 30% in 2001 [13]. A survey of successive adenovirus outbreaks in children during an 11-year period indicated that mortality was 3.6%[14].

This investigation demonstrated that acute respiratory infection caused by adenovirus type 3 caused the outbreak that occurred in over seventy schools in ten townships in 2004. The outbreak was very unusual because of the large number of children affected and the wide geographic areas involved. The most common manifestations resulting from adenovirus infection involved the respiratory and gastrointestinal tracts, but cardiac, neurological, cutaneous, urinary, and lymphatic manifestation also occurred[15]. In this outbreak, most of the cases had only signs and symptoms of respiratory diseases, some had manifestations of gastrointestinal tract diseases, but almost all of the cases had no extrapulmonary manifestation such as conjunctivitis, otitis media, lymphadenopathy, bleeding diathesis, anemia, hepatomegaly, splenomegaly, exanthema, neurologic signs and renal signs[16].

The results of the epidemiological study indicated that contact with a case, sharing towels among members of a family, and contact with poultry before onset was the main risk factors. Infection was probably transmitted through close contact and or droplet transmission, but fecal-oral transmission can not be excluded with evidences.

A large poultry farm with a hennery was located in Township A, the first township to be infected. The number of poultry was estimated at over 10 million in total. Most of the poultry houses were constructed near human residences and the poultry manure was piled on the road. Adenovirus infection can be seen in people, poultry, cattle, dog, rats, pigs and monkeys and be classified as Mastadenovirus and Aviadenovirus. Aviadenovirus or fowl adenovirus(FAV) was once thought impossible to infect mammals including hu-man beings for the reason of strict host specificity [17]. The results of the case-control study identified contact with poultry as one of the risk factors even after the effect of contact with cases was removed.(OR_{Adjust} = 5.4381, 95% CI:1.5297-21.9120). However, we did not detect out viral DNA from 57 animal specimens and five flies specimens using the fluorescence quantitation PCR assay. The lack of identifying virus in the poultry specimens could have been due to an insufficient number of poultry in our sample. Also possibly, an outbreak had occurred in the poultry population but was over by the time when investigators began testing poultry. If infection in humans did result from close proximity to poultry farms, this has implications for avian influenza transmission as well. Determining what specific kinds of contact with poultry put humans at greatest risk for such infections is very important for prevention.

The etiologic agent causing the outbreak was verified by three different laboratories, but some risk factors are still unknown. A single infectious source was not identified through field epidemiological investigation and not enough evidence supports the idea that cases from all other townships were infected from Township A.

Each adenovirus serotype can be further divided into genome types based on the patterns of digestion of their DNA with restriction enzymes, and Ad3 has been shown to have at least seven genome types with DNA restriction analysis [14]. Among genome types of Ad3 that have been identified to date, Ad3p are the prototype, and there are Ad3a through Ad3x and their variants. In China from 1962 to 1988, the dominant genome types were Ad3a2, with occasional isolates of Ad3a4, Ad3a5, and Ad3a6 [18]. The viral etiology study of an acute conjunctivitis outbreak that happened in Shenzhen Areas in the summer of 1997 was performed by He Yaqing, et al[19] and also demonstrated that the main viral strain was Ad3a2. In many outbreaks, the adenovirus viral strain can not be isolated and the genome type can not be identified[20]. Genome types may be useful for epidemiological studies. It is important to identify the variability in epidemic viral strains on time and relate specific strains with clinical manifestation and severity. Analysis of genome types may be of clinical as well as epidemiological importance. Future studies of adenovirus in poultry should examine if unique or similar strains circulate in that population.

Initially, a bacterium, such as streptococcus was suspected as the etiologic agent in this outbreak. Support for a bacterial etiology included fever, sore throat, redness and swelling of the tonsil. Also the majority of cases admitted to hospital had a white blood count presentation of normal to elevated(mainly neutrophils) and some of the cases presented ASO rise in convalescent period(6 positive of 17 specimens) compared to acute period(only 1 positive of 8 specimens). Furthermore, streptococcus A(α -hemolysis) was cultured and isolated from all 7 pharyngeal swab specimens collected on May 29, 2004. Another three persons were cultured positive for Streptococcus viridans by means of blood plate culture in the laboratory of Shanghai Fudan University. Therefore, use of oral antibiotics as prophylaxis was suggested for the key population in early June. However, cases occurred in people who were given antibiotics. We eliminated Streptococcus as a cause of illness based on the carrier rate of ASO of 30% in healthy people, no cases had signs of rash, 5%~10% of cases

had a recurrent fever some time after recovery, and Streptococcus would be a very unusual cause of a large community outbreak[21]. Also the course of illness for most cases was still over 5 days after use of antibiotics. Concurrent infection of adenovirus and other pathogens has been reported[22].

Prevention measures included screening the students every morning and surveillance in clinics to identify patients as early as possible. Patients were then isolated for at least two days after their symptoms disappeared and temperature returned to normal ranges in a hospital or at home and the epidemic was under control. Before using antibiotics as prophylaxis the cause of illness should be verified through laboratory testing. Before the confirmation of pathogen through viral culture, PCR detection of relating viral DNA and serology methods, it was very difficult to distinguish adenoviral diseases from a bacterial diseases based on clinical manifestation, white blood cell count, and chest X-ray findings. Although normal WBC count or leucopenia may be seen after a viral infection, the WBC count may be normal or may be elevated leukocytosis consisting mainly of neutrophils in the adenovirus infection. Progression of the clinical course despite antibiotic therapy and presence of a normal or elevated WBC count suggests the possibility of adenovirus infection[23].

In conclusion, the big outbreak caused by adenovirus type 3 was confirmed first based on epidemiological characteristics and laboratory test results as performed in Jiangsu province, China. Our finding that contact with poultry may be one risk factor is possibly important because of our increased awareness of avian influenza and the risk of transmission from poultry.

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