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## An outbreak of salmonellosis traced to consumption of cream cakes

Non-typhoidal salmonellosis is an acute food-borne disease caused by multiple serotypes of the bacterium, *Salmonella*. The infection has a short incubation period which ranges from 6-72 hours. Clinical features commonly manifest as fever and gastroenteritis with sudden onset of abdominal pain, headache, diarrhoea, nausea, and/or vomiting.<sup>1</sup> One of the commonest causative agents of this disease is *Salmonella* Enteritidis.<sup>2</sup> The incidence of gastroenteritis caused by *Salmonella* Enteritidis has increased in the last decade while outbreaks have been reported since the 1970s.<sup>3</sup>

In Nov-Dec 2007, the Ministry of Health noted an unusual increase in clusters of cases reporting gastroenteritis associated with the consumption of cakes. The first cluster was notified on 23 Nov 2007 and involved 15 people who developed symptoms after eating cream cake on 21 Nov 2007.

### Epidemiological investigations

As soon as the unusual increase in gastroenteritis was noted, epidemiological investigations were stepped up to determine the source of infection and mode of transmission. Stool analyses in a number of cases had detected the presence of *Salmonella* Enteritidis. We defined a case as a person who suffered symptoms of gastroenteritis with or without fever within 72 hours after having consumed cake in the period of Nov-Dec 2007. All reported cases were interviewed and information obtained on their clinical symptoms, date of onset of illness, food items eaten, food establishments visited, medical treatment sought, and other relevant epidemiological data.

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Preliminary findings from the investigations showed that cases were distributed all over Singapore and not restricted to any particular locality. They had purchased and consumed a variety of cakes from different retail establishments located throughout the island, and no specific cake outlet could account for all the cases. One important observation was that these establishments were all franchisees of a large and well known local confectionery.

A case-control study was initiated to determine the specific vehicle of transmission. Besides a detailed food history on the cakes that were eaten, enquiries were also made on the cases' exposure to other risk factors such as contact with pets or anyone who was ill, consumption of poultry or dairy products, and involvement in food preparation. For every case, attempts were made to obtain controls from the same household, workplace, school and community and they were asked identical questions. Differences in food-specific attack rates between cases and controls were analysed using SPSS version 15.0 (SPSS Inc., Chicago, IL). Chi square and Fisher's exact tests were used to determine significant differences between proportions. To quantify the extent of risk, odds ratio and the corresponding 95% confidence intervals were computed.

Further investigations and inspections were made into the food supply and methods of food preparation in various establishments and the confectionery. Special attention was paid to the cake factory and its line of production and distribution. Samples of the implicated cakes and raw ingredients were collected from the factory as well as cake outlets for microbiological examination. Two remnant samples of cake were also obtained from the cases and sent for analysis. All the food samples were tested in the Singapore General Hospital's

Food and Water Laboratory. Samples that were positive for *Salmonella* were subsequently serotyped, and phage typed in the molecular laboratory.

### Outbreak control

Outbreak control measures were concurrently implemented during epidemiological investigations. The factory was ordered by the licensing authority (Agri-Food & Veterinary Authority) to cease production and recall all implicated cake products from their franchisees on 30 Nov 2007. The factory was subsequently ordered to cease all manufacturing activities on 4 Dec 08 and retail establishments were closed on 5 Dec 08. Both factory and retail establishments underwent thorough disinfection. All food handlers from the factory and cake outlets were considered suspects and referred to the Communicable Disease Centre for stool screening. Legal orders were served on those found to be positive for food-borne pathogens to require them to refrain from engaging in the preparation of food until certified fit. They were allowed to return to work only after they had been re-screened and confirmed to be no longer positive.

The public was alerted of the outbreak and advised to discard all cake products purchased from the implicated retail establishments. At the same time, they were advised to observe proper food and personal hygiene, to store high-risk food items appropriately, and not to consume food items that had passed their expiry or use-by dates. An advisory on salmonellosis was also made available on the Ministry of Health's website.

Transmission ceased following closure of the factory, and the last case reported onset of illness on 4 Dec 2007.



## Results

A total of 216 cases of gastroenteritis were identified with onset of illness between 20 Nov and 4 Dec 2007 (Fig. 1). 14 tested positive for *Salmonella* Enteritidis, while the rest were culture negative or not tested but epidemiologically linked to the outbreak. The main presenting symptoms were: diarrhoea (96%), fever (63%), vomiting (60%) and headache (16%). The median incubation period was 12.3 hours (reported range, 3-139 hours). The cases were aged 1-78 years (median age, 29 years) with a male to female ratio of 1. Among the major ethnic groups, Indians were not represented while Chinese accounted for 70.4% of cases; Malays, 27.3%; and others, 2.3%. 18(8.3%) of the cases were hospitalised while the remainder had outpatient treatment or self medicated.

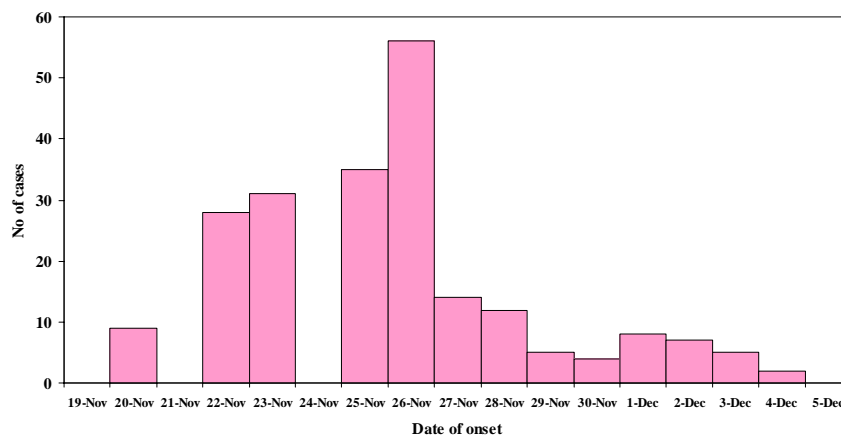
These cases comprised a total of 38 separate incidents that occurred either in small clusters, or singly and sporadically. In our case-control study of the exposure risk factors among 55 cases (from four clusters) and 39 controls, a statistically significant association was found between their illness and consumption of cream cakes from one specific confectionery

(Table 1). No other risk factors such as contact with family members who were ill, or pets, and consumption of poultry or dairy products were implicated.

Health inspections uncovered a number of hygiene irregularities in the factory. High-risk food items such as cream were left at ambient temperature for prolonged periods. Ready-to-serve cakes were also not immediately kept in chiller conditions with temperature display to prevent bacterial contamination and multiplication. In addition, the layout of the kitchen was such that the manufacture of semi-processed and finished food products was not separated. Utensils and working surfaces were also not cleaned frequently enough and posed risks of cross-contamination.

The food samples analysed for food-borne pathogens comprised 44 raw ingredients, semi-finished products and finished products obtained from the factory, 23 finished products sampled from nine of 38 cake outlets, and two cake remnants provided by the cases. Of the factory samples, five semi-processed and finished products (chocolate cream, truffle chocolate cream, whole hazelnuts and two hazelnut pastes taken on different days) tested positive for *Salmonella* En-

**Figure 1**  
Onset of symptoms of 216 cases linked to consumption of cream cakes, 20 Nov - 4 Dec 07



teritidis. Laboratory investigations also showed other coincidental bacterial contamination. One food sample showed high bacterial count and another tested positive for *Bacillus cereus*. Eight cake samples taken from five outlets also tested positive for *Salmonella* Enteritidis and showed concomitant high bacterial count. Of the cake remnants provided by the cases, one was positive for *Salmonella* Enteritidis and the other for *Salmonella* Group C.

Six (3.4%) of 176 food handlers and staff from the factory and four (1.7%) of 232 from the cake outlets also tested positive for *Salmonella* Enteritidis. As an incidental finding, three other food handlers (two from the factory, one from the cake outlets) were positive for *Salmonella* Group C, and another food handler (from cake outlet) for *Salmonella* Group E.

The *Salmonella* Enteritidis isolates were further analysed by phage typing (Table 2). Typing results of isolates from the food handlers, food samples and cases

were either phage type 1 or RDNC (reagents did not conform). The antibiogram pattern for each phage type was the same. Ribotyping results also showed that the *Salmonella* Enteritidis isolates were closely related genetically.

## Comments

The epidemiological features in this outbreak were consistent with a pattern of common source infection. The onset dates of the cases were between 20 Nov and 4 Dec 2007 and based on the known incubation period of 6-72 hours, the period of exposure was narrowed down to between 20 Nov and 1 Dec 2007. Supply of contaminated raw eggs or other raw ingredients from a source farm during this time was ruled out as it would have affected non-cake products and led to a more generalized outbreak. The implication of multiple retail establishments suggested that contamination had occurred somewhere after the farm but prior to the point of distribution and sale, and hence

**Table 1**  
**Analysis of risk factors in an outbreak of salmonellosis, Nov-Dec 2007**

Risk factors	Cases (n=54)			Controls (N=39)			P value	OR	95% CI
	Exposed	Not exposed	% exposed	Exposed	Not exposed	% exposed			
Cream cakes (from one specific confectionery)	52	2	96.3	6	33	15.4	<0.001	143	27.23-759.10
Contact with family members who were ill	11	43	20.4	9	30	23.1	0.754	NS	NS
Contacts with pets	10	44	18.5	5	34	12.8	0.573	NS	NS
Involvement in food preparation	13	41	24.1	8	31	14.5	0.685	NS	NS
Consumption of chicken	20	63	37.0	27	12	69.2	0.002	NS	NS
Consumption of dairy products	9	45	16.7	25	14	64.1	<0.001	NS	NS
Consumption of eggs	6	48	11.1	23	16	59.0	<0.001	NS	NS

NS – not statistically significant



probably in the factory. Our case-control study implicated consumption of cream cakes produced from one specific confectionery and supported the likelihood that contamination had indeed occurred in the factory.

The microbiological findings of *Salmonella* Enteritidis in the factory cake products as well as semi-processed products (ie truffle chocolate cream, hazelnuts and hazelnuts paste) pointed to widespread contamination in the cake and decoration area of the factory. Three of the six food handlers who tested positive for *Salmonella* Enteritidis had worked in this area and two routinely taste-tested the quality of the ingredients. Since they were asymptomatic and did not report experiencing illness previously, it could not be ascertained if they were victims rather than the cause of the outbreak. Nonetheless, these infected food handlers could spread the infection via the work surfaces, utensils and food ingredients if hygiene practices were insufficiently observed. The molecular phage typing results re-affirmed this important

epidemiological link between the contaminated cake products, cake ingredients and food handlers at the factory.

In the preparation of the cream cakes at the factory, it was noted that the 'Truffle Cake', 'Truffle Divine Cake' and 'Hazelnut Indulgence' used in-house processed butter cream while for the other cakes, ready-to-add fresh cream was used. Butter cream was a key semi-processed ingredient used in the icing of the cream cakes. A closer inspection into the production of the butter cream revealed that the butter cream was made from sugar syrup boiled at high temperature (120°C), half-whisked liquid egg-white and butter. Subsequently, other ingredients such as chocolate paste or hazelnut paste were mixed into the butter cream to form chocolate cream or hazelnut cream, respectively. The cream was then iced onto the chocolate sponge bases and the cakes were decorated to form the final product. There was no further baking of the cakes. Hence, any *Salmonella* introduced would have multiplied if there was a lapse in temperature control

**Table 2**  
**Phage typing of isolates of *Salmonella* Enteritidis**

	Number of isolates analysed	No. of phage type 1 isolates	No. of RDNC isolates
<b>Food Handlers</b>			
Factory	6	3	3
Outlets	3	2	1
<b>Food samples</b>			
Factory	5	3	2
Outlets	8	4	4
Home	1	1	0
Cases	13	11	2
Total	36	24	12

RDNC – reagents did not confirm



at the boiling stage. Moreover, exposing the iced cakes at the open preparation area at room temperature for a substantial period of time would have led to further multiplication of *Salmonella*. Closure of the factory on 4 Dec 07 effectively broke the chain of infection and stopped further transmission of the outbreak.

This outbreak has highlighted three important points in the manufacture and sale of cream cakes by any confectionery. Firstly, all food handlers in the fac-

tory should constantly be reminded to observe proper food hygiene practices and not to report for work if they are ill with gastroenteritis. Secondly, as cream cakes are considered high-risk food items, more stringent microbiological testing of raw ingredients and finished products should be put in place. Finally, in the event of a need for product recall, there must be a proper inventory system for tracing batch numbers of raw ingredients and the movement of finished products right up to the retail outlets.

*(Reported by Chan PP, Suhana S, Lalitha K, Foong BH, Ooi PL, Surveillance & Response Branch, Communicable Diseases Division, Ministry of Health)*

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## A review of the avian influenza situation in 2007

### Introduction

Avian influenza is caused by influenza viruses which mainly occur in birds. While many wild bird species carry these viruses with no apparent signs of harm, it is highly contagious among flocks of poultry and can be deadly to some domesticated birds<sup>1</sup>.

Although many avian influenza viruses do not usually infect people, the highly pathogenic H5N1 strain is known to be directly transmissible to humans<sup>2</sup>. An epidemic of highly pathogenic avian influenza caused by influenza A (H5N1) has been reported since mid-December 2003 in several Asian and African

countries. In 2007, human cases of H5N1 were reported in Cambodia, China, Egypt, Indonesia, Lao People's Democratic Republic (PDR), Myanmar, Nigeria, Pakistan and Vietnam<sup>3</sup>. Human cases of influenza A (H5N1) have also occurred through direct or close contact with infected poultry or contaminated surfaces. Thus far, no sustained human-human transmission has been reported.

### Situation overview

#### Highly pathogenic avian influenza in birds

During the period 1 Jan to 31 Dec 2007, the occurrence of the disease in the avian population con-



tinued globally (Fig 2). Afghanistan, Bangladesh, Benin, Cambodia, China, Czech Republic, Egypt, Germany, Ghana, Hungary, India, Japan, Kuwait, Lao PDR, Malaysia, Myanmar, Pakistan, Poland, Romania, Russia, Saudi Arabia, Thailand, Togo, Turkey, United Kingdom, Vietnam have confirmed new H5N1 outbreaks in poultry stocks. Most of the confirmed outbreaks occurred in domestic poultry, including chickens, turkeys, geese and ducks<sup>4</sup>. A few cases in wild birds were also reported in Czech Republic, France, Germany, Hong Kong and Poland<sup>5</sup>. The occurrence of the disease in these areas showed that the virus is not only spreading among the Asian countries but had also reached the African continent.

#### Human cases of avian influenza

Coupled with the spread of the virus among the domestic poultry populations, more countries are reporting human cases of H5N1 avian influenza infection (Fig 3). In 2007, 86 human cases were confirmed by the World Health Organization, a decrease of 25%

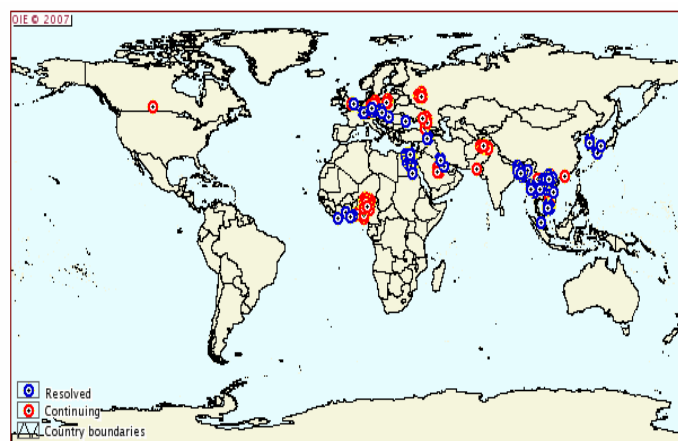
compared to the 115 human cases reported in 2006<sup>6</sup>. The overall case fatality remained high at 69% in 2007. The largest number of reported cases occurred in Indonesia (42 cases, a decrease of 24% from 55 cases reported in 2006), followed by Egypt (25 cases, an increase of 39% from 18 cases reported in 2006). After a year of no reported human cases since November 2005, human infections resurfaced in Vietnam since June 2007. Lao PDR, Myanmar, Nigeria and Pakistan were the latest countries that have reported their first human cases (Table 3).

Clusters of cases in China, Pakistan and Indonesia involving family members and relatives who lived together or near each other were also reported in 2007. While human-human transmission might have possibly occurred, they appeared limited.

#### Indonesia

Avian flu continues its spread across Indonesia with the H5N1 bird flu virus being entrenched in most

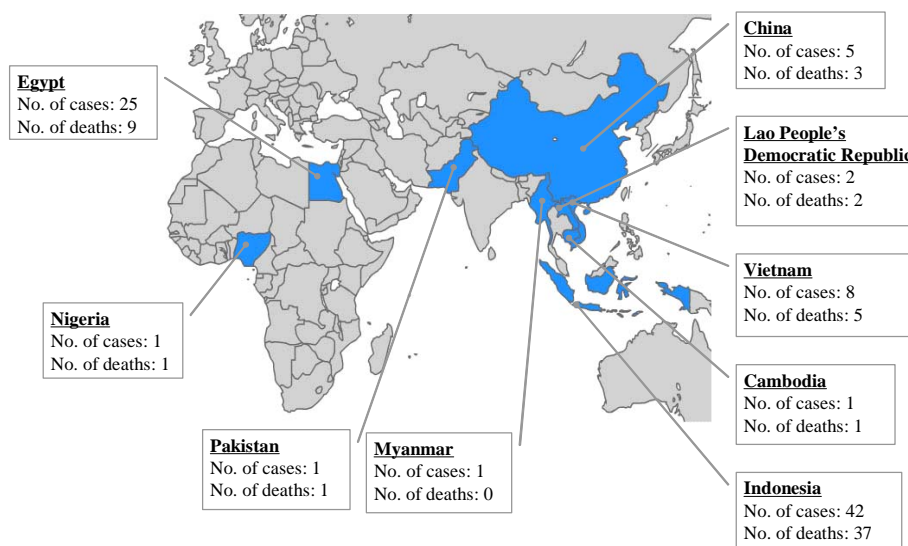
**Figure 2**  
Geographical distribution of highly pathogenic avian influenza, 2007



Source: World Organisation for Animal Health (OIE)



**Figure 3**  
**Geographical distribution of human cases of avian influenza A/(H5N1)\*, 2007**



\* Based on cases reported to the World Health Organization (WHO)

**Table 3.**  
**Human cases of influenza A (H5N1)\*, 2006-2007**

Country	2006		2007		Total (since 2003)	
	Cases	Deaths	Cases	Deaths	Cases	Deaths
Azerbaijan	8	5	0	0	8	5
Cambodia	2	2	1	1	7	7
China	13	8	5	3	27	17
Djibouti	1	0	0	0	1	0
Egypt	18	10	25	9	41	16
Indonesia	55	45	42	37	116	94
Iraq	3	2	0	0	3	2
Laos	0	0	2	2	2	2
Myanmar	0	0	1	0	1	0
Nigeria	0	0	1	1	1	1
Pakistan	0	0	1	1	1	1
Thailand	3	3	0	0	25	17
Turkey	12	4	0	0	12	4
Viet Nam	0	0	8	5	101	47
<b>Total</b>	<b>115</b>	<b>79</b>	<b>86</b>	<b>59</b>	<b>346</b>	<b>213</b>

\* Based on cases reported to the World Health Organization (WHO)





of the country's provinces. In 2007, a total of 42 cases including 37 deaths had occurred compared to 55 cases including 45 deaths in 2006. Cases occurred throughout the year (Fig 4a) and were reported from nine provinces across the country with the majority of the cases clustered around the western part of Java. The occurrence was highest in Jakarta (31.0%), followed by Banten province (26.2%), Riau province (14.3%) and West Java (11.9%). Sporadic cases were also reported in other parts of the country. Among them, two had occurred in the popular tourist destination of Bali for the first time. Occurrence of cases was observed to be the highest in the 15-34 years age group (Fig 4b). The female to male ratio was 1.8:1.

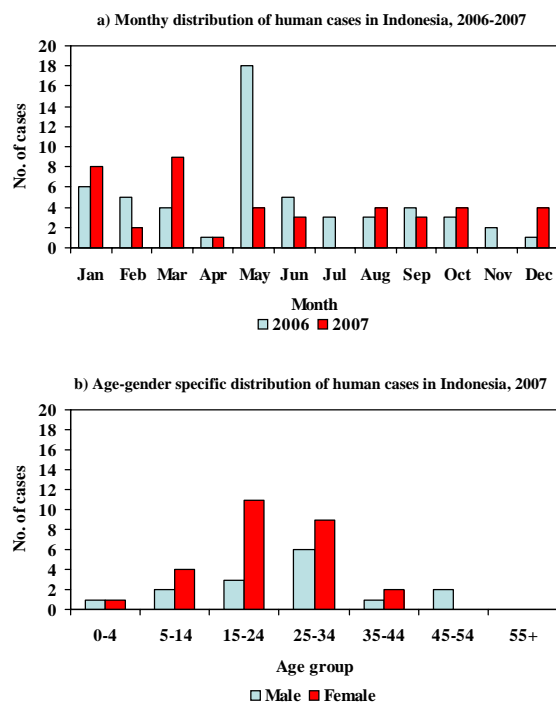
In early January 2007, a mother and son cluster of cases was reported in Tangerang in Banten Prov-

ince. The 37-year-old female developed symptoms on 1 January 2007 and died on 11 January 2007. Her 18-year-old son was also confirmed as infected with H5N1 avian influenza on 15 January 2007. Investigations into the source of their infections indicated that both had similar environmental exposure with sick poultry in their neighbourhood.

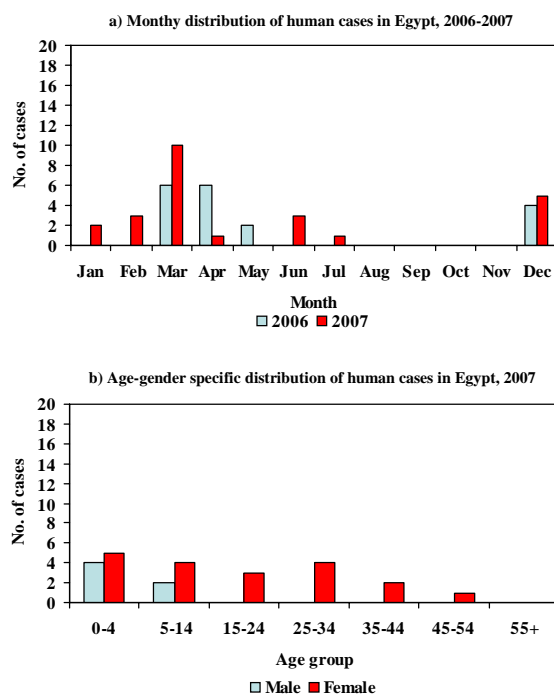
### Egypt

Egypt was the second worst country hit by bird flu after Indonesia in 2007. A total of 25 human cases including nine deaths were reported across the country. Cases tended to occur during the cooler months and in all age groups with young children below 15 years of age being the most affected (Figs 5a and 5b). The disease was also predominant in females. The

**Figure 4**  
Overall situation in Indonesia, 2007



**Figure 5**  
Overall situation in Egypt, 2007



female to male ratio was 3.2:1. A pair of siblings (a four-year-old boy and a six-year-old girl) from Qena Governorate was involved. Both had documented history of contact with dead birds prior to their onset of illness.

**Vietnam**

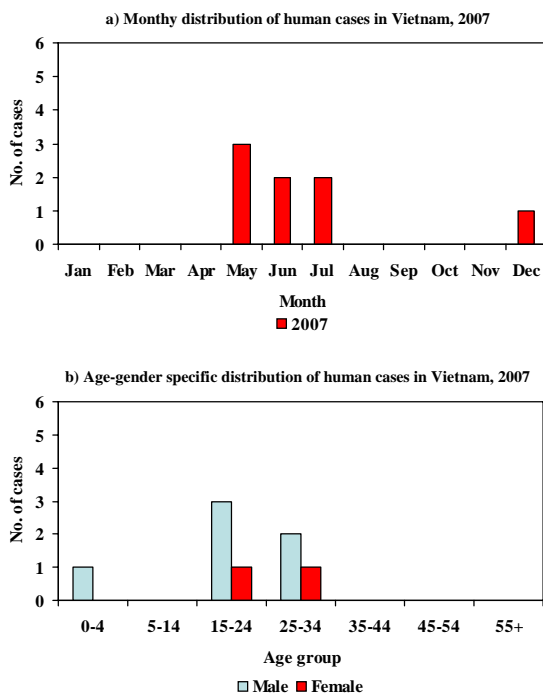
No cases were reported in 2006. However, human cases resurfaced in the country around mid-May to July in 2007 (Fig 6a). A total of eight laboratory-confirmed H5N1 human cases, including five deaths were reported. Cases were reported sporadically in six provinces and all had occurred in northern Vietnam. On 31 Aug 2007, WHO introduced an external quality assessment project for national reference laboratories for the detection of subtype influenza A vi-

ruses by polymerase chain reaction (PCR) testing and has now amended the criteria for accepting confirmed cases of A(H5) infection. Based on the amended criteria, five additional cases from Vietnam were included retrospectively to the official tally. The most affected age group was young working adults 15-34 years (Fig 6b). The male to female ratio was 3:1.

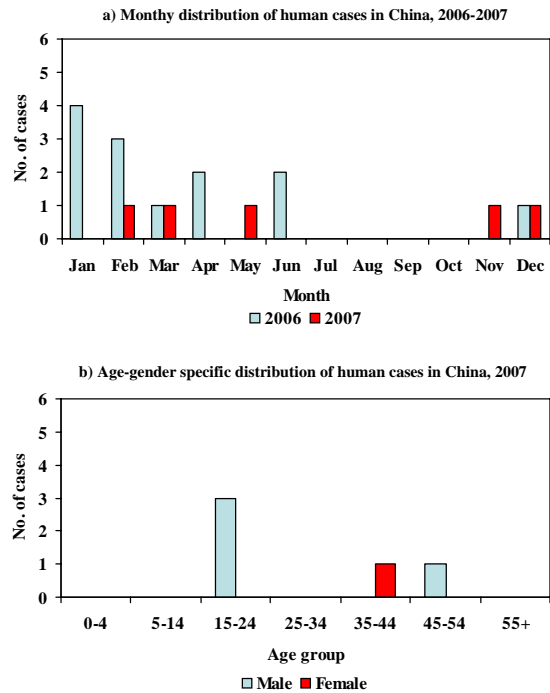
**China**

A total of five human cases, including three deaths with ages ranging from 16 to 52 years were reported in 2007. Similar to the situation in Egypt, cases also tended to occur during the cooler months (Fig 7a). Case occurrence was predominant in male with a male to female ratio of 4:1 (Fig 7b). In 2007, cases were reported in three provinces: one case in

**Figure 6**  
Overall situation in Vietnam, 2007



**Figure 7**  
Overall situation in China, 2007



Anhui province, two cases in Fujian and Jiangsu province, respectively. From the information provided to WHO, it was observed that four out of the five reported cases had no initial indication to suggest that they had contact with sick birds prior to becoming ill. Among the reported cases, there was a family cluster of two, involving a 24-year-old male and his 52-year-old father from Jiangsu Province. The 52-year-old man was hospitalised soon after his son died from the H5N1 infection on 2 December 2007. Although there had been unofficial reports of relating these two cases to possible common source of exposure, the means of transmission of these two cases remained unknown.

#### **Lao People's Democratic Republic**

In 2007, Lao People's Democratic Republic reported a total of two human cases of H5N1 infection. Both patients were from Vientiane province and had died after treatment in hospital. The first case involving a 15-year-old girl was reported to the WHO on 27 February 2007. She developed influenza-like symptoms on 10 February 2007 and was hospitalized in Vientiane with fever and respiratory symptoms on 15 February 2007. She had then sought medical care in neighbouring Thailand on 17 February 2007 and subsequently died on 7 March 2007. Poultry deaths had occurred in the village where she lived. The second case was reported to the WHO in March 2007, involving a 42-year-old female also from Vientiane. A duck in the woman's household tested positive for H5 avian influenza virus.

#### **Cambodia**

On 10 April 2007, the Ministry of Health in Cambodia had confirmed the country's seventh case of human infection with the H5N1 avian influenza virus. It was the only human case reported for 2007. The case

occurred in a 13-year-old girl from Ponhea Kreak district in Kampong Cham province. She developed symptoms on 2 April 2007, was hospitalized the following day and died on 5 April 2007. Investigations into the source of the girl's infection indicated the presence of sick and dead poultry in the village and that she had consumed a sick chicken prior to onset of symptoms.

#### **Myanmar**

The Ministry of Health in Myanmar confirmed the country's first case of human infection with the H5N1 avian influenza virus on 14 December 2007. She was a seven-year-old girl from Kyaing Tone Township in Shan State. The case was detected through routine surveillance following an outbreak of H5N1 in poultry in the area in mid-November 2007. She developed symptoms of fever and headache on 21 November 2007 and was hospitalized on 27 November 2007. She subsequently recovered from the illness. Investigations into her source of infection indicated that poultry had died in the vicinity of her home in the week prior to the onset of her illness. No further cases were detected.

#### **Nigeria**

On 31 January 2007, the government of Nigeria announced that a 22-year-old female from Lagos had died from avian influenza infection on 16 January 2007. Her mother also died on 4 January with similar symptoms. However, no samples were taken from the mother for laboratory testing. The 22-year-old female was the only reported case in Nigeria so far.

#### **Pakistan**

On 15 December 2007, the Ministry of Health in Pakistan informed WHO of eight suspected hu-



man cases of H5N1 avian influenza infection in the Peshawar area of the country. These cases were detected following a series of culling operations in response to outbreaks of H5N1 in poultry. Two family clusters were affected in the outbreak, involving four brothers and two cousins. The other involved a ten-year-old girl and her father.

The first confirmed case was announced by WHO on 27 December 2007 when the WHO H5 Reference Laboratory in Cairo, Egypt, and WHO Collaborating Centre for Reference and Research on Influenza in London, United Kingdom, confirmed the presence of avian influenza A(H5N1) in samples collected from one case in the affected family. Investigations conducted by WHO into the outbreak involving the above-mentioned family clusters revealed no evidence of sustained or community human-human transmission. This was the only confirmed human case re-

ported in Pakistan in 2007. No follow-up reports on their laboratory-confirmation were available for the other seven suspected cases so far.

## Conclusion

Despite substantial progress in global efforts to bring avian influenza under control, the disease continues to spread to new countries and to new areas in countries where containment has not been successful. Cases continue to occur both in humans and poultry in 2008. The risk of an influenza pandemic from H5N1 is still of concern and the virus will continue to pose a threat to the lives of people living and working around poultry. Hence, the importance of poultry surveillance cannot be over-emphasised. Furthermore, vigilance by clinicians will also be necessary for early detection of human cases should one occur in the absence of reported poultry outbreak.

*(Reported by Li HY, Communicable Diseases Division, Ministry of Health)*

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## An outbreak of contact lens associated *Fusarium* keratitis in Singapore

### Introduction

*Fusarium* keratitis is a rare fungal infection of the cornea that is potentially blinding even if detected early and treated aggressively. In January 2006, clinicians at the Singapore National Eye Centre noticed a spike in the number of cases of *Fusarium* keratitis. What was unusual about this outbreak was that the cases were associated with contact lens use rather than trauma.

### Case findings

Epidemiological investigation was immediately conducted to identify all cases of fungal cornea infection that had occurred since 2005. Major eye hospitals were directed to notify Ministry of Health (MOH) of *Fusarium* keratitis cases with a history of contact lens use.

As of 20 Feb 06, a total of 39 cases were notified, majority from the Singapore National Eye Centre (23 cases) followed by Tan Tock Seng Hospital (9 cases), Changi General Hospital (4 cases) and National University Hospital (3 cases). Preliminary investigations of these cases showed that 34 (87.2%) had used a contact lens solution (Bausch & Lomb ReNu multipurpose solution), four were unsure of the brand of the solution they used and one other had used a different brand. Majority (92%) of the cases started developing infection in the second half of 2005 onwards. There were 20 males and 19 females. Most (74.4%) of the cases comprised teenagers and young adults. Half (48.7%) of all the cases were in the 15-24 years

age group while an additional quarter (25.6%) were aged 25-34 years. A review of the laboratory data of the Department of Pathology, Singapore General Hospital, showed that the spike in cases had in fact started in November 2004, coinciding with the introduction of the commercial contact lens product. (Fig 8).

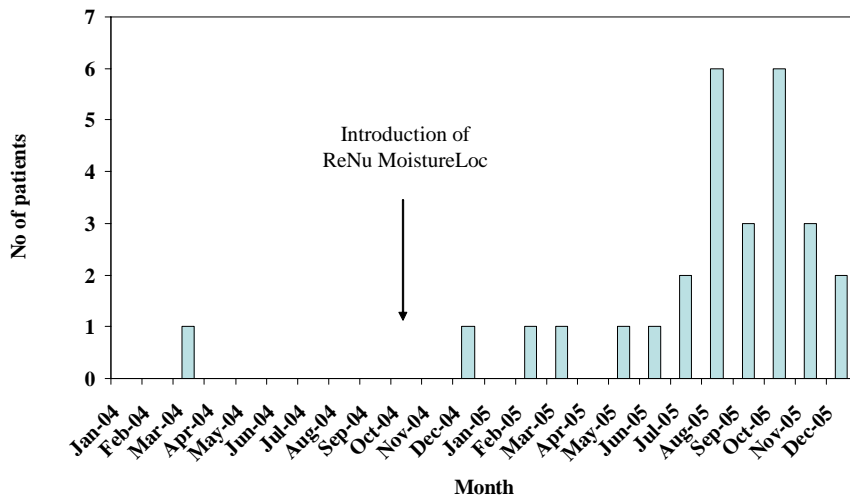
### Health alert

In view of the potentially serious adverse visual consequences of fungal corneal infection, on 17 Feb 06, MOH as a precautionary measure, strongly advised all contact lens users to discontinue the use of ReNu multipurpose contact lens solution, until the causes behind the sudden increase in infections could be more clearly ascertained. In the meanwhile, contact lens users were advised to practise proper contact lens care as recommended by their contact lens practitioners. For those who had experienced any symptoms of infection such as redness of eyes or pain or itchiness, they should consult a doctor immediately. As a precautionary measure, the manufacturer of ReNu multipurpose solution, voluntarily suspended sales of its products and cooperated with MOH and Health Science Authority in the investigations.

Following these public health measures, the number of recently acquired cases fell to 4 in Mar 06, as compared to 14 cases in the months of Jan and Feb 06, respectively (Fig 9). A total of 75 cases of *Fusarium* keratitis with a history of contact lens use were reported between 1 Nov 04 and 12 April 06, compared with two reported cases from 1 Jan -31 Oct



**Figure 8**  
Number of patients with *Fusarium* isolates from eye by month, 2004 and 2005



Source: Department of Pathology, Singapore General Hospital

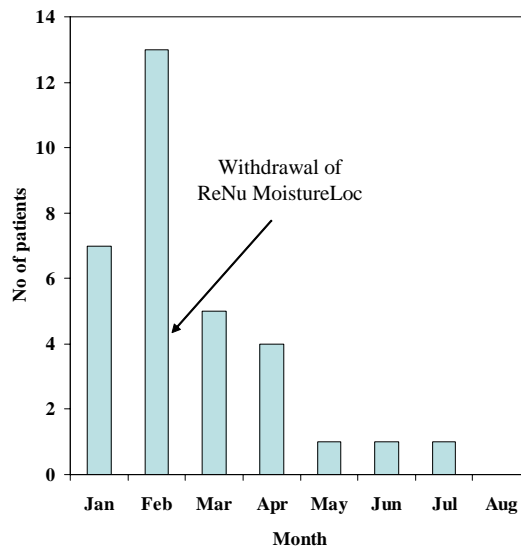
04. All the 4 cases with onset in Mar 06 used ReNu contact lens solution and had decided to finish the remaining solution before switching brands. Out of the 75 cases investigated, 59 were sure of the contact lens solution used. Of these, 56 (94.9%) said that they had used ReNu.

Following an alert issued from Singapore, outbreaks of *Fusarium* keratitis associated with the implicated contact lens solution were subsequently reported in the US and Hong Kong. In May 2006, as a precautionary measure, Bausch & Lomb announced a worldwide recall of ReNu with MoistureLoc

**Case-control study**

A nation-wide retrospective case-control study using a standardised form with two sets of controls (hospital-based controls and community-based con-

**Figure 9**  
Number of patients with *fusarium* isolates from eye associated with contact lens wear by month, 2006



Source: Department of Pathology, Singapore General Hospital



trols) was undertaken to investigate risk factors for the spike of fungal corneal infection. The selection of two sets of controls allowed the evaluation and replication of results in controls selected from two different populations. Details about the type and brand of contact lens and contact lens solution used in the past 3 months were elicited, as were the duration of use of the current brand of contact lens and contact lens solution. The study was approved by the Institutional Review Board, Singapore Eye Research Institute.

Sixty-one cases who presented with *Fusarium* keratitis and who wore contact lenses at least once a week for refractive error correction seen in Singapore between 1 Mar 2005 and 31 May 2006 were successfully recruited from all major eye hospitals in Singapore and one private ophthalmology clinic. Patients who were wearing bandage contact lenses for therapeutic reasons, were excluded.

*Fusarium* keratitis was defined as any infective corneal lesion that had *Fusarium* species cultured from the cornea (n=54). Patients that had a clinical picture of fungal keratitis, were treated with and responded to antifungal medications, but with negative corneal cultures were also included, if the *Fusarium* species could be cultured from the contact lens (n=7), or contact lens case (n=3).

The two sets of controls were chosen from individuals who wore contact lenses for the correction of refractive error, and had been wearing lenses at least once a week for the three months prior to their inclusion in the study.

Hospital controls (n= 179) were contact lens wearers at two major eye hospitals who presented for consultation at the refractive surgery clinics, or for

the management of ophthalmic complaints unrelated to infective keratitis (e.g., dry eyes). Population controls (n= 188) were contact lens wearers who attended private optometry practices in Singapore.

## Results

### Epidemiological features

The average age of all participants was 29 years (range 13 to 59), and 26.8% were male. The median number of years of contact lens use was 7.5 years (range 0.1 to 30 years), and 82.4% wore soft disposable contact lenses. The proportion that wore contact lenses past replacement date was 38.6%, and 25.3% wore contact lenses overnight. Overall, 41.7% used ReNu solution (Bausch & Lomb), 19.4% used Complete solution (Advanced Medical Optics), 8.8% used Solocare solution (Ciba-Vision), 5.6% used AOSep solution (Ciba-Vision), 4.4% used Opti-Free solution (Alcon), 2.7% used saline or tap water, 2.2% used OxySept solution (Advanced Medical Optics), and 0.5% used Boston solution (Bausch & Lomb). A further 6.6% had used two or more non-ReNu brands and 1% were not sure of the exact contact lens solution brand.

For all controls combined, males and individuals of Malay ethnic group had significantly higher risks of *Fusarium* keratitis. The risk of *Fusarium* keratitis for users of both types of ReNu contact lens solution use was significantly higher in cases compared with both controls [OR=40.6 (95% CI 12.4, 132.3)]. The risk was much higher for ReNu with MoistureLoc [OR=50.9 (95% CI 14.7, 176.2)] than for ReNu MultiPlus [OR=18.9 (95% CI 5.2, 68.5)]. Soft monthly disposable contact lenses increased the risk of *Fusarium* keratitis in all comparisons (OR=4.8; 95%



CI 2.3, 10.0). The use of contact lenses past replacement date increased the risk of *Fusarium* keratitis (OR=2.2; 95% CI 1.3, 3.8), after controls were combined.

The age-gender-ethnicity adjusted ORs were 34.2 (95% CI 12.8, 168.4) for ReNu with MoistureLoc, and 13.7 (95% CI 3.3, 49.2) for ReNu MultiPlus, while the multivariate adjusted OR for ReNu with MoistureLoc [98.0 (95% CI 18.3, 524.0)] and ReNu MultiPlus [20.9 (95% CI 3.9, 112.1)] were stronger.

The use of lenses past the replacement date increased the risk of *Fusarium* keratitis in population (OR=6.1; 95% CI 1.9, 20.3) and all controls (OR=4.7; 95% CI 1.7, 13.5). Females had lower risk (OR=0.3; 95% CI 0.1, 0.9), while Malays (OR=10.3; 95% CI 1.9, 54.9) had higher risk of *Fusarium* keratitis in multivariate models. Wearing monthly soft disposable lenses did not remain significant in multivariate models. The multivariate OR of ReNu solution use versus non-ReNu solution use was 48.9 (95% CI 9.8, 245.2) for population controls, 65.4 (95% CI 13.0, 329.2) for hospital controls, and 47.7 (95% CI 10.9, 209.8) for all controls, after adjusting for similar factors.

### Clinical features

Amongst the cases, bilateral involvement was present in 3 patients, and 31 patients (50.8%) were affected in the left eye. Eight were documented to have received topical corticosteroid eye drops prior to the diagnosis of *Fusarium* keratitis. Most patients were treated with anti-fungal medications; topical natamycin, amphotericin, and/or voriconazole were commonly used, often in combination. Eight eyes with

impending perforation or threatened scleral involvement also received systemic therapy; one received oral fluconazole (200mg b.i.d), four received systemic voriconazole (intravenous loading dose of 400mg given twice in 24 hours, followed by an oral dose of 200mg b.i.d), and one had a trial of oral itraconazole (400mg b.i.d) before conversion to systemic voriconazole. Eventually, five eyes required a therapeutic or tectonic corneal transplant in the acute stages of the infection – one had an acute corneal perforation, whereas the other four had disease progression despite maximal medical therapy.

### Laboratory findings

Further genetic studies were also performed on cultured *Fusarium* samples. Species identification was confirmed using 28S rRNA sequencing. The isolates from the eye specimens were studied for genetic relatedness to establish if there was a common source of infection; strain typing was done using amplified fragment length polymorphism (AFLP).

Thirty-eight patients had isolates available for 28S rRNA sequencing and 37 had a 100% match with the sequence from *Fusarium solani* CBS490.63 in Genbank. The remaining isolate matched *Fusarium oxysporum*. The results of AFLP typing for all the *Fusarium solani* isolates did not show the presence of a single identical clone, but showed 7 groups at 80% genetic similarity.

### Discussion

The case-control study demonstrated that the use of either ReNu with MoistureLoc or ReNu MultiPlus solutions significantly increased the risk of *Fusarium* keratitis, while controlling for age, gender, ethnicity,





income, and contact lens hygiene factors. The results were consistent with both population and hospital controls.

The study showed that ReNu MultiPlus was also linked to *Fusarium* keratitis, although this association was 5 times weaker compared with ReNu with MoistureLoc. However, the recent report of the case-control study in the US did not demonstrate a link between ReNu MultiPlus with *Fusarium* keratitis (OR = 0.7; 95% CI 0.2, 2.8). ReNu with MoistureLoc was introduced in Singapore in November 2004 to replace ReNu MultiPlus, which is an older formulation.

ReNu MultiPlus (8% of the ReNu contact lens solution market in 2006) has been available in the Singapore market for the past few years, but there were no reports of an increase of *Fusarium* keratitis cases prior to 2005. This may be explained by the fact that perhaps a proportion of prior *Fusarium* keratitis cases went unreported. However, it should be noted that in Japan, in which only ReNu MultiPlus is available and ReNu MoistureLoc is not sold, no cases of contact lens related *Fusarium* have been reported, and we are also unaware of any reported cases in Europe, where both ReNu products are sold.

It is also important to note that the overall contact lens hygiene practices of cases and controls were not optimal, and this clearly may play an important role in the pathogenesis of infectivity. A relatively high proportion used contact lenses past replacement date (55% of cases, 40% of population controls, 40% of hospital controls), and positive links with *Fusarium* keratitis were found in multivariate analysis. However, we found no significant interaction between ReNu

solution use, contact lenses past replacement date, and the risk of *Fusarium* keratitis.

We also found that men and people of Malay ethnicity had higher risks of *Fusarium* keratitis, even after controlling for ReNu contact lens solution use and other factors. The gender and ethnic differences may reflect differences in underlying biological mechanisms due to dissimilar genetic make-ups, or perhaps ethnicity may indicate differences in socioeconomic status, lifestyle or behaviour that increase the risk of *Fusarium* keratitis that we were not able to detect, although, surprisingly, *Fusarium* keratitis cases had higher socioeconomic status compared with community controls.

The microbiological typing results for the clinical isolates show multiple groups, hence a common source of contamination is unlikely. The precise mechanism by which the solution led to the increase in *Fusarium* keratitis could not be determined since microbiological evidence did not suggest a point source contamination. It is also intriguing that other areas where ReNu solution is widely used such as Europe did not report any increase in *Fusarium* keratitis. Investigations into the product have reportedly not revealed any issues relating to product contamination, sterility or other production related factors. The contact lens solution, however, may not perform as desired under in-use conditions, leading to a drop in the product's disinfecting capability. Another possibility is that the solution could induce a breach in the corneal epithelium and enhance the entry of *Fusarium* organisms into the cornea. Baush and Lomb concluded that MoistureLoc's formulation could create bio-films that shield the fungus from the sterilizing agent.

(Based on Saw SM, Ooi PL, Tan DTH et al. Risk factors for contact lens related *Fusarium* keratitis: a case-control study in Singapore. *Arch Ophthalmol* 2007; 125: 611-7; Annual Report 2006, Department of Pathology, Singapore General Hospital)



## Surveillance for enteric fevers and salmonellosis, 2006

### Enteric fevers

In 2006, a total of 83 cases of enteric fevers comprising 60 cases of typhoid and 23 cases of paratyphoid were reported (*Fig 10*). Of the reported typhoid cases, only 5 (8.3%) were classified as indigenous while the rest (91.7%) were all imported. The imported cases included 17 work permit/employment pass holders, 7 student pass holders, 17 foreigners seeking medical treatment in Singapore, 1 tourist from overseas and 1 other category of foreigners. There were 12 Singapore residents who contracted the disease when they travelled to the endemic countries for vacation, business, job assignment or social visit without taking adequate personal preventive measures. The countries of origin of were India (6 cases), Indonesia (4 cases), Bangladesh (1 case) and Malaysia (1 case).

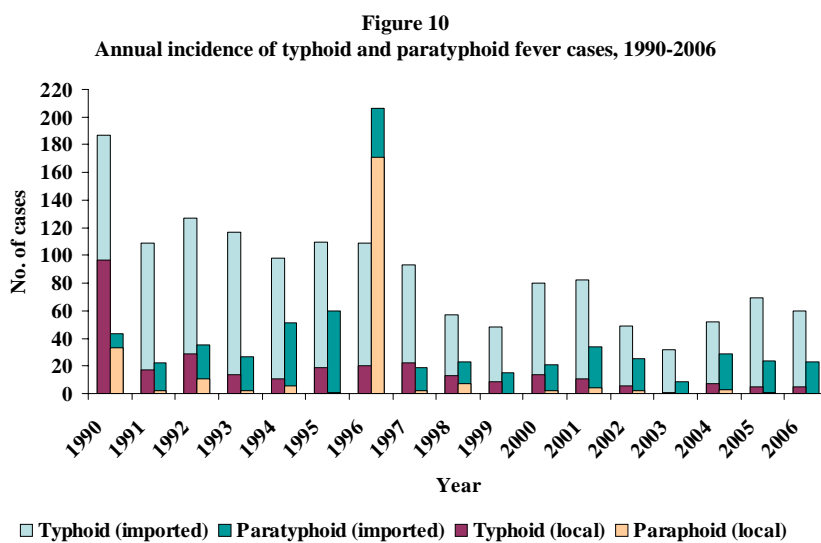
All the reported paratyphoid cases were imported; 5 were Singapore residents who contracted

the disease in India (2 cases), Indonesia (1 case), Myanmar (1 case) and Australia (1 case).

At the Department of Pathology, Singapore General Hospital, *Salmonella* Typhi and *Salmonella* Paratyphi isolates were phage typed and tested for four antibiotics; namely, ampicillin, ceftriaxone, ciprofloxacin and co-trimoxazole. There were 6 strains of *Salmonella* Typhi of phage types A, E1 and RDNC (react but does not confirm), and a case of *Salmonella* Paratyphi A (*Table 4*). The antibiogram of both species showed 100% susceptibility to these antibiotics.

### Salmonellosis

During the year, a total of 380 laboratory confirmed cases of salmonellosis were reported. Of these, 215 cases were caused by *Salmonella* Enteritidis. The incidence rate of S Enteritidis was highest in children below 5 years of age (8.1 per 100,000) and adults aged



above 55 years (10 per 100,000) (Table 5). There was a male predominance in all age groups. Among the three major ethnic groups, Malays had the highest incidence rate followed by Indians and Chinese (Table 6).

About one-third of the cases had other co-morbid conditions.

An outbreak of salmonellosis caused by *Salmonella* Enteritidis occurred in two adjoining institutions in May 2006 (Fig 11). A total of 77 cases who sought

medical treatment were identified. The main symptoms were watery diarrhoea (96.1%) and fever (88.3%). None were hospitalised. Dinner served on 18 May was implicated, but no specific food item could be identified. The mean and median incubation periods were 28.2 hours and 19 hours, respectively. Of 38 stool samples obtained from the cases cultured, 36 tested positive for *Salmonella* Enteritidis. Stool cultures of two of 36 asymptomatic foodhandlers were positive for *Salmonella* Enteritidis and *Salmonella*

**Table 4**

**Total number of isolates, phage types and antibiotic profile of *Salmonella* Typhi and *Salmonella* Paratyphi A**

Microorganism	Number isolated	Phage type (%)	Antibiotics	% sensitivity
S. Typhi	6	A (17)	AMP	100
		E1 (17)	CRO	100
		RDNC (67)	CIP	100
			SXT	100
S. Paratyphi A	1	RDNC (100)	AMP	100
			CRO	100
			CIP	100
			SXT	100

Key: AMP: ampicillin, CRO: ceftriaxone, CIP: ciprofloxacin, SXT: co-trimoxazole, RDNC: react but does not conform

**Table 5**

**Age-gender distribution and age-specific incidence rates of reported salmonellosis caused by *S. Enteritidis*<sup>^</sup>, 2006**

Age (yrs)	Male	Female	Total (%)	Incidence rate per 100,000 population*
0 – 4	11	6	17 ( 8.0)	8.1
5 – 14	5	1	6 ( 2.8)	1.1
15 – 24	13	9	22 ( 10.4)	3.2
25 – 34	35	7	42 ( 19.8)	4.6
35 – 44	22	8	30 ( 14.2)	3.8
45 – 54	13	11	24 ( 11.3)	3.8
55 +	41	30	71 ( 33.5)	10.0
Total	140	72	212 (100.0)	4.7

<sup>^</sup> Excluding three foreigners seeking medical treatment in Singapore

\*Rates are based on 2006 estimated mid-year population.

(Source: Department of Statistics, Singapore)



species, respectively. *Escherichia coli* was detected in a sample of fried vegetable and the swab taken from a food tray was heavily contaminated with bacteria. The main factor contributing to the outbreak was poor personal and food hygiene, in particular, cross-contamination between cooked and uncooked food.

At the Department of Pathology, Singapore General Hospital, a total of 92 *Salmonella* species were isolated. Of these, 83 were of serotype Enteritidis and nine of *Salmonella* Typhimurium. *Salmo-*

*nella* Enteritidis isolated were found to belong to 7 phage types (Table 7). Twenty-nine of the strains had PT 6a, which remained the most commonly isolated phage type for the past years. All the *Salmonella* Enteritidis strains were susceptible to ceftriaxone as in previous years.

In addition, 9 strains of *Salmonella* Typhimurium were cultured from stool, urine and wound samples, yielding 5 phage types. They were all susceptible to ceftriaxone (Table 7).

**Table 6**  
Ethnic-gender distribution and ethnic-specific incidence rates of reported salmonellosis caused by *S. Enteritidis*<sup>^</sup>, 2006

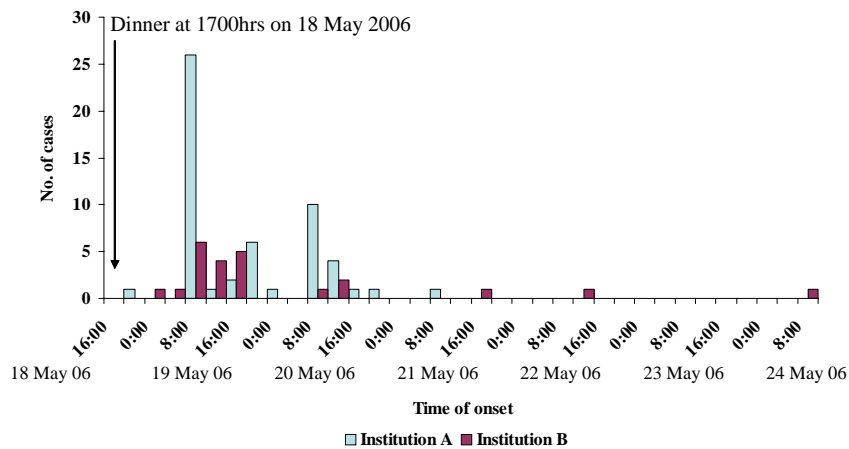
	Male	Female	Total (%)	Incidence rate per 100,000 population*
Singapore resident				
Chinese	68	42	110 (51.9)	4.1
Malay	20	14	34 (16.0)	6.9
Indian	9	5	14 (6.6)	4.4
Other	7	2	9 (4.2)	10.5
Foreigner	36	9	45 (21.2)	5.1
<b>Total</b>	<b>140</b>	<b>72</b>	<b>212 (100.0)</b>	<b>4.7</b>

<sup>^</sup> Excluding three foreigners seeking medical treatment in Singapore

\*Rates are based on 2006 estimated mid-year population

(Source: Department of Statistics Singapore)

**Figure 11**  
Time distribution of 77 cases of salmonellosis in two adjoining institutions, 19-24 May 2006



**Table 7**  
**Total number of isolates, phage types and antibiotic profile of *Salmonella* Enteritidis and *Salmonella* Typhimurium**

Microorganism	Number isolated	Phage type (%)	Antibiotics	% sensitivity
S. Enteritidis	83	1 (28)	AMP	64
		4 (10)	CRO	100
		6a (35)	CIP	98
		21 (5)	SXT	83
		37 (2)		
		RDNC (17%)		
S. Typhimurium	9	1 (11)	AMP	67
		104b (44)	CRO	100
		U302 (11)	CIP	78
		RDNC (22)	SXT	89
		UNITY (11)		

AMP: ampicillin, CRO: ceftriaxone, CIP: ciprofloxacin, SXT: co-trimoxazole, RDNC: react but does not conform, UNTY: no reaction at all

*(Based on Communicable Disease Surveillance in Singapore 2006, Ministry of Health, and Annual Report 2006, Department of Pathology, Singapore General Hospital)*

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## Laboratory surveillance for enteroviruses and respiratory viruses in Singapore, 2006

### Enteroviruses

In 2006, the number of requests for enterovirus isolation at the Department of Pathology, Singapore General Hospital, increased by 55.4%, a result of more hand-foot-mouth disease (HFMD) cases investigated by Ministry of Health (MOH). An increase in incidence was seen from March to October, peak-

ing in August and September (*Fig 12*). Coxsackievirus A(CA) 6 was the most common cause (accounting for 59 cases) followed by enterovirus (EV) 71 (16 cases) and CA16 (8 cases). Other enteroviruses isolated in fewer numbers from HFMD patients, in descending order of detection, were CA10, CA2, CA4, echo 25, CA9, echo 6 and echo 9.



Most of the patients were, unsurprisingly, young children ranging from one month to nine years of age, although most were under four years old. An exception was a 29-year-old pregnant woman from whose pustules CA6 was isolated.

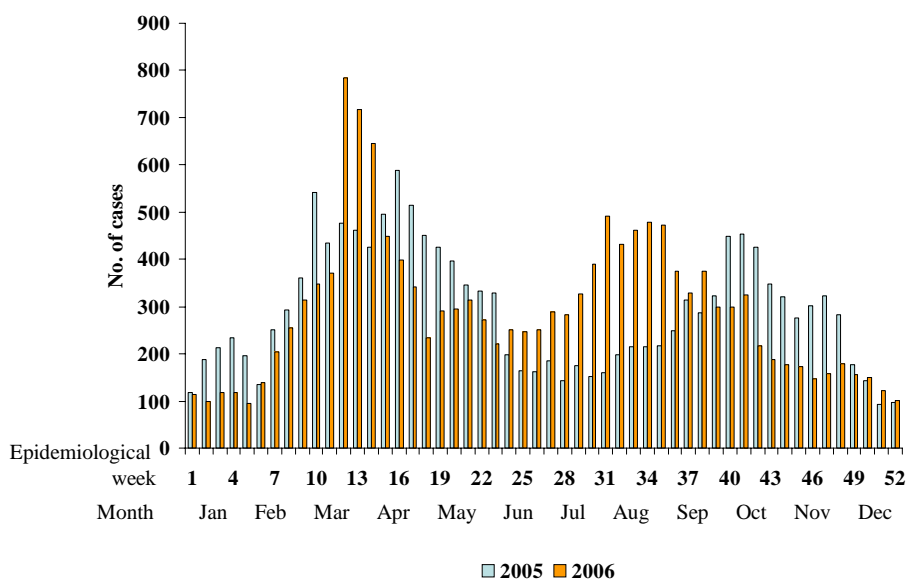
Enteroviruses also caused other diseases. Echo 6 was isolated from the cerebrospinal fluid of a seven-year-old boy with viral meningitis, and echo 9 from the stool of an eight year-old boy with meningitis. Coxsackievirus B (CB) 3 was the cause of neonatal pyrexia in one case and CA5 caused an upper respiratory tract infection in a six-month-old boy.

The WHO National Polio Laboratory (NPL) isolated a type 1 poliovirus from a stool sample of a two-and-a-half-year-old girl with acute flaccid paralysis. The child developed acute left leg weakness in her home in northern Nigeria on 21 April 2006 and was brought by

her family to Mt. Elizabeth Hospital in Singapore for medical treatment, where she was clinically diagnosed to have polyradiculitis. The type 1 poliovirus isolated from her stool sample collected on 28 April was determined to be a wild type by enzyme immunoassay and polymerase chain reaction by the NPL. The World Health Organisation (WHO) and Ministry of Health (MOH) were notified on 19 May. In accordance with WHO protocol, the isolate was then sent to the Polio Global Specialised Laboratory in Japan, where sequencing showed closest VP1 match (99.0%) with an isolate from Katsina State in northern Nigeria (NIE-KTS-DJA-05-003; Danja, 14 Jul 05) and the next closest match (98.9%) with an isolate from Gombe State (NIE-GMS-GME-05-012; Gombe, 27 Jul 05).

MOH sent a public health alert to all paediatricians, neurologists and internists in both public and private sectors. Epidemiological investigation showed

**Figure 12**  
Weekly distribution of reported cases of hand, foot and mouth disease, 2005 - 2006



that of the eight children with whom the patient had direct contact, two were Indonesians and one, a Russian, who had since returned to their home countries. No virus-positive cases were detected from follow-up of contacts with stool samples for poliovirus isolation in Singapore, Indonesia, and the Russian Federation.

Although Singapore continues to maintain its polio-free status, this case highlighted the need for continued vigilance for the importation of infectious disease like polio, especially as Singapore experiences a tremendous volume of human traffic into the country

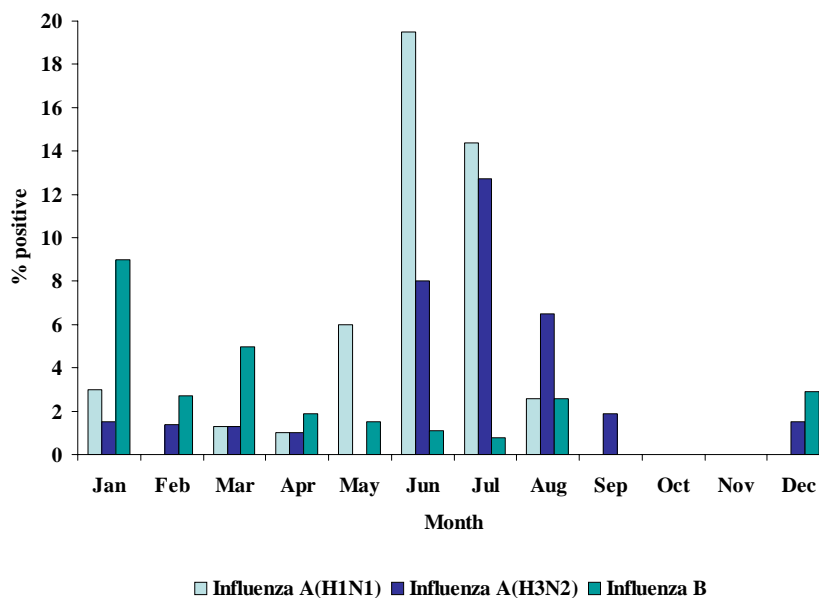
Apart from the wild poliovirus 1 mentioned earlier, polioviruses were isolated on two other occasions: Sabin-like poliovirus 3 was recovered from a

four-month-old boy and Sabin-like poliovirus 1, 2 and 3 from a four-month-old baby girl with a clinical diagnosis of HFMD. In these two cases, the polioviruses were most probably incidentally detected after immunisation with live poliovirus vaccine and were not the cause for their illness.

### Influenza virus

Influenza A continued to dominate over influenza B. However, influenza A H3N2 subtype made way for influenza A H1N1 subtype. Of the 78 influenza A isolates, 56.4% belonged to H1N1 subtype. Another 38 cases of influenza A were detected by antigen assay and were not subtyped. Influenza B was responsible for a significant proportion (40.2%) of influenza cases. While influenza A accounted for the

**Figure 13**  
Influenza virus isolates, 2006



bigger peak from May to August, influenza B was mainly active from January through March (*Fig 13*). A/California/7/2004(H3N2)-like strains that circulated in 2005 were replaced by A/Wisconsin/67/2005(H3N2)-like strains. A/New Caledonia/20/99-like strains continued to be detected for the first half of the year until they were replaced in May and June by A/Hawaii/15/2001(H1N1)-like viruses. After this brief period, the latter was no longer detected as they were supplanted by A/Solomon Islands/03/2006(H1N1)-like variants. B/Ohio/1/05, which made its appearance towards the end of 2005, remained active throughout 2006, with only the very occasional detection of B/Shanghai/361/2002-like and B/Florida/07/2004-like

strains. No H5N1 virus was detected from suspected avian influenza cases.

### Other respiratory viruses

After four years, the number of parainfluenza type 1 cases detected exceeded that of parainfluenza type 3 by four. Only two cases of parainfluenza type 2 were detected. Of the 10 cases from whom rhinovirus was isolated, only one was an infant, the rest being adults from 32 to 77 years old. Seven viruses were isolated from broncho-alveolar lavage, two from nasal aspirate samples and the last from a throat swab. Five of them were clinically diagnosed with pneumonia while four had unknown diagnoses.

*(Based on Communicable Diseases Surveillance in Singapore 2006, Ministry of Health; Annual Report 2006, Department of Pathology, Singapore General Hospital)*

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