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CONTENTS

Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome: a comparison

Background

In September 2012, a novel coronavirus currently known as the Middle East respiratory syndrome coronavirus (MERS-CoV), was isolated from the sputum of a 60-year-old Saudi man from Jeddah, Saudi Arabia.[1] This case subsequently died from acute pneumonia and renal failure. It was later retrospectively found that human cases of MERS-CoV infection had occurred earlier in April 2012, in a cluster of pneumonia cases among healthcare workers in Jordan. Since then, 114 cases of human infection with MERS-CoV, including 54 deaths, had been reported as of 9 September 2013 (*Fig. 1*).[2]

The emergence of MERS-CoV bears uncanny resemblance to the severe acute respiratory syndrome coronavirus (SARS-CoV), which caused a global pandemic ten years ago. Through retrospective investigation, the index case of SARS-CoV was believed to have been infected in Guangdong Province, China, in November 2002. By the end of the global outbreak on 5 July 2003, a total of 8098 human cases of SARS-CoV infection were reported in 26 countries, including 774 deaths.[3]
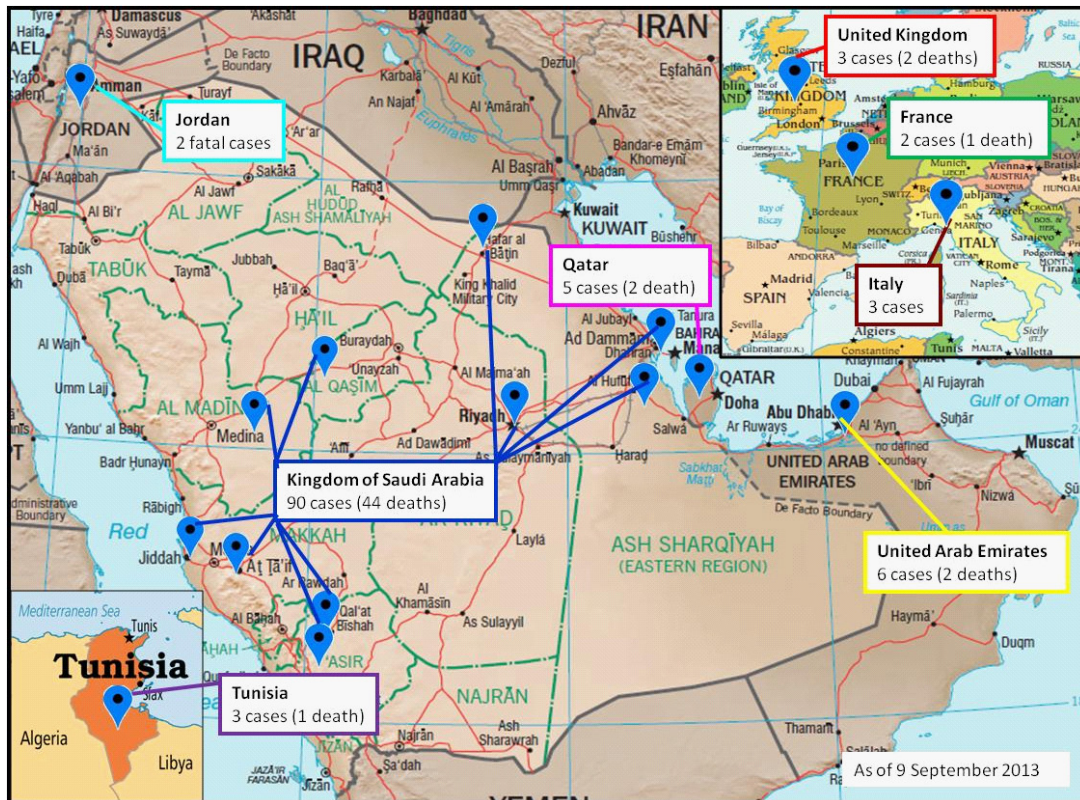
Virological and epidemiological characteristics

Both MERS-CoV and SARS-CoV belong to a large family of coronaviruses, which infect many species of animals, including humans. The virological and epidemiological characteristics of both viruses are summarized in *Table 1*.

Figure 1
Human cases of MERS-CoV infection reported by country of residence

Source of map: US Central Intelligence Agency

Natural reservoirs

Both MERS-CoV and SARS-CoV are believed to originate from a "spill over" from a wildlife reservoir (probably bats) to humans, possibly via an intermediate host(s). The Himalayan masked palm civet (*Paguma larvata*) was thought to be an intermediate host, and the wild-animal markets in Guangdong were suspected to have provided the interface for animal-to-human transmission.[11]

MERS-CoV may also have arisen from a "spill over" event. Genetic analysis suggested either a European or African bat origin for MERS-CoV[4,5] In Saudi Arabia, a coronavirus fragment found in a faecal sample from an Egyptian tomb bat (*Taphozous perforatus*) captured 12 kilometres from the home of a human case of MERS-CoV was found to be a 100% match for the virus isolated from the human case.[9] Currently, the limited information makes it difficult to rule out the presence of other intermediate hosts. *In vitro* studies showed that MERS-CoV could infect cells from different species, including monkeys, humans, pigs and bats.[6,7] Exposures to farm animals such as sick camel or goat were reported in a few cases. One serological study in blood samples from

Table 1

Virological and epidemiological characteristics of MERS-CoV and SARS-CoV

	MERS-CoV	SARS-CoV
Virological characteristics		
Phylogenetic origins	First human virus within betacoronavirus lineage C; common ancestor for MERS-CoV might have appeared halfway through 2011[,]	Betacoronavirus lineage B; SARS-like virus crossed animal-to-human species barrier and adapted to human host in late 2002
Estimated rate of evolution	$1·6 \times 10^{-3}$ substitutions per site per year (n=5)[4]	2×10^{-3} substitutions per site per year
Receptors on host cells	Dipeptidyl peptidase 4 (DPP4)	Angiotensin-converting enzyme 2 (ACE-2)[6]
Tissue distribution of receptors	Epithelial cells in kidney, small intestine, liver, prostate, activated leukocytes[8]	Type 1 and 2 pneumocytes, enterocytes of small intestine, proximal tubular cells of kidney[6]
Epidemiological characteristics		
Probable natural reservoirs	*Taphozous perforates* bats in Middle East	*Rhinolophus blasii* bats in China[6]
Probable intermediate animal host	Camels (*Camelus dromedaries*) suspected, other intermediate hosts cannot be excluded	Himalayan masked palm civet (*Paguma larvata*), Chinese ferret badger (*Melogale moschata*), raccoon dog (*Nyctereutes procyonoides*)
Incubation period	Up to 14 days[2]	1-14 days, with a median of 4-5 days, and a mean of 4-6 days
Route of transmission	Contact & airborne?	Contact & airborne[6]
Estimated basic reproduction number R$_0$	0.69 at worst-case scenario (high introduction rate of the virus into population with a moderate transmissibility); 0.6 at best-case scenario (low introduction rate of the virus with higher transmissibility)	0.8 at pre-pandemic stage; 2-4 at pandemic stage[15]
Case-fatality rate (CFR)	Age-dependent, 76% for cases aged 60 and over, 38% for cases younger than 60 years, with an overall CFR of approximately 55%[15]	Age-dependent, less than 1% in persons aged 24 years or younger, 6% in persons aged 25 to 44 years, 15% in persons aged 45 to 64 years, and greater than 50% in persons aged 65 years and older with an overall CFR of approximately 15%[12]
Age	Median 50 years (n=89) (range: 14 months to 94 years)[2]	Median age varies by countries from 15 to 59 (range: less than 1 year to 100 years)
Gender	61% male, 39% female (n=90)[2]	47% male, 53% female (n=8050)[16]
Co-morbidities	85%[2]	Information unavailable

livestock animals identified antibodies against MERS or MERS-like viruses in Omani and Spanish camels, suggesting that camels could be an intermediate host for MERS-CoV.[10] Camels are closely associated with human activity in the Middle East, including transport, food and racing, which may provide ample opportunities for "spill over" events. However, the exact route of exposure that leads to human infection remains to be established.[8]

Routes of transmission and transmissibility

The primary route of transmission of SARS appeared to be droplet infection, aerosolization and fomites. Majority of the cases were infected via close contact with severely ill patients in healthcare and household settings. Compared to SARS-CoV, the route of transmission for MERS-CoV remains less clear. Reports on nosocomial transmissions prompted the European Centre for Disease Prevention and Control (ECDC) and several international experts to advise healthcare workers caring for suspected and confirmed cases of MERS-CoV infections to practise both contact and airborne transmission precautions, in addition to standard precautions.[13,14]

The basic reproduction number R_0 for MERS-CoV was estimated to be lower than 1, and lower than that of SARS-CoV. [15] With the implementation of effective control measures, MERS-CoV is thought to be incapable of triggering a pandemic at the present time. However, the R_0 for MERS-CoV would change if the virus mutates or if there are mass gatherings like the pilgrimage in Saudi Arabia that facilitate the transmission of the virus through unprotected close contact. The proportion of asymptomatic and mild cases also affects the estimation of R_0. Increasing

asymptomatic and mild cases have been found during contact-tracing since mid-2013. Compared with the severe cases, these cases were younger without underlying co-morbidities, and with more even distribution between genders. The relative mildness of illness in secondary cases may reflect a difference in virulence between primary infections acquired from non-human exposure and secondary infections acquired from human-to-human transmission. However, the WHO conceded that the possibility of an artefact of surveillance and case-finding activities could not be ruled out.[9] Paired serological testing would be helpful to differentiate between infections and asymptomatic carriage.

Clinical presentations

MERS-CoV infection has presented with a wide disease spectrum thus far, ranging from asymptomatic infection, mild illness, to severe disease requiring mechanical ventilation and death.[4, 10, 11] The clinical presentation of MERS-CoV infection is reminiscent of that of SARS-CoV infection, including abrupt onset of high fever, rigors, and malaise, which deteriorate to a productive cough and pneumonia. Hematological abnormalities, in particular lymphocytopenia, were also observed frequently in both SARS-CoV and MERS-CoV infections. A proportion of SARS-CoV and MERS-CoV cases also experienced gastrointestinal symptoms. A detailed comparison of clinical features of MERS-CoV and SARS-CoV infections was described by Assiri et al.[12]

Compared to SARS-CoV, MERS-CoV infections had a much higher case-fatality rate (50% vs 15%), a higher prevalence in males (61% vs 47%), a high prevalence of cases with co-morbidities (85%) and an under-representation of children. It is unclear

if the higher fatality rate of MERS-CoV infection is attributed to under-reporting of asymptomatic or mild cases, or underlying higher prevalence of co-morbidities. For SARS-CoV infection, the case fatality rate (CFR) ranged from 0% to 50%, with an increasing CFR associated with increasing age. The age dependence of disease severity and mortality associated with the SARS-CoV infection was not completely attributed to the presence of other co-morbidities and the reason remains unknown, although it has been generally associated with a progressive age-related senescence of the immune system and perhaps other immune-mediated response to virus-host interactions and disease.[13]

Pulmonary involvement

Both MERS-CoV and SARS-CoV caused severe respiratory diseases in human. The viral load for both viruses was significantly higher in the lower respiratory samples than upper respiratory samples. Low viral load in the upper respiratory tract was thought to contribute to the low transmissibility of the virus. Based on the initial data, the WHO strongly recommended lower respiratory specimens, rather than nasopharyngeal swabs, to be used for diagnosis of MERS-CoV infection.[14]

Studies using *ex vivo* organ cultures revealed that MERS-CoV replicated well in both human bronchial and lung tissues, in contrast to SARS-CoV which replicated efficiently in lung tissues, but limitedly in bronchial tissues.[15] The tropism and replication competence of MERS-CoV suggested that MERS-CoV replicates as well as, if not better than SARS-CoV in lung and bronchial tissues, highlighting the potential threat posed by this virus to humans. Similar to SARS-CoV, MERS-CoV also targets type II alveolar epithelial cells, which play a key role in the regeneration of the alveolar epithelium after its damage as a result of viral infection.[24] This is in line with the disease severity and lung pathology observed in both SARS-CoV and MERS-CoV infected patients.

Extrapulmonary involvement

Besides the respiratory system, SARS-CoV also infected other organs, as high viral load was recorded in faeces, urine and serum samples.[16] The pathogenic mechanism underlying the watery diarrhoea observed in a quarter of patients with SARS remains unknown. Similarly, diarrhoea was also reported in some cases with MERS-CoV infection. In a study of 47 MERS patients, gastrointestinal symptoms were observed in many cases, including 12 cases with diarrhoea (26%), ten cases with vomiting (21%), and eight cases with abdominal pain (17%).[21] However, the causal relation between the virus and gastrointestinal symptoms has not been established. MERS-CoV with a concentration close to the lowest detection limit was identified in the faeces of a case on day 12 and 16 of illness[4] (the case was not reported to have gastrointestinal symptoms).

The presence of MERS-CoV in blood samples remains controversial, as the virus was not detected in blood samples in some cases,[4,18] but detected at a low level in one case.[17] In addition, e*x vivo* lung cultures demonstrated that both MERS-CoV and SARS-CoV infected endothelial cells of medium sized interstitial blood vessels of the lung, suggesting potential dissemination of the virus to other organs systematically.[24]

One clinical symptom commonly observed in many MERS-CoV cases, but less frequently in SARS-CoV cases was acute renal failure. Surprisingly, only

low viral load was detected in the urine samples of one case[4]. However, the case had received potentially nephrotoxic antimicrobial treatment for underlying multiple myeloma, and thus it was not clear whether renal failure in this case was due to viral infection.

Animal models

Development of animal models is crucial in the development of drugs and vaccines against a pathogen. A variety of animal models have been developed for SARS-CoV, including cynomolgous macaques, ferrets, cats, golden Syrian hamsters, mice and African green monkeys.[18] Unfortunately, they do not recapitulate the human disease fully, with shorter infection period, less severity and absence of gastrointestinal symptoms. MERS-CoV replicated well in a rhesus macaque model, causing varying degrees of acute pneumonia and mild-to-moderate clinical symptoms that lasted for a few days.[19] Similar to the observations made in human, MERS-CoV was found to infect the lower respiratory tract of the animals. However, no systemic infection was detected in the animals, as compared to the infection in humans. Nevertheless, the animal model would enable further studies on the pathogenesis and potential intervention strategies for MERS-CoV.

Treatment

Similar to SARS-CoV, MERS-CoV also evades innate immune responses, reflected by the poor interferon (IFN) and pro-inflammatory chemokine responses observed in *in vitro* human airway epithelium (HAE) cultures and *ex vivo* bronchus or lung cultures.[24,20] Various interventions were attempted to treat the SARS-CoV infected patients during the outbreak, including interferon alfacon-1 (a synthetic interferon) combined with steroid, protease inhibitors together with ribavirin, or convalescent plasma containing neutralizing antibody; however, a definitive treatment regime has yet to be established.[21] IFN therapy has been shown to have therapeutic potential in SARS-CoV infection *in vitro*, animal models and several human studies based on retrospective controls. Similarly, both IFN alpha and beta significantly inhibited viral replication of MERS-CoV in *ex vivo* cultures, and both IFN I and III suppressed viral replications of MERS-CoV in *in vitro* HAE cultures, suggesting a potential therapeutic application of IFNs in treating MERS-CoV infections.[28,22] Combination of IFNa-2b and ribavirin was shown to inhibit viral replication of MERS-CoV *in vitro*, at a concentration likely achievable in humans.[23]

Risk assessment

One major risk factor for SARS-CoV infection was the rearing and slaughtering of wildlife for human consumption in the wet markets of southern China.[12] Like SARS-CoV, MERS-CoV is thought to be of animal origin and transmitted to humans sporadically through a yet unknown route. At present, there are several gaps of critical information including the geographic distribution of the virus, the potential source of infection, routes of transmission and main exposures. The appearance of cases in multiple locations in the Middle East suggests that the virus may be widespread throughout the region, or that the virus was present in something that was distributed throughout the region.[17]

For both viruses, human-to-human transmissions have occurred with considerable mortality. Close contact with cases in hospitals posed one of the major risks for the spread of SARS-CoV. Air travel also amplified a local outbreak to a potential global pandemic. These risk factors may apply for

MERS-CoV as well. There was clear evidence that MERS-CoV could be transmitted between humans of close contacts in the healthcare and household settings, and secondary transmission from imported cases had occurred in the United Kingdom, France, Tunisia and Italy. An analysis of commercial airline traffic out of the Kingdom of Saudi Arabia (KSA), Qatar, United Arab Emirates (UAE) and Jordan in 2012 revealed that only about 7% of the passengers from these countries travelled to the four countries that have imported cases thus far, raising questions on whether the virus might already be imported into low and lower-middle income countries which constituted 65% of the traffic and are not well equipped to detect or contain the virus.[24] In light of the current development, the European Centre for Disease Prevention and Control (ECDC) assessed that a future SARS-like scenario could not be excluded.[14]

Underlying immuno-suppression and co-morbidities may increase the susceptibility of the cases to MERS-CoV infection, and result in atypical clinical presentation which may delay the diagnosis. Unrecognized asymptomatic or mild cases in the community or healthcare setting may be a significant contributor to undetected on-going disease transmission, as an index case in a family cluster in KSA was suspected to have acquired MERS-CoV while in hospital from an unidentified asymptomatic or mild case.[25] SARS-CoV evolved to acquire increasing transmissibility in humans during the course of the outbreak. Although no sustained transmission in the community has been observed thus far for MERS-CoV, similar adaptation of the virus may also occur, and warrants constant monitoring and assessment.

With the ongoing presence of the source(s) of infection in the Middle East region and the increased awareness and surveillance efforts, additional cases of the infection in the region, as well as imported cases from the region can be expected. In view of the presence of air traffic between Singapore and the affected areas in the Middle East, the importation of the MERS-CoV infection is possible. Although subsequent spread of the infection in the community as a result of an imported case is unlikely given its limited human-to-human transmissibility, there is a possibility of limited transmission to close contacts. The Ministry of Health (MOH) and healthcare institutions remain vigilant against possible imported cases of the MERS-CoV infection and have stepped up operational readiness in the event of an imported case. The situation will be monitored closely for further developments.

(Contributed by Public Health Intelligence Unit, Epidemiology & Disease Control Division, and Communicable Diseases Division, Ministry of Health, Singapore)

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A genetic insight into the novel avian influenza A(H7N9) virus outbreak in China, March to August 2013

Introduction

Influenza A viruses belong to the family *Orthomyxoviridae*. Its virus genome comprises eight genes - heamagglutinin (HA), neuraminidase (NA), basic polymerase 1 (PB1), basic polymerase 2 (PB2), acidic polymerase (PA), matrix (M), nucleoprotein (NP) and non-structural protein (NS) - encoding for 11 proteins. On the basis of antigenic differences in the two surface proteins HA and NA, influenza A viruses are categorised into different subtypes.[1] 17 HA and 10 NA subtypes are known to exist; wild waterfowl are natural hosts for all known influenza subtypes except H17 and N10 which have only been found in bats.[2]

On 31 March 2013, the National Health and Family Planning Commission of China reported three human infections in Shanghai and Anhui with a previously undescribed avian influenza A(H7N9) virus. Additional infections were subsequently detected; as of August 2013, a total of 135 cases of human infection with avian influenza A(H7N9) including 45 deaths were reported in twelve provinces/municipalities of China. One imported case was also reported in Taiwan. This case had worked in Jiangsu, China, prior to his illness.

Sporadic human cases of avian influenza A(H7) virus infection including A(H7N2), A(H7N3) and A(H7N7), linked with outbreaks in poultry have previously been reported. These infections in humans have mostly been mild, ranging from conjunctivitis to mild upper respiratory illness, with the exception of one fatal avian influenza A(H7N7) case in the Netherlands who developed pneumonia and acute respiratory distress syndrome.[1] However, the avian influenza A(H7N9) outbreak in China was characterised by rapidly progressive pneumonia, respiratory failure, acute respiratory distress syndrome and a recorded case fatality ratio of 33% as at August 2013.[3]

Origins of the novel influenza A(H7N9) virus

Phylogenetic analyses of the avian influenza A(H7N9) viruses causing human infection showed that the viruses originated from multiple reassortment events, and was genetically distinct from previously reported avian influenza A(H7N9) lineages found in wild birds.[3-6] The time, location and host species in which the reassortment events took place to give rise to the novel avian influenza A(H7N9) virus remain uncertain, although domestic ducks have been proposed as key intermediates for the acquisition of diverse influenza virus from migratory birds and reassortment events with subsequent transmission to chickens.[4,7] These events were likely to have occurred in Shanghai, or the adjacent provinces of Zhejiang or Anhui.[4]

The virus is wholly of avian origin. Based on comparison with publicly available sequences, the H7 gene is most closely related to the HA gene from avian influenza A(H7N3) isolated from ducks in Zhejiang in 2011. While earlier analyses suggested that the N9 gene is most closely related to avian influenza A(H7N9) viruses isolated from wild ducks in South

Korea in 2011, recent data have proposed that the N9 is, instead, closer to avian influenza A(H11N9) and A(H2N9) viruses found in migratory wild birds in Hong Kong from 2010 to 2011.[7]

The remaining six internal genes appeared to have been most related to those from at least two different origins of avian influenza A(H9N2) viruses from China, including a 2012 isolate from a brambling in Beijing (PA, PB1 and PB2 genes), and a 2011 and a 2012 isolate from chickens in Zhejiang and Shanghai, respectively (M, NP and NS genes).[4,5,9] There is sufficient diversity between the current avian influenza A(H7N9) isolates obtained from human cases to imply that the novel virus has further diversified since its emergence into at least two different lineages.[4] Also, the internal genes of the avian influenza A(H7N9) viruses are more diverse than their HA and NA genes, indicating that the viruses are still subject to frequent reassortment and rapid evolution.[8]

Source of human infection

While the source of the virus remains unknown, the direct source of human infection by this novel virus is likely to be infected poultry and contaminated environments in live poultry markets.[9,10] The majority of human cases reported exposure to live animals such as poultry, including during visits to live animal markets.[11] Genetically similar avian influenza A(H7N9) viruses to the human isolates were simultaneously present in chickens, pigeons and the environment in the live poultry markets of the affected regions, suggesting direct avian transmission as the source of human infection.[5,9,12,13] With the exception of one sample from a wild pigeon in Nanjing, and one sample from a racing pigeon from a pigeon farm in Jiangsu, all positive avian influenza A(H7N9) samples tested

by the Ministry of Agriculture of China were from live-poultry markets.[14] This strongly suggests that live poultry markets may have been the origin of emergence of the novel avian influenza A(H7N9) virus; live poultry markets bring together a high density of bird and poultry species from various sources provide an ideal environment for reassortment of various avian influenza virus subtypes.[9]

Molecular features of the novel avian influenza A(H7N9) virus

The cleavage of HA is crucial for influenza infectivity and virus spread. In humans, infection and replication of the influenza virus is restricted to the respiratory tract, where proteases that cleave HA are known to be present. In avian hosts, however, proteases that cleave HA are present in many different tissues. Low pathogenic (for birds) avian influenza viruses possess monobasic amino acid residues at the HA cleavage site, which can be cleaved in the gastrointestinal tract of birds, resulting in a local infection. In contrast, highly pathogenic avian influenza viruses have multibasic amino acid residues at the HA cleavage site that are cleaved in a broad range of tissues, expanding the range of organs they can infect and consequently causing systemic infection in birds. The avian influenza A(H7N9) viruses isolated from human cases, poultry and environmental samples contain monobasic amino acid residues at the HA cleavage site, characteristic of low pathogenicity in avian hosts.[5,6,9] This is likely to allow the virus to spread silently in domestic and wild birds. A unique feature of the avian influenza A(H7N9) outbreak in humans was the apparent absence of preceding die-offs in poultry or wild birds, a sharp contrast to avian influenza A(H5N1) or other A(H7) subtype outbreaks.[1,15] Preliminary laboratory testing showed

that avian influenza A(H7N9) infected chickens and quail did not show signs of illness but were shedding virus.[8,16] Another feature favouring adaptation in poultry that was found in all sequenced isolates is a five amino acid deletion in the stalk region of NA which might improve virus adaptation to land-based poultry.[5,15] This deletion distinguishes the novel avian influenza A(H7N9) virus from the previously noted lineages in wild birds, and suggests that the virus was already circulating among terrestrial birds prior to the cases of human infection.[4,6]

Genetic analyses of the avian influenza A(H7N9) isolates have also revealed molecular markers predicted to be associated to human adaptation. Glycan receptor binding specificities of HA govern host tropism. Avian influenza viruses generally prefer α-2,3 sialic acid receptors which are abundant in the avian alimentary tract, while human influenza viruses preferentially bind α-2,6 sialic acid receptors which are predominant on the human respiratory tract. The different receptor affinities between human and avian influenza viruses act as a barrier to cross-species infections. For instance, although the avian influenza A(H5N1) virus may infect humans and undergo limited replication due to a minor population of α-2,3 sialic acid receptors present only in the lower respiratory tract, its lack of affinity for the α-2,6 sialic acid receptors limits bird-to-human and also secondary human-to-human transmission. However, the rapid increase in the number of human infections with avian influenza A(H7N9) from March to May was unprecedented for avian influenza viruses, hinting at a propensity for human infection or due to enhanced surveillance.[15] Accordingly, a variety of HA mutations including Ser138Ala, Thr160Ala, Gly186Val, Gln226Ile and Gln226Leu which have been experimentally associated with a switch from α-2,3 to α-2,6 receptor

specificity, were found in most of the sequenced avian influenza A(H7N9) human, environmental and avian isolates. This implies that these viruses have partially acquired human-type receptor binding specificities which had facilitated cross species infection.[5,6,9,12,15] Additionally, a Ile368Val substitution in PB2, which enables droplet transmission in mammalian models, is present in majority of these isolates.[15]

Human influenza viruses replicate in the upper respiratory tract at the nasal body temperature of 33°C, markedly lower than avian influenza viruses which replicate in the intestinal tract at 41°C. Interestingly, a Glu627Lys substitution in PB2, which allows viral replication at lower temperatures, was found in many of the avian influenza A(H7N9) isolates from human cases, but not in the poultry and environmental isolates.[8,9] This substitution has also been demonstrated to be a principal determinant of virulence for avian influenza A(H5N1) isolates.[17] While a direct comparison of the fatality risk of admitted patients with avian influenza A(H7N9) and A(H5N1) suggest a substantially milder clinical course for A(H7N9), corroborated by observations in infected ferrets, the ability of avian influenza A(H7N9) to cause severe pneumonia in a significant proportion of cases was unexpected for avian influenza A(H7) subtype viruses.[1,18,19] Notably, the PB2 Glu627Lys adaptation was identified in the avian influenza A(H7N7) isolate that caused the only known fatal infection of the avian influenza A(H7) subtype prior to the current A(H7N9) outbreak.[20] Studies have also found that avian influenza A(H7N9) virus isolates from human cases replicated more efficiently and were more lethal in mice compared to those isolated from birds, indicating that PB2 Glu627Lys likely contributed to the increased virulence, as was observed for avian influenza A(H5N1) viruses.[8,21]

One human isolate which did not have Glu-627Lys, expressed the PB2 Asp701Asn mammalian signature associated with mammalian adaptation, transmission and virulence.[5,8,15] The absence of the Gln727Lys and Asp701Asn in the poultry and environmental isolates implies that these genetic adaptations were acquired within the human host during replication following the cross species jump. In comparison, the adaptability of avian influenza A(H5N1) viruses to humans may be inferior to avian influenza A(H7N9) as the former appear to acquire these PB2 substitutions less frequently.[5,9] Additional mutations detected in all sequenced avian influenza A(H7N9) isolates include Asn30Asp and Thr215Ala in M2, and Pro42Ser in NS1, all previously associated with increased virulence in the mouse infection models.[15]

In the absence of an effective vaccine, antiviral compounds are the frontline therapeutic options against infection with novel influenza viruses. Two classes of antivirals are currently approved for the treatment of influenza A infections: the M2 blockers adamantanes and NA inhibitors.[22] Based on sequence analysis, the novel avian influenza A(H7N9) virus is likely to be resistant to adamantanes as all the isolates sequenced to date possess the M2 Ser31Asn substituition.[6] This mutation is also present in influenza A(H1N1)pdm09 and seasonal influenza strains.[23]

With the exception of three known human isolates carrying the NA Arg292Lys substitution, most strains of avian influenza A(H7N9) are predicted be sensitive to the NA inhibitors.[6] NA Arg292Lys is known clinically to confer high-level resistance to oseltamivir, intermediate resistance to peramivir and reduced sensitivity to zanamivir, but at a cost to viral fitness and transmissibility.[23] *In vitro* phenotypic testing, however, demonstrated susceptibility to both oseltamivir and zanamivir in all tested isolates, including the strain A/Shanghai/1/2013 containing the NA Arg292Lys substitution. Further analysis revealed that the A/Shanghai/1/2013 isolate contained a mixed population of virus with (35%) or without (65%) the Arg292Lys substitution, which likely masked the anti-viral resistant phenotype. As predicted, enrichment of the Arg292Lys virus population conferred the properties of oseltamivir, zanamivir and peramivir resistance.[3,22] In most avian influenza A(H7N9) patients, oseltamivir treatment was associated with falling viral loads in the respiratory tracts which correlated with improved outcome. Notably, treatment failure and adverse clinical outcomes were observed in the three patients in whom NA Arg292Lys substitution emerged within days of initiating oseltamivir and corticosteroid treatment.[3,24] There was evidence suggesting that in at least one patient, the mutation had emerged *de novo*, likely as a result of selection pressure from oseltamivir therapy.[24] Arg292 is a highly conserved residue across all NA subtypes and to date, the Arg292Lys is a rare substitution that has only been reported in human isolates from patients treated with oseltamivir; the apparent ease with which antiviral resistance emerges in avian influenza A(H7N9) is therefore concerning.[23,24] Key genetic mutations identified in avian influenza A(H7N9) viruses are shown in *Table 2*.

Pandemic potential

A key concern of the avian influenza A(H7N9) outbreak has been its potential to cause a human pandemic. Reports of family clusters for which human-to-human transmission cannot be ruled out suggest the potential for virus spread between close contacts.[11,25] Although preliminary genetic analyses of the avian influenza A(H7N9) virus have highlighted hallmark genetic signatures governing human host

Table 2

Key genetic mutations identified in avian influenza A(H7N9) viruses

Viral protein	Key molecular features	Associations
HA	Monobasic cleavage site	Low pathogenicity in avian hosts
	Ser138Ala	Switch from avian-like α-2,3 to human-like α-2,6 receptor specificity
	Thr160Ala	
	Gly186Val	Expected to be necessary for human transmission
	Gln226Ile	
	Gln226Leu	
NA	Five amino acid stalk deletion	Adaptation to land based poultry
	Arg292Lys	Resistance to NA inhibitors
		Reduced viral fitness and transmissibility
PB2	Ile368Val	Enables droplet transmission in mammalian models
	Glu627Lys	Allows adaptation to lower temperature of human upper respiratory tract
	Asp701Asn	
		Increased virulence in mammalian host
M2	Asn30Asp	Increased virulence in mice
	Thr215Ala	
	Ser31Asn	Adamantane resistance
NS1	Pro42Ser	Increased virulence in mice

binding and adaptation, there is currently no evidence of widespread infection in humans resulting from human-to-human transmission. Similar to human infection with avian influenza A(H5N1), avian influenza A(H7N9) patients presented primarily with infection of the lower respiratory tract, and sputum and endotracheal aspirates were better than nasopharyngeal and throat swabs for the detection of the virus.[5,26] These suggested that the virus might replicate more efficiently where both α-2,3 and α-2,6 sialic receptors were located.[5] Hence, while the novel virus has a postulated increased affinity for α-2,6 sialic receptors, binding to α-2,3 is likely to be retained. Glycan arrays have showed mixed α-2,3 and α-2,6 glycans

binding preferences, although the specific affinity for glycan type was variable among the avian influenza A(H7N9) isolates.[8]

Structural studies with human tracheal epithelium have subsequently demonstrated that the current avian influenza A(H7N9) isolates have weak binding to the upper respiratory tract compared to human adapted viruses, which might have restricted aerosol transmissibility of the virus.[27] Transmission studies in animal models have shown that while avian influenza A(H7N9) virus were readily transmitted via direct contact, transmission via respiratory droplets and aerosols was limited, and was intermediate to that

of typical human and avian influenza viruses.[8,28-30] Residual binding to α-2,3 sialic receptors and the lower stability of the avian influenza A(H7N9) HA in the acidic mammalian nasal mucosa were suggested to be barriers to increased virus transmission via the respiratory route.[28] Although the avian influenza A(H7N9) viruses were not readily transmissible via respiratory droplets, the efficient virus transmission via the direct contact route which had not been observed with avian influenza A(H5N1) may explain its increased propensity for human infection.[30] Animal models and *in vitro* studies have indicated that the avian influenza A(H7N9) viruses have the capacity for efficient replication in mammals and human airway cells.[28,30]

Conclusion

The genetic changes observed in the avian influenza A(H7N9) virus, coupled with experimental data in cells and animal models help to explain some of the epidemiological features of the outbreak in China, including transmission and disease severity in humans and birds.

Surveillance since 1918 has yet to identify any poultry-adapted influenza virus with the ability to cause stable, widespread infection in humans.[31] Currently, the avian influenza A(H7N9) virus exhibits features of a poorly adapted avian influenza virus that remains unable to sustain its spread among human hosts. Yet, influenza viruses constantly change, and the presence of several important mammalian signatures in the current avian influenza A(H7N9) strains arguably suggests that this virus may be more likely than other avian viruses to become human adapted.

While knowing the presence of mutations involved in mammalian transmission would theoretically aid in determining if there is evolution in the direction of a pandemic virus, it is currently not possible to predict how an influenza virus would behave in humans just from monitoring genetic changes. The exact determinants of efficient transmissibility in humans or virulence do not hinge on single critical mutations in the virus, but are instead multifactoral and multigenetic. Predictions of how particular influenza strains will evolve also remain highly speculative.[32] In addition, virus independent factors including host susceptibility such as the presence of underlying comorbidities, extremes of age and genetic predisposition can influence interspecies jumping of avian influenza virus to humans, as well as the severity of human infection.[15]

Continued surveillance and monitoring of the global developments in influenza viral evolution as well as pandemic preparedness and planning will serve to prepare us against the emergence of the next influenza pandemic.

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Outbreak of *Salmonella* Enteritidis in several childcare centres in Singapore

Introduction

On 11 May 2011, the Ministry of Health (MOH) was notified of two separate food poisoning incidents involving pre-school children from two childcare centres after consuming a lunch meal provided by a caterer on 10 May 2011.

Field investigations were carried out immediately at the childcare centres and the implicated caterer's premises. We report the epidemiological investigations and findings of the outbreak.

Methods

As part of our investigations, we asked the implicated caterer if they had also catered lunches for other childcare centres. If so, we would follow up with the management of these centres to ask if any of the children and staff had come down with food poisoning symptoms.

A case was defined as a child or staff of the affected childcare centres who developed at least two of the following signs and symptoms of gastroenteritis:

fever, diarrhoea, vomiting or abdominal pains, after consuming lunch provided by the implicated caterer on 10 May 2011.

Epidemiological and demographic data of the affected cases, such as age, gender, ethnicity and date of onset of illness were recorded. Clinical signs and symptoms of those who were ill and the types of medical treatment sought were also obtained.

Stool samples were collected from reported cases and the caterer's food handlers for testing of enteropathogens (*Shigella, Campylobacter, Vibrio, Salmonella, rotavirus* and *norovirus*). Food and environmental samples were also taken from the food premises for microbial analyses.

Findings

We found that the caterer had provided lunch on 10 May 2011 to 17 other childcare centres. Of these,

10 childcare centres reported at least two or more cases of food poisoning. A total of 271 cases were identified, giving an overall attack rate of 18.5%. They comprised 228 children and 5 teachers from eight childcare centres under Management A, 20 children and 8 teachers under Management B, and 10 children under Management C. The attack rates for children and staff were 18.8% and 14.9% respectively. The children were aged between 2 and 6 years while the teachers were aged between 21 and 51 years. The median incubation period was 24 hours and ranged from 6 – 96 hours. The epidemic curve is shown in *Fig. 2*. The clinical features comprised fever (98.2%), diarrhoea (88.2%), vomiting (53.9%) and abdominal pain (8.5%). Of the reported cases, 42 (15.5%) required hospitalization while the rest sought outpatient treatment. All recovered uneventfully.

Our investigations revealed that seafood pasta served during lunch on 10 May 2011 was the only

Figure2
Onset of illness of 271 food poisoning cases at 10 affected centres , 10 - 14 May 2011
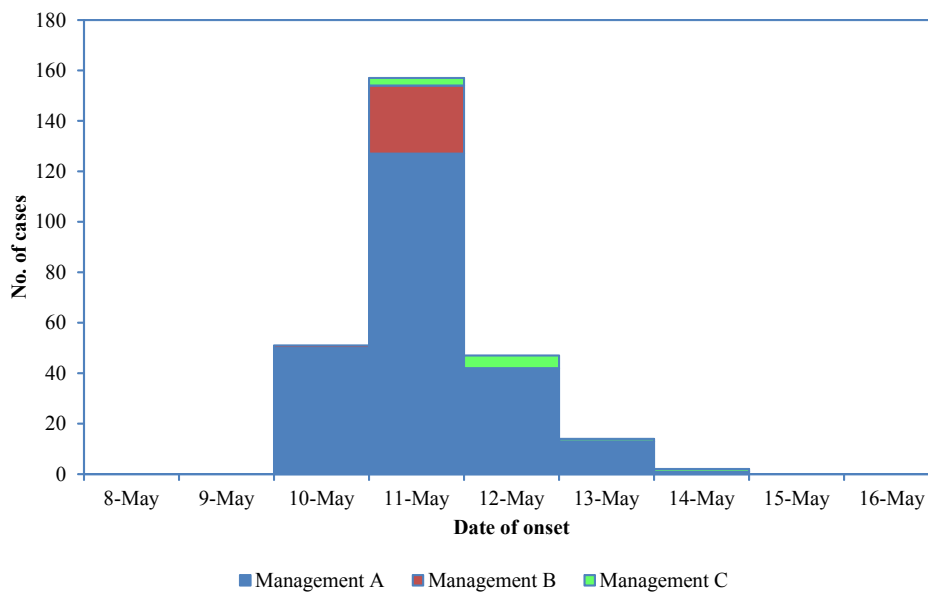
common food item that had been served to all 10 affected childcare centres. The seafood pasta, which consisted of pasta, tomato sauce, prawns and fish, was prepared on the same day. The pasta and tomato sauce with prawns and fish were packed separately in food trays and covered with cling wrap. The seafood pasta was subsequently delivered by vans to the childcare centres.

A number of food hygiene lapses in the caterer's premises were observed such as dirty food trays, no separate trays for storage of raw or cooked food items, chopping boards for raw and cooked food washed together in a big pot of hot water, and improper storage of leftover ice cubes.

A total of 48 stool samples were obtained from the reported cases of seven affected childcare centres.

Of these, 45 were positive for *Salmonella* Group D which was further identified as *Salmonella* Enteritidis. Of 28 isolates obtained from reported cases in 6 childcare centres, all were found to have identical Multiple-Locus Variable number of tandem repeat Analysis (MLVA) (*Fig. 2*).

Of the 30 food handlers screened, one of them was found to be positive for *Aeromonas*. This was deemed to be an incidental finding not related to the outbreak. He was asymptomatic prior to the screening. No food remnants were available for microbial testing and 19 other food samples obtained during visits to the caterer's premises on 12 and 13 May 2011 were negative for food-borne pathogens. However, two of the ten environmental swabs tested showed presence of *E. coli* in the blender (used for making the pasta sauce) and in the basin at the washing area.

Figure 3
MLVA typing of three *Salmonella*-positive cases
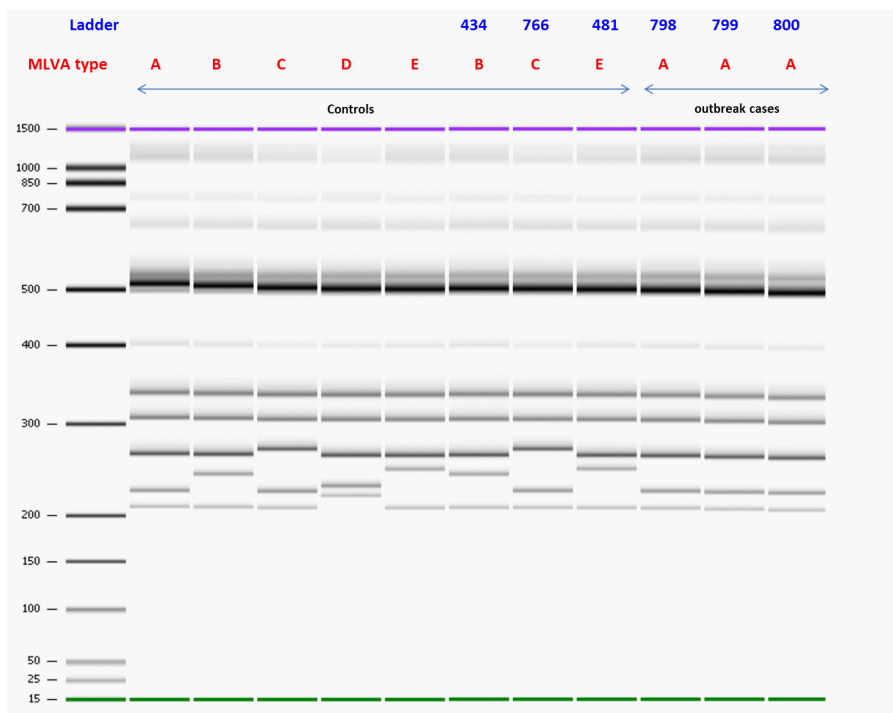
Discussion

This is a point-source outbreak of salmonellosis caused by *Salmonella* Enteritidis, with identical MLVA type A. We did not carry out a case-control study to determine the vehicle of transmission as these preschool children would not be able to provide a reliable food history.

In Singapore, salmonellosis was made a legally notifiable disease in December 2008 under the Infectious Diseases Act. Since then, the incidence rate of salmonellosis cases has shown an increasing trend from 22.9 per 100,000 population in 2009 to 28.2 per 100,000 population in 2012[1]. *Salmonella* Enteritidis associated food-borne outbreaks have been reported in egg-based pancake[2], cream cakes[3], bread[4], and tiramisu[5].

Salmonella Enteritidis is not native to seafood but is commonly found in items such as poultry and eggs[6]. While the exact mechanisms of bacterial contamination of the seafood pasta lunch meal supplied by the caterer and consumed on 10 May 2011 are uncertain, it may be possible that cross-contamination with raw foods had occurred during preparation and storage at the caterer's premises. There is supportive evidence of this likelihood in the finding of *E. coli* in the blender (indicating improper cleaning after previous use), and the mixing of trays and chopping boards for raw and cooked foods (showing poor food hygiene practices).

The caterer's licence was temporarily suspended by the National Environment Agency (NEA) from 12 May 2011 to 6 July 2011. The suspension was lifted by NEA after the caterer had carried out a thorough review of its food safety management system and made improvements to enhance its processes, with particular emphasis on cross-contamination prevention. All the food handlers were also required to re-attend and pass the basic food hygiene course before they were allowed to resume work.

(Contributed by Toh HY, La MV, Hishamuddin P and Tay J, Communicable Diseases Division, Ministry of Health)

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Outbreak of norovirus gastroenteritis at a nursing home in Singapore

Introduction

On 24 Dec 2012, the Ministry of Health (MOH) was notified of an outbreak of gastrointestinal illness at a nursing home, involving 18 residents and 6 staff who had developed diarrhoea and vomiting between 21 and 24 Dec 2012.

The 4-storey nursing home, which is licensed by MOH, has a total of 242 residents. The residents are housed in rooms situated on the first, second and third storeys. Meals for all staff and residents are prepared and cooked in an in-house kitchen with separate Muslim and non-Muslim facilities. The home also provides day-care services to 160 clients (80 clients in the Day Rehabilitation Centre, 39 clients on home-help programmes and 41 clients in the Dementia Care Centre), some of whom are also provided with meals while attending day-care services there. There are 202 staff, 100 of whom reside in its premises.

An epidemiological investigation, including an inspection at the nursing home, was carried out immediately. This report summarizes the findings of the outbreak investigation.

Methods and materials

Active case detection through interviews with staff and review of patient records was carried out to identify other unreported cases. Demographic data such as age, gender and ethnicity were recorded with the assistance of the nursing manager. Signs and symptoms of those who were affected, dates of onset of illness, and the type of medical treatment sought were also obtained.

Stool samples were obtained from the reported cases and tested for the more common enteropathogens (*Shigella*, *Campylobacter*, *Vibrio*, *Salmonella*, rotavirus and norovirus). Implicated food handlers were referred to the Communicable Disease Centre, Tan Tock Seng Hospital, for medical screening and stool testing. Food and environmental samples were also collected for microbiological analyses.

A case was defined as an individual who resided or worked or attended services at the nursing home and who developed one or more of the following symptoms: watery diarrhoea (two or more episodes within 24 hours), vomiting, with or without accompanying abdominal pain, fever and nausea between 17 Dec 2012 and 6 Jan 2013.

Results

Epidemiological findings

A total of 60 cases, who consisted of 42 residents, 15 staff and 3 day-care services clients, were identified, giving an overall attack rate of 10.6%. The attack rate was 17.4% for residents, 7.4% for staff and 1.9% for clients. Among the affected residents, the attack rate was higher in those on oral feeding (17.8 %) compared to those on tube feeding (15.0%) (*Table 3*). The mean ages of affected residents, staff and day-care clients were 73 years, 39 years and 68

Table 3
Attack rates by residents, staff and clients in an outbreak of gastroenteritis in a nursing home, 17 Dec 12 to 6 Jan 2013

		Total	No of affected cases	Attack rate (%)
Residents		242	42	17.4
Oral feeding	Non-Muslim	162	32	19.8
	Muslim	40	4	10
Tube feeding	Non-Muslim	40	6	15
	Muslim	0	0	0
Staff		202	15	7.4
Clients		160	3	1.9
Total		604	60	9.9

years, respectively. Their symptoms comprised watery diarrhoea (72.4%), vomiting (65.5%), fever (6.9%) and abdominal pain (5.0%). A total of 9 cases (15%) were hospitalized for observation, 17 cases (28.3%) sought treatment at a polyclinic or private clinics, 8 cases (13.3%) were treated by the in-house volunteer doctor and the remaining 26 cases (43.4%) either self-medicated or recovered without treatment.

The epidemic curve is shown in *Fig. 4*.

The majority of the affected residents in this outbreak resided on the first and second floor (21 cases residing on first floor and 19 cases on the second floor); only two residents on the third floor were affected. The initial wave of affected residents in this outbreak was predominantly non-Muslim residents and staff who consumed food prepared at the non-Muslim kitchen. Subsequently, residents who were on tube-feeding and Muslim residents were also affected.

The kitchen was satisfactorily maintained during site inspection on 26 December 2012. No major hygiene lapses were observed.

Laboratory findings

Six stool samples obtained from four ill residents and two staff were tested positive for norovirus. Further genetic analyses of the viruses carried out by the National Public Health Laboratory showed that all were of the same genotype GII.

Four of 20 food handlers, who were involved in daily food preparation or feeding duties of residents, were tested positive for norovirus. One of them had symptoms of gastroenteritis on 18 Dec 2012, but continued working in the non-Muslim kitchen on 19 Dec 2012 and throughout the duration of the outbreak. Another food handler was tested positive for *Aeromonas* species but negative for norovirus. He was the

Figure 4
Onset of illness of 60 reported cases of gastroenteritis in a nursing home,
17 December 2012 to 6 January 2013
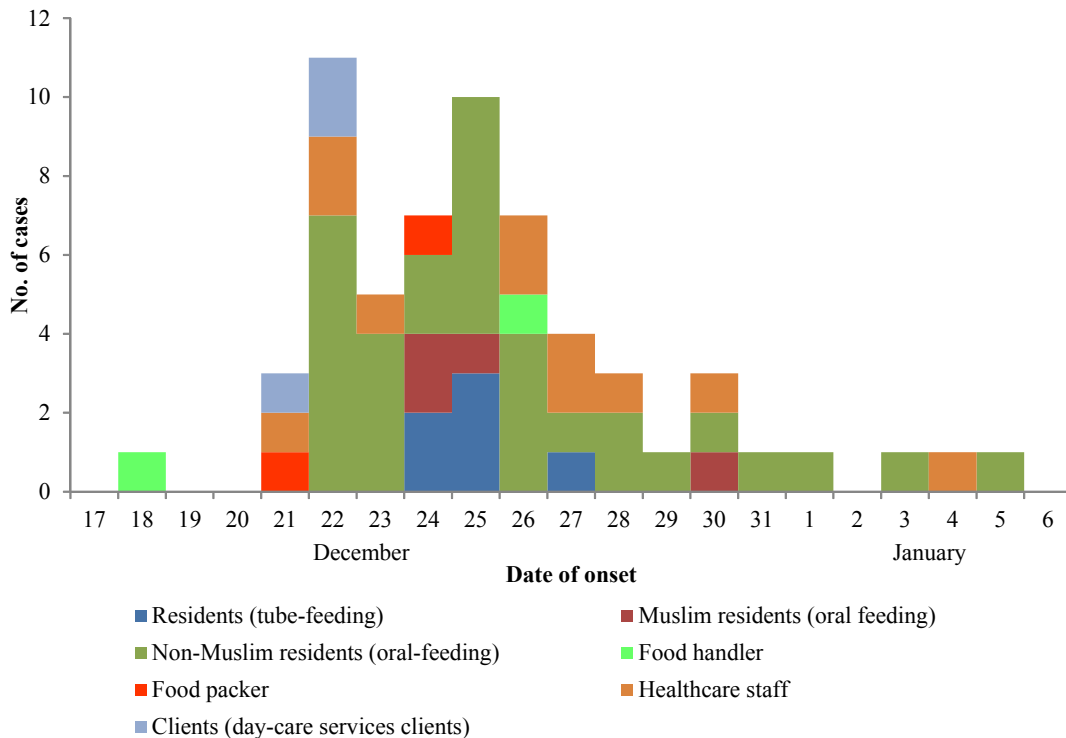
head chef of the Muslim and non-Muslim kitchens and was asymptomatic throughout the outbreak.

Of three food samples tested, one (mixed vegetables) was found to have high total coliforms (460 MPN/g) and faecal coliforms (460 MPN/g); the safety limit for both is <50 MPN/g. This indicates poor handling practices.

Two of the four environmental swabs were positive for norovirus genogroup I. One was obtained from the cabinet top beside an affected resident's bed while the other was from the table top of the adult diaper trolley.

Prevention and control

At the time of outbreak investigation, the home had already stepped up infection control procedures to prevent further on-going transmission especially at the second and third storeys where majority of the affected residents were housed. In addition, MOH advised the home on the following measures:

- Identify and isolate sick residents and staff early;

- Set up isolation rooms for cases.

- Promote frequent and proper hand washing among healthcare workers and other staff, es-

pecially after attending to patients, toilet visits and before eating or preparing food;

- Carry out regular disinfection with diluted bleach of frequently touched surfaces such as door handles, knobs, staircase railings and lift buttons;

- Use a different trolley to dispose of diapers for affected cases and to disinfect it after use;

- Ensure that toilets are in a sanitary condition and adequately equipped with soap and toilet paper;

- Ensure adequate ventilation in places of congregation, such as the wards and avoid overcrowding;

- Remind food handlers to observe good food and personal hygiene, and to refrain from handling food if they are unwell;

- Remind healthcare workers who are unwell to refrain from taking care of residents and feeding duties.

- Advise healthcare workers to double bag vomitus for disposal and to clean and disinfect the area with diluted bleach.

Following strict observation of prevention and control measures, the outbreak subsided with the onset of illness of the last reported case on 5 Jan 2013.

Discussion

The epidemiological and clinical features of this outbreak, with watery diarrhoea and vomiting as the predominant symptoms, suggest that this was probably an outbreak of viral gastroenteritis. The isolation of norovirus genogroup GII from the stools of six cases and four food handlers pointed to this viral pathogen as the cause of the outbreak.

Noroviruses are highly contagious and are usually transmitted directly from person to person by faecal-oral spread, indirectly through contaminated food and water, or through environmental contact[1]. Good evidence exists for transmission due to aerosolisation of vomitus that presumably results in droplets contaminating environmental surfaces or entering the oral mucosa and being swallowed[2]. There is no evidence to suggest that infection occurs through the respiratory system[3].

Seroprevalence studies in Mexico, Turkey, Spain, Korea, and Brazil have shown that norovirus infections are common throughout the world, and most children would have experienced at least one infection by the age of 5 years[4-8]. In both developing and developed countries, noroviruses are estimated to cause 12% of all severe diarrheal diseases[9]. Norovirus is the leading cause of gastroenteritis in the United States constituting over 50% of all foodborne-disease outbreaks due to a known cause that were reported to the US Centers for Disease Control and Prevention from 2006 to 2008[10]. In Germany, according to the Robert-Koch-Institute in Berlin, the number of norovirus outbreaks has increased by 20% between 2009 and 2010 and is among the top reportable diseases[11]. In Singapore, a recent study carried out by the Ministry of Health between 2009 and 2011 highlighted an increasing trend of detection of norovirus in reported gastroenteritis outbreaks (2.8% to 6.3%) as well as in implicated food handlers (10.8% to 23.1%)[12].

The incubation period for norovirus is 12-48 hours and symptoms may last 24-72 hours. The virus is usually present in very high amounts in the stool and vomitus of those who are unwell, and the infection can be transmitted rapidly in crowded, closed settings.

Healthcare facilities, including nursing homes and hospitals, are the most commonly reported settings for norovirus outbreaks in the United States and other industrialized countries. Nearly two-thirds of all norovirus outbreaks reported in the United States occur in long-term care facilities[13]. Similar outbreaks of norovirus gastroenteritis have also been documented to occur in educational institutions and nursing homes in Singapore[14-16].

Noroviruses in human belong to one of three norovirus genogroups (GI, GII, or GIV), which are further divided into more than 25 genetic clusters. Over 75% of confirmed human norovirus infections are associated with genotype GII[13]. Of the documented outbreaks in Singapore, more than half have been associated with genotype GII[14-16].

The index case for this outbreak is likely to be the symptomatic food handler who was ill on the night of 18 Dec 2012 and tested positive for norovirus.

His duties were mainly confined to the non-Muslim kitchen such as washing of dishes and preparation of food. Despite having gastrointestinal symptoms, he continued working the next day on 19 Dec 2012. He could have contaminated the utensils or food in the non-Muslim kitchen. This could explain why Muslim staff and residents were not affected in the initial phase of the outbreak.

Subsequent spread of the infection to the Muslim and other residents and staff could have occurred through person-to-person transmission or via contaminated environmental surfaces, as evidenced by the detection of norovirus in an adult diapers trolley and a cabinet of one of the cases. The affected healthcare staff were probably infected through close contact with the infected residents when attending to their daily needs such as changing of soiled diapers, bathing and feeding. These staff would then further spread the infection to other unaffected residents if they did not practise proper personal hygiene.

(Contributed by Pang QY, Han HK, Hishamuddin P and Tay J, Communicable Diseases Division, Ministry of Health)

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