

**Investigation of an influenza A outbreak with an unusual presentation
in Kyaingtone Township during October 2005**

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An investigation of an influenza outbreak, presenting with encephalopathy, in the family quarters of a military training school, Kyaingtone Township, was carried out in October 2005. Clinical, laboratory and epidemiological investigations were performed in children from the military family quarters as well as from three nearby villages. Sera samples and nasopharyngeal swabs from children, as well as sera from pigs in that area were collected. On-site rapid tests for influenza A were performed and four of nine nasopharyngeal samples were positive for influenza A. Blood samples from the children were also tested for malaria and dengue infection using near-patient tests and were negative. Timely instigation of control measures in the outbreak area averted further spread of the disease. The nasopharyngeal samples were transported to Yangon, where they were tested for avian influenza A (H5N1) employing a reverse transcriptase polymerase chain reaction and for influenza A, (H1, H3 and H5) by the indirect immunofluorescent assay. None of the samples were positive. Japanese encephalitis (JE) IgM enzyme immunoassay was performed on both human and pig sera and were negative. Japanese encephalitis haemagglutination inhibition (HAI) tests on pig serum samples showed two of the five pig sera to be positive. The nasopharyngeal samples were sent to the WHO National Influenza Centre for confirmation and four nasopharyngeal samples were positive for influenza A (H1N1).

INTRODUCTION

Nowadays, a lot of attention has been directed towards avian influenza. However, we should keep in mind that outbreaks caused by the human influenza subtypes can occur and are occurring. Human influenza can cause repeated infections throughout the life of an individual. It occurs worldwide and outbreaks happen every year; pandemics are usually followed by annual epidemics or sporadic outbreaks. Influenza is usually a seasonal winter illness in temperate climates, but it is less seasonal in tropics. Since 1977, there have been three

families of influenza circulating (and are varying by antigenic drift) in the human population. They are influenza A (H1N1), influenza A (H3N2) and influenza B. Avian influenza, at present wreaking havoc among birds and humans, is caused by influenza type A, subtype H5N1.

Although the clinical entity of influenza-associated encephalopathy has not gained universal recognition, it has been reported as a complication of influenza in Japanese children. Influenza type A was detected in most cases. Most of the patients had been young children. Influenza encephalopathy is

typically associated with a sudden onset of high fever, severe convulsions, rapidly progressive coma, and death within 2 or 3 days [1]. Similar encephalopathic symptoms associated with influenza infection has been reported from North American and European countries [2, 3].

A cluster of child deaths associated with fever and convulsions was reported from Kyaingtone Township, in Eastern Shan State during October, 2005 and a team comprising of a microbiologist and epidemiologists was dispatched to the area to investigate and control the outbreak. This study is the account of the investigation and control of the outbreak.

MATERIALS AND METHODS

Area of outbreak

The outbreak occurred in the family quarters of a military training school in Kyaingtone Township, Eastern Shan State, approximately 3 miles from the centre of Kyaingtone. Clinical, laboratory and epidemiological investigations were carried out in children from the military family quarters as well as from the nearby three villages.

Clinical cases

Before the arrival of the investigation team, a medical officer screened the children from the military family quarters and admitted 13 children who had fever and or respiratory symptoms to the 300-bedded No. 3 Military Hospital, Kyaingtone. Clinical cases admitted were examined clinically. Blood samples were collected from all 13 patients and nasopharyngeal swabs were taken from 5 children with respiratory symptoms. A lumbar puncture was done on one child showing neurological symptoms. Nasopharyngeal swabs were also collected from 4 Wanyan Village children who were neighbors of two clinical cases and were showing respiratory symptoms. One of these children from the Wanyan Village was also admitted to the Eastern Shan State General Hospital and

a blood sample was also collected from him. Clinical examination and collection of clinical samples were performed by the clinicians and the microbiologist using protective personnel equipment (PPE) like, caps, goggles, masks, gown, apron and boots followed by appropriate disposal of PPE and proper hand washing. The case charts of the three fatal cases were reviewed and discussed with the attending medical officers.

Animals

Blood samples were collected from five pigs in the area of outbreak.

Laboratory investigations

Malaria Rapid Test

Finger prick capillary blood samples from the 13 clinical cases were tested for malaria infection utilizing the Paracheck[®] near-patient test on-site.

Dengue Rapid Test

Dengue IgM and IgG antibodies were searched for in serum samples of the 13 clinical cases by the Panbio[®] dengue immunochromatographic near-patient test on-site.

Japanese encephalitis (JE) IgM EIA

Thirteen human and 5 pig serum samples were assayed for Japanese encephalitis IgM antibodies by IgM capture enzyme immunoassay (EIA) at the Virology Research Division of the Department of Medical Research (Lower Myanmar). Briefly, 96-well microtitre EIA plates were coated with anti-IgM antibody and serum samples, weak positive control and negative control samples were added in duplicate and incubated. The wells were then washed and acetone extracted JE antigen was added to the wells and incubated. After washing, anti-JE antibody conjugated to horseradish - peroxidase enzyme was added and incubated. After washing, substrate was added and colour was developed. The absorbance (optical density OD) in the wells was read in an EIA

reader and cut-off values were calculated. Wells showing OD higher than the cut-off value were regarded as positive.

Japanese encephalitis (JE) Haemagglutination Inhibition Assay (JE-HAI)

The five pig serum samples were tested for JE haemagglutination Inhibition antibodies by the haemagglutination inhibition test as described by Clark and Casals [4].

Influenza A Rapid Test

After collection, the 9 nasopharyngeal swab samples were immersed in phosphate-buffered saline (PBS) and the nasopharyngeal secretions from swabs were expressed into PBS. The nasopharyngeal secretions were then tested on-site, for the presence of influenza A viral antigen by the Directigen Flu A[®], a near-patient test, according to manufacturer's instructions.

Influenza A (H5) Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Nasopharyngeal swab samples positive for influenza A by the rapid test were assayed for the presence of influenza A (H5) by the RT-PCR assay [5]. Briefly, the nasopharyngeal aspirates were centrifuged to obtain a cell-free supernatant. Viral RNA was extracted from the nasopharyngeal supernatant and subjected to RT-PCR employing influenza A (H5) specific primers. The RT-PCR products were subjected to agarose gel electrophoresis and the cDNA bands were viewed under UV light and photographed. A molecular weight marker and a positive control were also included.

Influenza A (H1, H3 and H5) Subtype-specific Immunofluorescent Assay

Nasopharyngeal swab samples positive for influenza A by the rapid test were tested for influenza A (H1), (H3) and (H5) antigen by the indirect immunofluorescent test utilizing subtype-specific monoclonal antibodies [5]. Briefly, cell deposits from centrifuged nasopharyngeal samples were spotted onto glass slides and fixed with chilled acetone. The fixed cells were incubated with

influenza A (H1), (H3) and (H5) subtype-specific monoclonal antibodies. The slides were then washed and anti-mouse antibody conjugated to fluoresceine isothiocyanate was added and incubated. The slides were washed, dried and viewed under a fluorescent microscope. Observation of intracellular fluorescence denotes the presence of the corresponding subtype-specific influenza A antigen in the clinical samples.

Rapid Latex Agglutination Test for meningitis

The CSF fluid sample from one child presenting with severe headache was tested on-site by the BioMuerix Latex Agglutination Test[®] for detection of *Haemophilus influenza B*, *Streptococcus pneumoniae*, *Neisseria meningitidis* a, b, & c antigens, according to the manufacturer's instructions. Routine cytological and biochemical examinations of the CSF sample were also done at the Pathology Laboratory of the Eastern Shan State General Hospital.

Confirmation at WHO National Influenza Centre

All 9 nasopharyngeal samples were sent to the WHO National Influenza Centre at the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. There the nasopharyngeal samples were tested for influenza type A (H1, H3 and H5) and influenza type B by the RT-PCR method.

Epidemiological investigations

The military family quarters, a primary school, and three nearby villages were inspected to determine the potential source of illness. The school attendance register was also examined to look for absenteeism. Inspections included environmental sanitation and breeding of animals as well. History of any illnesses in children in the military family quarters and villages before the outbreak was investigated and the possible route of spread of illnesses from one area to another was construed.

Control measures

Immediately after onsite diagnosis of influenza A was obtained, home quarantine was imposed on the military family quarters and Wanyan Village for 10 days. Cleaning and disinfection of the houses and environment of the fatal and clinical cases were initiated. The primary school where the children from the military families and nearby villages attend, was not allowed to reopen at the end of Thadingyut Holidays and was temporarily closed for two weeks. The school was also cleaned and disinfected.

Health education

A talk on influenza pandemic preparedness was given to responsible officers of the State, districts and townships of the Eastern Shan State at the Triangle Command Headquarters, during the four-monthly Departmental Co-ordination Meeting of the Eastern Shan State. Health education talks on influenza pandemic preparedness was also provided to Kyaingtone Township government person-nel and to the household members of the family quarters and villages.

RESULTS

Fatal cases (Table 1)

Clinical history of the 3 cases that expired before the arrival of the investigation team revealed that all 3 cases had either tonic-clonic or generalized convulsions on admission to the No.3 Base Military Hospital. All three children expired within 18 to 28 hours of admission. A blood sample was received from only one case and a post mortem examination was also done on this case. The blood sample was negative for malaria, dengue and JE. Relevant post mortem findings were marked cerebral oedema with normal meninges.

Of the 13 children that were later admitted to the hospital, a seven-year-old girl died after the arrival of the investigation team.

She had fever and respiratory symptoms and then she became hypotensive and gradually went into stupor and coma. Despite intensive management, she expired on the next day of admission. Blood and nasopharyngeal samples were collected from her and a postmortem examination was also performed. The blood sample was negative for malaria, dengue and JE. The nasopharyngeal sample was positive for influenza A by the rapid near-patient test and confirmed to be influenza A (H1N1) by the WHO National Influenza Centre. Post mortem revealed massive cerebral oedema with normal meninges.

Recovered cases (Table 2)

Of the 13 admitted children, 12 recovered completely and were discharged from the hospital. Blood samples from all these children were negative for malaria, dengue and JE. Of the nasopharyngeal samples from four children showing respiratory signs and symptoms, one was positive for influenza A by the near-patient rapid test. This child is a 5-year-old sister of the fourth fatal case who was also positive for influenza A. However, her nasopharyngeal sample was tested negative for influenza A at the WHO National Influenza Centre. The nasopharyngeal sample of this child was negative for influenza A (H5) by the RT-PCR test as well as negative for influenza A (H1, H3 and H5) by the immunofluorescent test at the Virology Research Division of the Department of Medical Research (Lower Myanmar). The elder sister of one of the fatal cases, showed neurologic symptoms like severe headache without any fever. The CSF sample of this girl was clear and cytology and biochemical parameters were within normal limits. Near-patient tests for detection of antigens of bacterial meningitis agents were negative. She recovered without any sequelae.

Wanyan Village children

Two of the 4 nasopharyngeal samples collected from Wanyan Village children

Table 1. Case summaries of fatal cases

Sr No.	Name	Age/ Sex	Symptoms	Outcome	Laboratory investigations							
					Directigen Flu A	Malaria Paracheck	Dengue ICT	JE IgM ELISA	H5N1 RT-PCR	IFA test	H1,H3, H5 PCR	Autopsy
1	EEK	5 F	afebrile/ tonic-clonic convulsions	Expired within 24 hrs of admission	ND	ND	ND	ND	ND	ND	ND	ND
2	TTA	6 F	fever/ tonic-clonic convulsions	Expired within 24 hrs of admission	ND	ND	ND	ND	ND	ND	ND	ND
3	SSM	3 F	fever/ generalized convulsions	Expired after 30 hrs of admission	ND	ND	ND	Neg	ND	ND	ND	Marked cerebral oedema
4	SMN	7 F	fever & vomiting	Expired after going into coma	Pos	Neg	Neg	Neg	Neg	Neg	H1N1	Marked cerebral oedema

ND = not done Pos = positive Neg = negative

Table 2. Case summaries of recovered cases

Sr No.	Name	Age/ Sex	Symptoms	Outcome	Laboratory investigations							
					Directigen Flu A	Malaria Paracheck	Dengue ICT	JE IgM ELISA	H5N1 RT-PCR	IFA test	H1,H3, H5 PCR	
1	HEP	12 F	fever	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
2	MCS	6 F	fever & cough	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
3	MKS	10 M	fever & cough	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
4	SLN	4 M	fever & cough	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
5	WTH	6 F	fever & cough	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
6	WPA	1 M	fever & cough	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
7	YNM	3 F	fever & cough	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
8	MKH	9 F	fever & cough	Discharged	Neg	Neg	Neg	Neg	ND	ND	Neg	
9	AAM	10 F	fever	Discharged	ND	Neg	Neg	Neg	ND	ND	ND	
10	KKM	8 F	afebrile severe headache	Discharged	Neg	Neg	Neg	Neg	ND	ND	Neg	
11	TES	7 F	vomiting	Discharged	Neg	Neg	Neg	Neg	ND	ND	Neg	
12	STTH	5 F	fever & cough	Discharged	Pos	Neg	Neg	Neg	Neg	Neg	Neg	

ND = not done Pos = positive Neg = negative

Table 3. Case summaries from Wan Yan Village children

Sr No.	Name	Age/Sex	Symptoms	Laboratory investigations				
				Directigen Flu A	H5N1 RT-PCR	IFA test	H1,H3, H5 PCR	JE IgM ELISA
1	AY	5 M	fever & cough	Neg	ND	ND	Neg	ND
2	BG	3 F	fever & cough	Neg	ND	ND	H1N1	ND
3	AGL	5 M	fever & cough	Pos	Neg	Neg	H1N1	ND
4	BD	13 F	fever & cough	Pos	Neg	Neg	H1N1	Neg

ND = not done Pos = positive Neg = negative

were positive for influenza A by the near-patient rapid test. They were negative for influenza A (H5) by the RT-PCR test as well as negative for influenza A (H1, H3 and H5) by the immunofluorescent test at the Virology Research Division of the Department of Medical Research (LM). However, three of these four nasopharyngeal samples were tested positive for influenza A (H1N1) by the RT-PCR test at the WHO National Influenza Centre (Table 3).

Attack rates

The age specific attack rates were:

Under 5 years	25%
5 – 11 years	65%
12 years and above	10%
Female / Male ratio	3:1

Epidemiological investigation

Epidemiological investigations indicated that influenza-like illnesses started to occur in many children in the Wanyan Village since the first two weeks of October 2005. Thirty-two children from the Wanyan Village attended the primary school and during the first two weeks of October 2005 there was high absenteeism of village children. Children of military personnel living in the military family quarters also attend this school and it was deduced that the illness spread from village children to children from the military family quarters within this school. The minimum incubation period of the illness was estimated to be 3-4 days with a median period of 5-6 days. Weakness in the reporting of influenza-like illness outbreak in the Wanyan Village led to the spread of the disease through the primary school. Investigations into the death of animals, such as chicken and pigs revealed that there were no large outbreaks of animal deaths or diseases and they were attributable to usual occurrences probably not related to the human outbreak.

DISCUSSION

Unusual clinical presentations of influenza infection may mislead clinicians in making

a clinical diagnosis. Presence of convulsions in the first three fatal cases made clinicians to consider neurological infections like encephalitis and meningitis. Although the two blood samples from five pigs had JE-HAI antibodies (IgG), the blood samples of all children and the pigs had no JE-IgM antibodies, excluding the possibility of recent Japanese encephalitis infection. Furthermore, in this outbreak, positive results of a rapid near-patient diagnosis of influenza A in some clinical cases pointed towards influenza A infection. Consequently, timely initiation of control measures led to the containment of the outbreak with no more occurrences of new cases.

The immunofluorescent assay using subtype-specific monoclonal antibodies, employed in this study was unable to detect influenza antigen in the influenza A positive nasopharyngeal samples. This was probably due to the time taken to transport the nasopharyngeal samples from Kyaingtone to Yangon. Nasopharyngeal cells are fragile and intact cells are required for the diagnosis and the samples must be processed within a few hours of collection for the method to be sensitive.

There are very few reports of influenza A infection with the unusual presentation of encephalopathy. An influenza epidemic in Nagasaki in 1995 was associated with twelve cases of influenza encephalopathy with a mortality rate of 50% [6]. The pathogenesis of influenza encephalopathy is poorly understood. Although influenza virus could be demonstrated in respiratory tract samples from patients with encephalopathy, the presence of influenza virus was not detected in the cerebrospinal fluid samples of these patients [7]. Recently it has been reported that inflammatory cytokines, inducible nitric oxide synthase (iNOS), adhesion molecules and mini-plasmin may play a role in the development of influenza encephalopathy [8]. It has been suggested that direct invasion by influenza A virus and inflammation are unlikely to be the causes

of encephalopathy [9]. Moreover, histological abnormalities of the brain are often absent and despite the occurrence of massive brain oedema, no influenza antigen has been detected [10]. In this outbreak marked cerebral oedema was observed in two fatal cases. Females outnumbered males and the 5 to 11 year age group predominate in this outbreak. Studies from Japan also reported a higher prevalence of influenza encephalopathy in young children [11]. In this outbreak, four fatal cases and one recovered case exhibit neurologic symptoms and signs suggestive of influenza encephalopathy.

In outbreaks of infectious diseases, it should be stressed that prompt and early reporting by the local responsible personnel is vital for an early and effective control of the outbreak. An early reporting of a potential influenza outbreak, on-site laboratory confirmation with field detection kits, and rapid implementation of mass vaccination had limited the magnitude of an outbreak in confined settings [12]. Rapid recognition of the aetiological agent is essential for timely and prompt initiation of control measures as infection outbreaks with different aetiologies require different control measures. In this outbreak, a rapid near-patient diagnostic test, the influenza A rapid test provided immediate indication of the causal agent of the outbreak enabling the team to introduce rapid and appropriate control measures to curb the outbreak. Moreover, this study exemplifies that, despite the unavailability of influenza vaccines or antivirals, the outbreak could be controlled by conventional epidemiologic interventions.

It should also be emphasized that for the protection of the outbreak investigation team, health care workers and laboratory personnel, it is imperative for them to wear personnel protective equipment, followed by correct disposal and proper hand washing.

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