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Field Epidemiology Training Program

Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health

Tiwanond Road, Muang District, Nonthaburi 11000, Thailand

Tel: +662-5901734, Fax: +662-5918581

Email: osireditor@osirjournal.net

Website: <**http://www.osirjournal.net**>

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Field Epidemiology Training Program, Bureau of Epidemiology
Department of Disease Control, Ministry of Public Health, Thailand
Tel: +6625901734-5, Fax: +6625918581, Email: osireditor@osirjournal.net, http://www.osirjournal.net

Epidemiology of Leptospirosis from Thai National Disease Surveillance System, 2003-2012

Soawapak Hinjoy*

Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand

* Corresponding author, email address: soawapak@gmail.com

Abstract

Leptospirosis is a major public health problem in Thailand. A description on epidemiology of the disease could increase understanding of leptospirosis and suggest potential prevention and control measures. Data on human leptospirosis cases from all 77 provinces in Thailand were collected based on the national surveillance criteria. Univariate analysis and multiple logistic regression were conducted to describe occurrence of leptospirosis cases and risk factors for mortality. From 2003 to 2012, there were 41,089 cases of leptospirosis reported to Bureau of Epidemiology, Ministry of Public Health, Thailand. Average annual incidence rate was 6.6 per 100,000 population. Northeastern region showed the highest incidence (12.5 per 100,000 population). There were 606 deaths, with case fatality rate 1.5%. Seasonal variation was observed, with the highest incidence during rainy season from August to October. Ratio of male to female cases was 3.5:1. Adults aged 55-64 years had the highest incidence rate (9.9 per 100,000 population). There were 72.3% of the cases that worked in agricultural sector. Delay in seeking treatment, which was beyond three days after onset, significantly increased the risk of death (adjusted odds ratio = 1.8, 95% CI = 1.53-2.19).

Key words: leptospirosis, epidemiology, mortality determinants, Thailand

Introduction

Leptospirosis is a zoonotic disease, caused by spirochetes of genus *Leptospira* with worldwide distribution. *Leptospira* bacteria are grouped into serovars according to their antigenic relatedness. There are currently over 250 recognized serovars.[1,2] Some pathogenic serovars can infect many species of both wild and domestic animals. The most common serovars infecting humans include Autumnalis, Bratislava and Pyrogenes[3] which were similar serovars found among animals. All mammalian species can harbor *Leptospira* in their kidneys and transmit the disease through urine, acting as a source of infection to humans and other animals. Rodents are the main reservoir because rodents can shed *Leptospira* throughout their lifespan without any clinical manifestations.[4]

Humans are rarely chronic carriers and are, therefore, considered as accidental hosts. Two forms of disease, icteric and anicteric, are found. Anicteric leptospirosis is a mild disease and typically self-limiting while icteric form is more severe. Icteric form occurs in 5-10% of all patients, which is often rapidly progressive, and may be associated with severe jaundice, liver failure, renal failure and even death. [5]

From 1986 to 1996, there were 200-300 reported cases of leptospirosis and less than 10 deaths each year in Thailand. In 2000, epidemics of leptospirosis occurred, following severe flooding in the northeast and the south. There were more than 14,000 cases reported, with incidence rate of 23.1 per 100,000 population, and 362 deaths, with mortality rate of 0.6 per 100,000 population.[6] During the outbreaks in 2000, case fatality rate was 2.5%. Most cases (85.2%) were found in the northeastern region, followed by 7.4% in the north, 4.8% in the south and 2.7% in the central regions. An intensive prevention and control campaign, including integrated pest control, was implemented nationwide since 2000 and cases of leptospirosis decreased to 4,500-5,000 cases annually since 2003, with incidence rate of 5-9 per 100,000 population.[6] Purpose of this study was to describe epidemiology of leptospirosis in Thailand from 2003-2012. Understanding epidemiological patterns could provide better information to guide prevention and control measures.

Methods

We analyzed information of leptospirosis cases from 2003 to 2012, which were reported through the national disease surveillance system to the information center at Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand. The surveillance case definition for a suspected leptospirosis case used by Bureau of

Epidemiology was a person with high fever, chills and severe headache, followed by at least one of the following clinical symptoms: myalgia, red eyes, jaundice, neurologic signs, respiratory failure or kidney failure with an epidemiologic link to occupational factors such as being farmers, sewage workers, slaughterhouse workers, or veterinary surgeons.[7]

Exploratory data analysis and management was performed using Epi Info Version 2000[8] and statistical analysis was performed using Stata SE10 (Stata Corporation, College Station, Texas). Two-sided p-value less than 0.05 was considered as statistically significant. Simple tabulation was used to describe proportions of cases in each category of exposure variables. Univariate analysis was performed by calculating odds ratio (OR) and 95% confidence intervals (CI) to evaluate each risk factor for mortality. In order to investigate whether any association was caused by confounding factors, multiple logistic regression analysis was performed. The model was reduced in backward elimination procedure. The 10% change in coefficient was considered as evidence of possible confounding. Any variables that remained significant were kept in the model. Adjusted OR and 95% CI were also calculated.

Results

During 2003-2012, Bureau of Epidemiology received 41,089 reports of suspected leptospirosis cases across all 77 provinces in Thailand while 67% or 27,503 cases were reported from the northeastern region. Average incidence rate was 6.6 per 100,000 population. The northeastern region had the highest incidence rate per 100,000 population (12.5), followed by the south (5.8), the north (5.0) and the central (1.0). There were 606 deaths recorded during 2003-2012, and 56.9% were from the northeastern region (Table 1). Overall case fatality rate was 1.5% and mortality rate was 0.1 per 100,000 population.

Seasonal variation was evident. Number of cases was highest during August to October which coincided with rainy season in Thailand (Figure 1).

Ratio of male to female cases was 3.5:1 (32,055 males and 9,034 females). Persons aged 55-64 years had the highest incidence rate, with 9.9 per 100,000 population. The most common occupation was farmer (60.6%), followed by labor (15.5%) and student (8.7%). More leptospirosis cases came from rural areas (85.8%) compared to urban areas (13.2%). Among the reported cases, 64.9% were reported from community hospitals and 32.8% from central or regional referral hospitals. Proportion of outpatient visits for leptospirosis was 70.5%. Median period between date of onset and date of seeking medical care was three days (range 0-60 days, interquartile range 1-5 days) while median duration between date of onset and date of death was five days (range 0-47 days, interquartile range 4-8 days).

In addition, 540 foreign workers who succumbed to leptospirosis were admitted to hospitals in Thailand and were reported into the surveillance system as well. These foreigners included Myanmar (423 cases), Laotian (45 cases), Cambodian (31 cases), Malaysian (one case) and others (34 cases). There were 29,149 cases that were loss to follow up. Thus, outcome status of only 11,940 cases (29%) among 41,089 cases could be included in the study (Table 1).

Table 1. Characteristics of human leptospirosis cases in national surveillance data of Thailand, 2003-2012 (n=11,940)

Characteristic	Number of dead case (%)	Number of survived case (%)
Region		
Central	88 (14.5)	834 (7.4)
South	79 (13.0)	1,328 (11.7)
Northeast	345 (56.9)	8,193 (72.3)
North	94 (15.5)	979 (8.6)
Age (year)		
≤14	41 (6.8)	824 (7.3)
15-54	436 (71.9)	8,282 (73.1)
≥55	129(21.3)	2,228 (19.7)
Gender		
Female	137 (22.6)	2,511 (22.2)
Male	469 (77.4)	8,823 (77.8)
Marital status		
Married	455 (75.2)	7,984 (70.7)
Single	150 (24.8)	3,301 (29.3)
Occupation		
Government service	6 (1.2)	134 (1.4)
Agricultural farmer	354 (67.9)	7,116 (72.5)
Student	29 (5.6)	957 (9.8)
Labor	132 (25.3)	1,606 (16.4)
Residence		
Urban	68 (11.3)	1,318 (11.7)
Rural	536 (88.7)	9,979 (88.3)
Nationality		
Thai	603 (99.5)	11,224 (99.1)
Foreigner	3 (0.5)	105 (0.9)

There were no significant differences between cases who survived and those who died, with regard to gender, age, area of residence and nationality. The

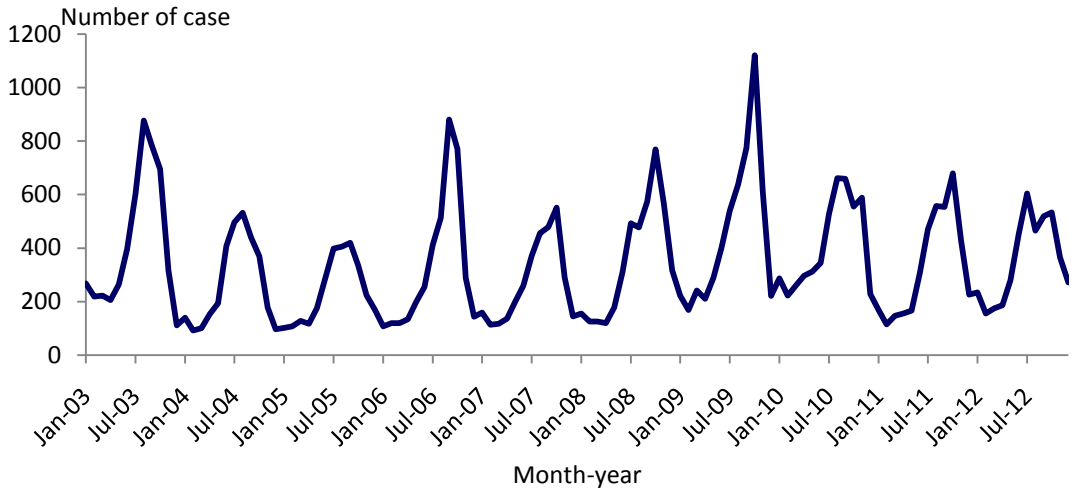

Figure 1. Number of leptospirosis cases by months in Thailand, 2003-2012

only significant risk factor for mortality was delay in seeking medical care of more than three days from onset (Table 2).

Table 2. Univariate and multivariate analyses of factors associated with leptospirosis death in Thailand, 2003-2012

Variable	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Duration between date of onset and date of seeking medical care (day)		
≤ 3	1	1
> 3	1.93 (1.64-2.28)	1.83 (1.53-2.19)
Age (year)		
≤14	1	1
15-54	1.06 (0.76-1.47)	1.16 (0.81-1.68)
≥55	1.16 (0.81-1.67)	1.24 (0.83-1.85)
Gender		
Female	1	1
Male	0.97 (0.80-1.18)	1.01 (0.81-1.25)
Occupation		
Government service	1	1
Agricultural farmer	1.11 (0.49-2.53)	1.18 (0.52-2.70)
Student	0.68 (0.28-1.66)	0.71 (0.29-1.75)
Laborer	1.84 (0.79-4.24)	1.89 (0.82-4.38)

Discussion

A 10-years retrospective review of leptospirosis cases did not show any change in disease pattern or distribution. However, incidence, mortality and case fatality rate had significantly decreased when compared to the cases occurred during 2000-2002, according to data from the national disease surveillance system.[6]

Cases were most commonly reported from the northeastern region. From a serological study of rodent population in Thailand, antibody against *Leptospira* was identified in 7.1% of rodents in the northeast, 4.9% in the north, 4.3% in the central and 3.0% in the south[9]. The highest proportion of leptospirosis infection among rodents in the northeast might relate to high number of human leptospirosis cases reported.

Another evidence of an association between rodent and human leptospirosis infections was finding of *L. interrogans* serovar Autumnalis as a predominant cause of epidemics among human cases in the northeastern region during 2000-2001 and bandicoot rat was found to be an important reservoir host of *L. interrogans* serovar Autumnalis.[10] Furthermore, environmental conditions in the northeast may offer a suitable habitat for *Leptospira*. Northeastern region was a relatively poor area, with most rubber plantations belonged to small holders[11] and a large source of rodent infestation.

Occurrence of leptospirosis cases showed a distinct seasonality. Most cases occurred during rainy season and several outbreaks could be associated with floods and hurricanes.[12,14] The rainy season in Thailand lasts from July to October. Incidence of leptospirosis in Thailand peak between August and October, which correlate directly with amount of rainfall[15] and frequent exposure of agricultural farmers to rice fields during seeding and planting of rice. Temperature is also a major factor influencing potential reproduction of rodents which tends to increase during rainy seasons. Therefore, humans may have more chance of exposure to water contaminated with urine of infected rodents.[16] Due to the facts mentioned above, rodent control should be strengthened before the rainy season to prevent and control leptospirosis more effectively. As some outbreaks have been associated with flood, prevention

and control measures during flood should be focused against direct contact with contaminated water by wearing protective clothing, especially boots.

Men commonly have frequent contact with *Leptospira* from occupational exposure or recreational activities compared to women.[17-19] From a study in Lao PDR, there were differences in certain daily activities between men and women.[20] For example, proportion of barefoot walkers was significantly higher among men than women. Swimming in streams and collecting woods were also more common among men. These activities have been considered as important risk factors for infection.

Multivariate logistic regression revealed that delay in seeking medical care beyond three days after onset significantly increased the risk of death. There was a clear correlation between leptospirosis death and delay in treatment. This result was consistent with many other studies[21-23]. Risk communication to raise people's and physicians' awareness on symptoms of leptospirosis should be promoted. Preventive health care should be conducted as a program to promote health in the areas. Community-driven campaign on raising public awareness may facilitate early treatment at health care facilities to reduce risk of developing complications.

As the data reported through the national surveillance system were data from passive surveillance on only suspected cases of leptospirosis and did not contain laboratory results, confirmed cases and pattern of serovar changes in each region could not be identified in this study.

In conclusion, epidemiological pattern of leptospirosis cases from the national disease surveillance system in Thailand during past 10 years remained stable. Early diagnosis and treatment of suspected cases could lower case fatality rate of leptospirosis.

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Department of Disease Control, Ministry of Public Health, Thailand
Tel: +6625901734-5, Fax: +6625918581, Email: osireditor@osirjournal.net, http://www.osirjournal.net

Influenza Viruses in Children Attending Yangon Children Hospital, Myanmar during Influenza Season in 2013

Htin Lin[1,*], Thu HM[1], Wai KT[1], Aye KM[1], Win MM[1], Aye KT[1], Mar W[1], Thu HM[1], Phyo AH[1], Win S[2], Thant KZ[1]

1 Department of Medical Research (Lower Myanmar), Ministry of Health, Myanmar

2 Department of Medical Science, Ministry of Health, Myanmar

* Corresponding author, email address: drhtinlin@gmail.com

Abstract

Globally, circulating subtypes of human and avian H5 influenza viruses occasionally cause epidemics. To determine the burden of influenza virus among children with influenza-like illness (ILI) who visited a hospital in Yangon, a cross-sectional study was conducted in Yangon Children Hospital from June to August 2013. Nasal swabs were taken from 100 children with ILI and viral RNA was tested by reverse transcription polymerase chain reaction (RT-PCR). Samples positive of influenza A virus were subtyped by multiplex RT-PCR. Of 100 ILI cases, six cases (6%) revealed matrix gene of influenza A virus. Five (83.3%) out of six influenza A cases were of seasonal H3 subtype and one case (16.7%) was of pandemic H1 subtype. In 2013, majority of influenza A viruses found in ILI children was seasonal H3 subtype that was different from the previous findings during 2010-2011 when pandemic H1 subtype was predominant. The results highlighted that subtyping of influenza viruses should be continued to determine trends of predominant influenza subtype and hence, to estimate the disease outbreaks.

Key words: influenza, children, respiratory diseases, Myanmar

Introduction

Influenza is a globally important disease that has occasionally caused epidemics and pandemics for centuries. Global burden of seasonal influenza is estimated to be 600 million cases, with 3 million cases of severe illness and 250,000-500,000 deaths per year.[1] In Myanmar, about 8-20% of influenza-like illness (ILI) cases were laboratory-confirmed to be influenza. The disease exhibits seasonality in rainy season, especially from June to August, with age prevalence of 5-9 years.[2,3]

Among many subtypes of influenza A virus, three hemagglutinin (HA), namely H1, H2 and H3, and two neuraminidase (NA), namely N1 and N2, have caused human epidemics. Due to genetic reassortment property of influenza A viruses, a new strain of a particular subtype usually emerges, resulting in influenza pandemics.[4] In 2009-2010, a new strain of H1 subtype caused pandemic, affecting about 50 million people with 10,000 deaths worldwide.[5] In Myanmar, about 90% of influenza A virus isolates in 2010 were of pandemic H1 subtype according to the data from National Health Laboratory, Myanmar.

Apart from these three HA subtypes, humans can also be infected with other subtypes of influenza A virus that mainly infect birds, which is known as avian influenza viruses. The first documented case of avian influenza H5N1 virus in human occurred in Hong Kong during 1997 while other avian influenza viruses have also caused human outbreaks in many countries.[6] Although H5N1 virus is limitedly transmitted from person to person, case fatality rate in humans is as high as 60%.[7]

During past five years, approximately 300-500 ILI cases visited Yangon Children Hospital (YCH) every year during the influenza season. It is important to determine predominant subtypes of influenza virus among people at risk in order to estimate the disease outbreaks. However, there had been limited data on subtypes of influenza virus among ILI children in Myanmar. Thus, we conducted this study to determine the predominant subtypes of influenza virus among children attending YCH in 2013. By doing so, outbreak of a particular subtype of influenza virus could be estimated and appearance of a new strain of virus could be notified timely.

Methods

The YCH is a major public children hospital in Yangon, Myanmar. A cross-sectional study was conducted at out-patient department (OPD) of YCH. We recruited all ILI OPD cases for two times per week on every Monday and Tuesday during the influenza season (June to August) in 2013.

ILI was defined as fever (oral temperature more than 100˚F) with cough or/and sore throat.[8] After a medical officer in OPD made clinical diagnosis of ILI cases, the investigators collected nasal swab specimens from them. We excluded children with respiratory distress such as severe pneumonia. During the study period, total 106 nasal swab specimens were collected from ILI children aged 6-132 months (mean age 32.7 months, SD 26.9). Among 106 samples, six specimens were excluded from the study due to inadequate amount. Thus, only 100 specimens were continued for influenza testing.

Ethical approval was obtained from Ethical Review Committee, Department of Medical Research (Lower Myanmar), Ministry of Health, Myanmar.

Transportation of Specimens

After collecting the specimens, we put them into the tubes containing viral transport media (VTM) and transported to the laboratory of Virology Research Division in Department of Medical Research (Lower Myanmar). Preparation of VTM was according to the guidelines provided by World Health Organization (WHO).[9] At the laboratory, the specimens were stored at (-70)°C until extraction of viral RNA was done.

Extraction of Viral RNA

We extracted viral RNA from nasal swab specimens by using one-step RNA extraction kit (QIAamp® viral RNA mini assay). Nasal swab specimen (140 µl), lysis buffer AVL (560 µl), absolute ethanol (560 µl), wash buffer AW1 (500 µl), wash buffer AW2 (500 µl) and elute buffer AVE (60 µl) were used according to manufacturer's instructions.

Detection of Influenza Viruses by Polymerase Chain Reaction

We detected influenza viruses in specimens by reverse transcription polymerase chain reaction (RT-PCR) using primers from matrix gene of influenza A and B viruses (Table 1). Reverse transcription and PCR amplification were done by using QIAGEN one-step RT-PCR kit. Reaction mixture contained distilled water (11 µl), 5X buffer (5 µl), deoxyribonucleotide triphosphate (dNTP) solution (1 µl), sense primer of influenza A (0.5 µl), anti-sense primer of influenza A (0.5 µl), sense primer of influenza B (0.5 µl), anti-sense primer of influenza B (0.5 µl), enzyme mixture (1 µl) and template RNA (5 µl). Temperature for reverse transcription was 95˚C for 45 minutes. Cycling temperature was 94˚C for 40 seconds, 55˚C for 40 seconds and 72˚C for one minute for a total of 40 cycles. After the amplification, we mixed 9 µl of PCR mixture with loading dye. Then, the mixture was subjected to 2% agarose gel electrophoresis at 100 volts for 45 minutes and reaction bands were visualized by Molecular Imager (Gel Doc™ XR+, Bio-Rad). RNA templates from positive control specimens of influenza A and B viruses were also amplified along with RNA of testing specimens. Samples positive of influenza A virus were included in subtyping.

Subtyping of Influenza Viruses by PCR

Subtyping of influenza A virus was done by multiplex RT-PCR using primers from HA genes of pathogenic influenza A subtypes, including pandemic H1, seasonal H1, seasonal H3 and avian H5 (Table 1).

Table 1. Primer sequences applied in typing and subtyping of influenza viruses[10,11]

No.	Primer	Sequence (5' to 3')
1	Influenza A (sense)	CTT CTA ACC gAg gAA ACg
2	Influenza A (anti-sense)	Agg gCA TTT Tgg ACA AA (g/T) CgT CTA
3	Influenza B (sense)	ATg TCg CTg TTT ggA gAC ACA AT
4	Influenza B (anti-sense)	TCA gCT AgA ATC AgR CCY TTC TT
5	Seasonal H1 (sense)	CTT gTC AgA CAC CCA Agg gTg
6	Seasonal H1 (anti-sense)	CAT CCA TCT ACC ATC CCT gTC CA
7	Pandemic H1 (sense)	CTT Agg AAA CCC AgA ATg Cg
8	Pandemic H1 (anti-sense)	ACg ggT gAT gAA CAC CCC A
9	Seasonal H3 (sense)	TgC TAC TgA gCT ggT TCA gAg T
10	Seasonal H3 (anti-sense)	Agg gTA ACA gTT gCT gTR ggC
11	Avian H5 (sense)	AAC AgA TTA gTC CTT gCg ACT g
12	Avian H5 (anti-sense)	CAT CTA CCA TTC CCT gCC ATC C

Table 2. Demographic characteristics of influenza-like illness cases in Yangon Children Hospital, Myanmar, June to August 2013 (n=100)

Characteristic	Influenza positive (n=6)		Influenza negative (n=94)		P-value
	Number	Percent	Number	Percent	
Age (year)					
< 5	6	100.0	80	85.1	Not applicable
5-9	0	0	12	12.8	
10-13	0	0	2	2.1	
Sex					
Male	4	66.7	53	56.4	0.7
Female	2	33.3	41	43.6	
Residence					
Urban	3	50.0	54	54.0	1.0
Peri-urban	3	50.0	46	46.0	

As for PCR amplification, QIAGEN one-step RT-PCR kit was also used. Reaction mixture contained distilled water (9 µl), 5X buffer (5 µl), dNTP solution (1 µl), sense primer of pandemic H1 (0.5 µl), anti-sense primer of pandemic H1 (0.5 µl), sense primer of seasonal H1 (0.5 µl), anti-sense primer of seasonal H1 (0.5 µl), sense primer of seasonal H3 (0.5 µl), anti-sense primer of seasonal H3 (0.5 µl), sense primer of avian H5 (0.5 µl), anti-sense primer of avian H5 (0.5 µl), enzyme mixture (1 µl) and template RNA (5 µl). Cycling temperature was 94˚C for 40 seconds, 55˚C for 40 seconds and 72˚C for one minute for total 40 cycles. RNA templates from each control subtype of influenza A virus (seasonal H1, pandemic H1, seasonal H3 and avian H5) were also amplified along with RNA of testing specimens.

Data Analysis

EpiData software was applied for data entry and cross-tabulation of the data. Fisher's Exact test was applied to determine significance of association between the variables and association was considered as significant when P-value was less than 0.01.

Results

A total of 100 children with ILI were included in the study and total 100 nasal swab specimens were tested for matrix gene of influenza A and B viruses by PCR. Six (6%) out of 100 cases showed positive reaction for influenza A virus and 94 cases (94%) showed negative reaction. There was no case of influenza B virus.

All six influenza positive cases were under five years of age, ranging 6-44 months (mean age 22.7 months, SD 12.7). Four cases (66.7%) were males and two (33.3%) were females. However, association of sex preponderance did not exist. Half of them were from urban area while half were from peri-urban (Table 2).

Both influenza positive and negative ILI cases presented with cough, fever and rhinorrhea. Other symptoms like vomiting, diarrhea or febrile convulsion were not seen in any ILI cases (Table 3).

Table 3. Clinical symptoms of influenza-like illness cases in Yangon Children Hospital, Myanmar, June to August 2013 (n=100)

Symptom	Number of influenza positive case (n=6)	Number of influenza negative case (n=94)
Cough	6	94
Fever	6	94
Rhinorrhea	6	94
Vomiting	0	0
Diarrhea	0	0
Convulsion	0	0

Among six cases of influenza A virus, five cases (83.3%) were of seasonal H3 subtype and one case (16.7%) was of pandemic H1 subtype. There was no case of seasonal H1 or avian H5 subtypes (Table 4).

Table 4. Subtypes of influenza virus among cases with influenza A virus in Yangon Children Hospital, Myanmar, June to August 2013 (n=6)

Subtype of influenza A virus	Number of case	Percent
Seasonal H1 subtype	0	0
Pandemic H1 subtype	1	16.7
Seasonal H3 subtype	5	83.3
Avian H5 subtype	0	0
Total	6	100.0

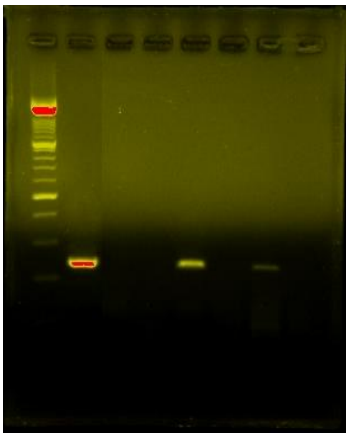

Figure 1. Gel images showing PCR bands of influenza A virus

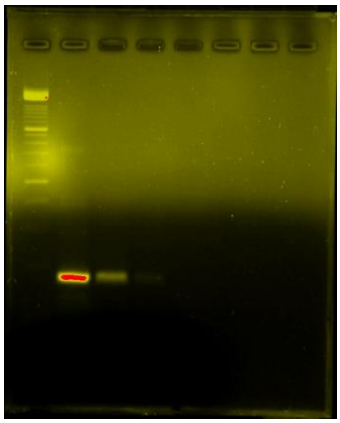

Figure 2. Gel images showing PCR bands of seasonal H3 subtype of influenza A virus

Discussion

Among 100 ILI cases, influenza viruses were detected in six cases (6%). Previous studies in Myanmar showed that influenza viruses were detected in about 8-20% of ILI cases.[2,3] This might be due to different in age prevalence of the study population. Most of the ILI cases included in this study were under five years of age (86%). There were less ILI cases in school-age which was the prevalent age of influenza.[12]

The remaining 94 influenza negative ILI cases might be due to other viral etiolgical agents such as respiratory syncytial virus, parainfluenza virus, adenovirus, rhinovirus, enteroviruses, metapneumovirus and coronavirus.[13-15] Certain bacteria species like *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae* and *Legionella pneumophila* might also be responsible for these cases although these infections are not so common in ILI cases.[14,16]

In this study, the clinical symptoms of influenza positive and negative cases were not different. Similar finding was observed in a study conducted in Myanmar during 2003-2004.[2] This proved that clinical manifestations of ILI are non-specific and accurate diagnosis of influenza needs laboratory confirmation.

All influenza positive cases in this study were due to influenza A virus and there was no case of influenza B virus. Thus, influenza virus type A could be the predominant type of influenza virus among children attending YCH in 2013. Another study conducted in Myanmar during 2003-2004 also revealed that influenza A virus was detected in majority of cases.[2] However, in a study conducted in Myanmar during 2005-2007, influenza virus type B was found to be more prevalent than type A.[3] In addition, a previous study conducted in YCH during 2010 showed that positivity rate of influenza A virus and influenza B virus were the same.[15,17] Therefore, it was noticed that prevalence of influenza virus types changed from time to time, even in the same population.

Among six cases with influenza A virus, five cases (83.3%) were of seasonal H3 subtype which could be regarded as the predominant subtype of influenza virus in the study population. Other studies conducted in Myanmar during 2003-2004 and 2005-2007 showed that seasonal H3 subtype was responsible for majority of influenza positive cases.[2,3] In agreement to these studies, a study in Alberta also found seasonal H3 subtype as the predominant subtype in January 2013 which was the peak influenza season.[18] Despite that, a previous study in YCH during 2010-2011 stated that all influenza cases were found to be pandemic H1 subtype.[19]

Limitation

Clinical presentations like myalgia and arthralgia could not be determined among ILI cases since the study population included very young children who could not complain about their illness.

Conclusion and Recommendation

In 2013, the predominant subtype of influenza A virus among children in YCH was found to be seasonal H3. The results highlighted that subtyping of influenza viruses should be continued to determine trends of predominant subtype of influenza virus and hence, to estimate the disease outbreaks.

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Suggested Citation

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Field Epidemiology Training Program, Bureau of Epidemiology
Department of Disease Control, Ministry of Public Health, Thailand
Tel: +6625901734-5, Fax: +6625918581, Email: osireditor@osirjournal.net, http://www.osirjournal.net

Investigation of Highly Pathogenic Avian Influenza (H5N1) Outbreaks among Poultry in Ningxia, China, 2012

Hualei Liu[1,2,*,§], Wang XL[3,§], Magalhães RJS[4,§], Zhou XY[5], Castellan D[6], Chanachai K[7], Wang JJ[1], Zhao YL[1], Zheng DX[1], Huang BX[1], Wang ZL[1]

1 China Animal Health and Epidemiology Center, Qingdao, China

2 Field Epidemiology Training Program for Veterinarians, Department of Livestock Development, Bangkok, Thailand

3 Ningxia Animal Disease Prevention and Control Center, China

4 University of Queensland, Infectious Disease Epidemiology Unit, School of Population Health, Herston, Queensland, Australia

5 HPAI H5N1 Programme, Food and Agriculture Organization of the United Nations, Beijing, China

6 Emergency Centre for Trans-boundary Animal Diseases, Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Thailand

7 Bureau of Disease Control and Veterinary Service, Department of Livestock Development, Thailand

* Corresponding author, email address: hualeiliuwy@163.com

§ These authors contributed equally to this paper.

Abstract

In April 2012, there were reports of suspected highly pathogenic avian influenza (HPAI) H5N1 among poultry layers in Ningxia. A retrospective investigation was conducted to describe epidemiological characteristics of the outbreak and identify risk factors for HPAI H5N1 infection. We administered a questionnaire to all 87 poultry layer farms in Touying County, Ningxia Province, within four weeks following the last reported outbreak. Samples of sick chickens were collected from 45 suspected farms to determine presence of H5N1 virus by reverse transcription polymerase chain reaction (RT-PCR) and confirmed by virus isolation. We used multiple logistic regression analysis to identify risk factors for infection and semivariogram analysis to demonstrate clustering of infected farms. Among 45 farms with positive PCR results, virus was isolated from four farms. Farms with improper waste disposal (Adjusted OR = 2.7, 95% CI = 1.20-8.30) and having visitors in farm within the past month (Adjusted OR = 5.5, 95% CI = 1.97-15.64) were significantly associated with HPAI infection. H5N1 was widespread in the county. Frequent human movement into farms and improper waste management increased the risk of infection. Biosecurity practices, including limiting number of visitors and proper waste disposal, should be enhanced to prevent further H5N1 infection.

Key words: HPAI, H5N1, outbreak investigation, poultry layer

Introduction

The H5N1 subtype of highly pathogenic avian influenza (HPAI) infection is endemic in many countries around the world, including China. The first outbreak of HPAI H5N1 was reported in China on 27 Jan 2004. During the following months, 49 outbreaks were reported in 2004, resulting in culling of 9.02 million poultry.[1,2]

HPAI H5N1 virus clades 7, 2.3.4 and 2.3.2 were the causes of most HPAI outbreaks in China since 2009.[3] Compulsory vaccination combined with stamping out of infected flocks had been used to prevent and control HPAI since 2004.[4] The H5N1 vaccines currently used in China, Re-4, Re-5 and Re-6, were derived from clades 7, 2.3.4 and 2.3.2 respectively.[5]

The H5N1 outbreaks declined dramatically, following mass vaccination in the country. However, H5N1 viruses can still be detected in live bird markets, causing sporadic outbreaks. Mutation frequency of H5N1 virus in China has increased because of

selection pressure caused by vaccination, antigenic shift and antigenic drift of H5N1 viruses.[6,7]

The first outbreak of H5N1 in Ningxia Province was reported in 2006 and the viruses involved in that outbreak belonged to clade 7. Since then, Re-4 vaccine has been used to prevent H5N1 outbreaks in Ningxia. On 13 Apr 2012, several poultry farms in Touying County, Ningxia Province reported suspected outbreaks of HPAI to the local government. The outbreak was confirmed by the Ministry of Agriculture on 18 Apr 2012. Based on phylogenetic analysis of hemagglutinin (HA) gene, the virus involved in this outbreak was HPAI H5N1 virus clade 7.2 which shared 94.1% of HA sequence with Re-4 vaccine strain.[8] A retrospective investigation was conducted in order to describe epidemiological characteristics of outbreak distribution in the affected county and identify risk factors associated with H5N1 infection.

Methods

Outbreak Investigation

We conducted an outbreak investigation in all 87 poultry layer farms within four weeks following the last report of an outbreak in Touying County, Ningxia during 2012. Trained investigators, consisting of provincial and local veterinarians, interviewed farmers using a structured questionnaire. The investigation team collected organs and cloacal swab samples from five sick chickens in each farm with suspected H5N1 infection. Presence of H5N1 virus was determined by reverse transcription polymerase chain reaction (RT-PCR) and virus isolation. Isolated HPAI H5N1 viruses were confirmed by hemagglutination inhibition (HI) assay.

Suspected infected farms were layer farms in Touying County with poultry showing at least one of the following clinical signs: sudden death, drop in egg production, neurological signs, swollen head, hemorrhage on foot scale or cyanosis of comb, and positive RT-PCR result for H5N1 viruses. Confirmed farms were suspected farms with isolated H5N1 virus.

Geographic location of all poultry layer farms in Touying County was obtained from outside the farms using handheld global positioning system devices (UniStrong, Beijing) and analyzed by geographic information system, ArcGIS 10 (ESRI).

Risk Factor Analysis

A cross-sectional study was conducted using H5N1 suspected poultry farms as cases and all other non-infected poultry layer farms in Touying County as non-cases. All risk factors involved in the study were tested by chi-square or Fisher's exact test.

Biologically meaningful first-order interaction terms were also tested. Correlation between predictors was assessed using Pearson's correlation coefficient. Then, all factors with p-value less than 0.2 were considered for inclusion in a multivariable Bernoulli logistic regression model. All analyses were conducted using statistical software Stata version 11 (Stata corporation, College Station, Texas).

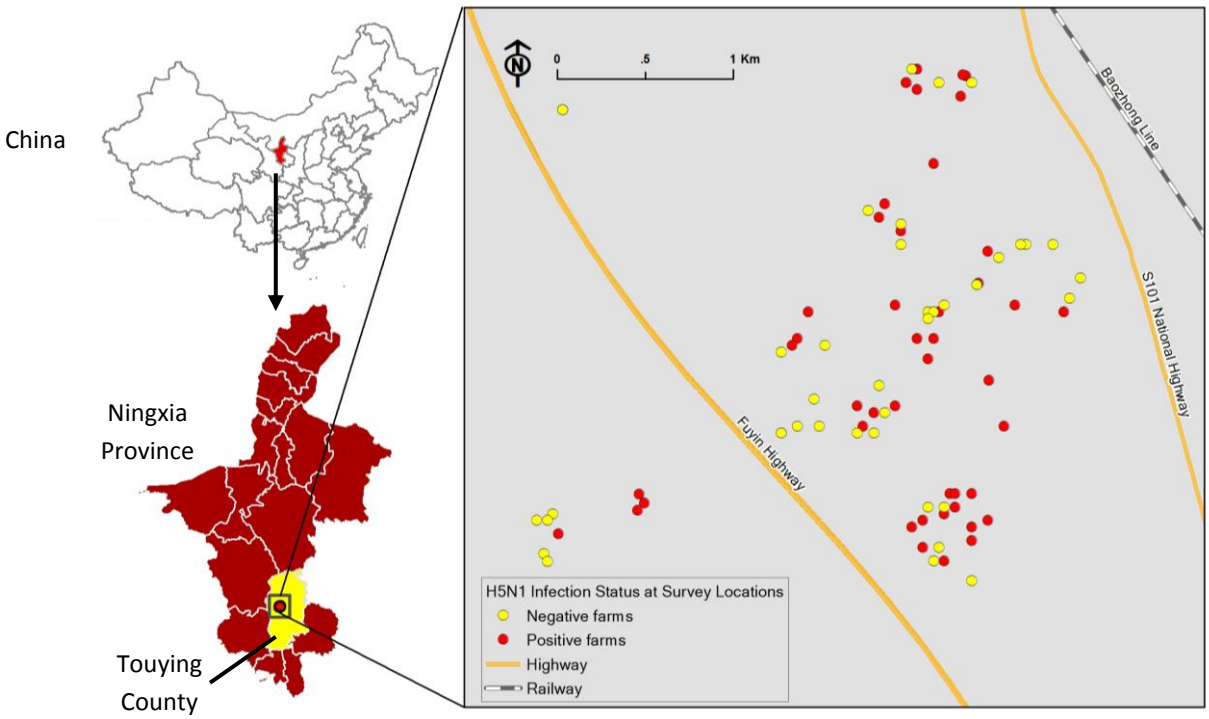

Figure 1. Geographical distribution of H5N1 farms in Ningxia Province, China, 2012

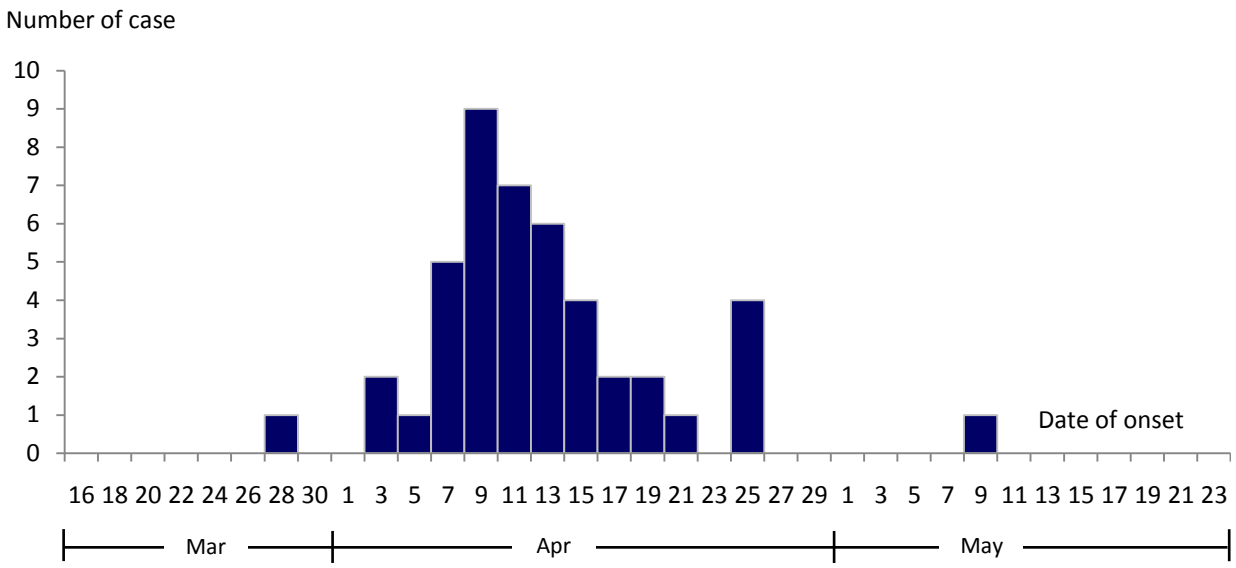

Figure 2. Epidemic curve of HPAI H5N1 outbreaks in 45 farms of Ningxia Province, China, 2012

Spatial Analysis

A semivariogram of residuals of final multivariable model (residual semivariogram) was used to quantify extent of spatial dependence left unaccounted by the variables in final multivariable model.[9] Semivariogram is a graphical representation of a mathematical function which describes variability within a variable depending on location by examining the variation in observations with distance between all pairs of sampled locations. Residual semivariograms were constructed using geoR package in R version 2.10.0.

Results

Descriptive Epidemiology

All poultry farms in Touying County were poultry layer farms. Out of total 87 poultry layer farms in Touying County, 45 farms were suspected farms (51.7%). H5N1 viruses were isolated from four layer farms. Phylogenetic analysis revealed that the H5N1 viruses were belonged to clade 7.2.[8] Geographical distribution of the 45 case farms and 42 non-case farms were shown in figure 1.

Clinical signs in the index farm began on 28 Mar 2012. There was a rapid increase in number of infected farms afterward. The number of newly suspected farms reached a peak on 9 Apr 2012, after which there was a reduction in number of suspected farms until 9 May 2012 when the last suspected farm was reported (Figure 2).

All infected flocks in the index farm were vaccinated at least once with Re-4 and Re-5 H5N1 vaccines, except in the first infected flock. The most common clinical signs in suspected farms were lethargy and lack of appetite, drop in egg production and sudden death (Table 1). Mean proportion of mortality in all case farms was 17% (SD 3.0%). The highest mortality (83.3%) was found in non-vaccinated flock.

Table 1. Clinical signs of H5N1 outbreaks in 45 farms, Ningxia Province, China, 2012 (n=45)

Clinical sign	Infected farm	
	Number	Percent
Lethargy and lack of appetite	45	100.0
Drop in egg production	40	88.9
Sudden death	38	84.4
Cyanosis of comb	23	51.1
Neurological signs	18	40.0
Swollen head	6	13.3
Hemorrhage on foot scale	6	13.3

Factors Associated with HPAI H5N1 Infection

Results of the univariate analysis (Table 2) indicated that H5N1 infection was significantly associated with improper waste or dead bird disposal, no disinfection of egg tray and having visitors in farm. Improper waste or dead bird disposal were highly correlated (r^2=0.9).

Variables of improper waste disposal and having visitor in farm were retained in the final multivariable model. Results indicated that HPAI H5N1 case farms were almost three times more likely to have improper waste disposal (Adjusted OR = 2.7, 95% CI = 1.20-8.30) and five times more likely to have visitors prior to the outbreak (Adjusted OR = 5.5, 95% CI = 1.97-15.64) compared to non-case farms.

Table 2. Univariate analysis of factors associated with H5N1 outbreak in Ningxia Province, China, 2012

Factor	Category	Number of non-infected farm	Infected farm		Odds ratio (95% CI)	P-value
			Number	Percent		
Age of farm owner (year)	≥ 44	21	27	56.3	1.0 (0.96-1.08)	0.464
	< 44	21	18	46.2		
Farm as the main source of owners' income	Yes	39	40	50.6	1.6 (0.37-7.19)	0.396
	No	3	5	62.5		
Operating year	≥ 5	24	29	54.7	1.4 (0.57-3.22)	0.490
	< 5	18	16	47.1		
Breeding density (birds/m^2)	≥ 10	15	24	61.5	2.1 (0.87-4.87)	0.099
	< 10	27	21	43.8		
Improper waste disposal	Yes	19	9	32.1	3.3 (1.30-8.38)	0.012
	No	23	36	61.0		
Improper disposal of dead bird	Yes	19	11	36.7	2.6 (1.04-6.29)	0.041
	No	23	34	59.6		
Disinfection of egg tray	Yes	28	19	40.4	0.4 (0.15, 0.87)	0.022
	No	14	26	65.0		
Disinfection of external vehicle	Yes	15	10	40.0	0.5 (0.20, 1.32)	0.165
	No	27	35	56.5		
Introduction of new flocks	Yes	4	1	20.0	4.6 (0.59-36.15)	0.159
	No	38	44	53.7		
Having visitors in the farm	Yes	8	24	75.0	4.8 (1.85-12.78)	0.001
	No	34	21	38.2		
Workers visited to live bird market	Yes	2	3	60.0	1.4 (0.23-9.00)	0.533
	No	40	42	51.2		
Workers exposed to dead bird	Yes	5	8	61.5	1.6 (0.41-6.79)	0.443
	No	37	37	50.0		

Residual Spatial Variation in HPAI H5N1 Infection

We found that the variables included in the final multivariable model accounted for only 20% of clustering of infected farms (Figure 3). This suggested that location of farms in close proximity might explain the transmission of H5N1 in these farms.

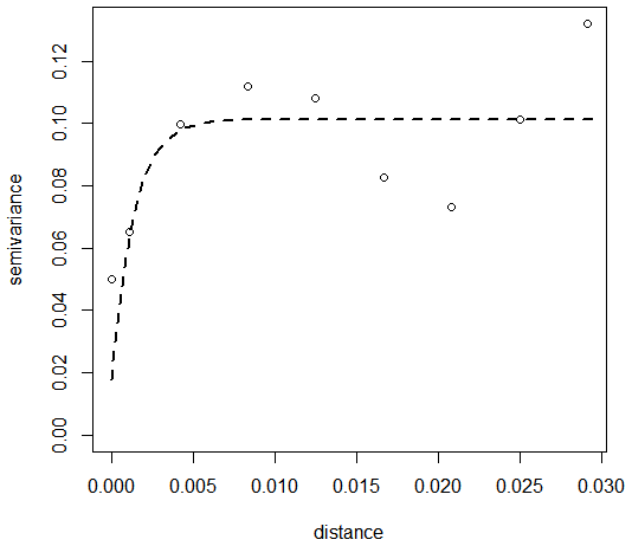

Figure 3. Semivariogram on residuals of the final multivariable model of HPAI H5N1 infection in Ningxia Province, China, 2012

Discussion

The outbreak of H5N1 HPAI in Touying County was confirmed by virus isolation. Four farms were proven to be H5N1 positive by virus isolation and 45 farms were H5N1 positive by RT-PCR. Recombinant events of H5N1 viruses with other avian influenza viruses had been reported in China in recent years.[10-13] Some of the new variants have resulted as sporadic cases of HPAI in China. However, we did not find any new recombinant strains isolated from this outbreak. Continuous evolving of clade 7 viruses was found in another study conducted in the same area. Nucleotide sequence of viral HA gene only shared 94.1% of homology with that of A/chicken/Shanxi/2/2006 (H5N1), which was the prototype strain of clade 7.2 and the corresponding Re-4 vaccine.[8] Some critical amino acid substitutions in viral HA1 domain occurred at antigenic sites resulted in variation between the field isolate and the vaccine strain. These substitutions such as K53E, K115E, S121H, E126N, A127T, G139E, K140N, T167A, D183N, K189M and T195N (H5 numbering), could change the antigenicity of the virus. In another study, the virus isolated was characterized by the HI assay using specific pathogen free (SPF) chicken serum against the vaccine strain prepared at China Animal Health and Epidemiology Center. HI titer of the vaccine strain was five folds more than the field isolate.[8] These results indicated that the virus causing this outbreak was distinguishable from the vaccine strain. Chinese authorities also recognized the evolution of HPAI H5N1 virus clade 7 and considered a new vaccine candidate to use against this new emerging strain.

Early warning and rapid response is the key to reduce risk of transmission during an outbreak. Delayed reporting of suspected cases also play a role in rapid transmission of the disease. Thus, awareness of farmers on rapid reporting of suspected events should be enhanced. Timely reporting and investigations of die-off would enable animal health agents to record key information, concerning the first signs of an outbreak.

There might be some recall bias in the investigation since it was conducted retrospectively.

The findings in this study demonstrated that biosecurity practices could be important determinants for spread of HPAI H5N1. Susceptibility of poultry flocks to HPAI H5N1 infection could be reduced by implementation of vaccination policy. Once a farm has been confirmed as infected, immediate measures must be taken, including depopulation. In this study, high mortality was observed among non-vaccinated flocks of the index farm. Improper disposal of vaccinated flocks with asymptomatic infection of HPAI H5N1 virus in the same farm might spread the outbreak. A barrier must be created and maintained between farms and the outside world in order to prevent spread of a disease in and out of the farms. Visitors such as veterinarians or traders were also risk factors for transmission of H5N1 in this outbreak. Limiting number of people to contact with poultry could also prevent potential exposure of the virus to the poultry.

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Suggested Citation

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Field Epidemiology Training Program
Bureau of Epidemiology, Department of Disease Control
Ministry of Public Health, Tiwanond Road
Muang District, Nonthaburi 11000, Thailand
Tel: +662-5901734, 5901735
Fax: +662-5918581
Email: osireditor@osirjournal.net

http://www.osirjournal.net