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Editorial

Can HIV/AIDS be Eliminated by the Current National Strategy?

Wiwat Rojanapithayakorn, Chief Editor

The conflict between humans and HIV virus, which has been very large since 1981, is now coming close to a close. In 2010, the Joint United Nations Programme on HIV/AIDS (UNAIDS) proposed a 5-year strategy (2011-2015) of getting to zero with the vision of 3-zeros: (1) zero new infections, (2) zero aids-related deaths, and (3) zero discrimination. Also, 10 goals were set as strategic directions of the multi-sectoral responses.[1] The proposal was endorsed by the global community and the issues of the 3-zeros had become the themes of the World AIDS Days' campaigns during 2011-2015.[2] This 3-zeros strategy has been translated into various key actions toward prevention, treatment and social acceptance of people affected by HIV.

In 2014, UNAIDS launched the 90-90-90 global targets to end the AIDS epidemic.[3] The targets are aimed to achieve 90% of people living with HIV know their status, 90% of people living with HIV who know their status are on treatment, and 90% of people on treatment are virally suppressed. The underlying reasons for proposing this strategy were the increasing access of people to antiretroviral (ARV) therapy and the practice of the policy to treat all HIV infected persons regardless of their immunity status (or regardless of the number of CD4 cells). This strategy to end AIDS has raised a serious question of whether we are going to achieve the ending of the epidemic.

With the 90-90-90 targets, countries are now investing their resources and efforts on treatment. For Thailand, the national AIDS program is now emphasizing on same-day HIV testing, treatment at any CD4 level, the Reach-Recruit-Test-Treat-Retain (RRTTR) strategy,[4] and the pre-exposure prophylaxis (PrEP)[5]. Other interventions have become hidden behind the testing and treating programs. This approach seems to underestimate the ability of people in improving their health literacy, avoiding risky behavior and practicing HIV prevention intervention such as the use of condoms. In other words, the approach treats people as passive subjects who are difficult or impossible to change behaviors, so the government has to regularly purchase high volume of ARV drugs to prevent and treat HIV.

In reality, there is a significantly high proportion of HIV infected people who are still reluctant to start treatment[6]. For those on ARV, many of them have poor compliance in taking the drugs[7]. Also, the huge resource for ARV drugs will result in limited resource for prevention programs such as the procurement of condoms.

An article entitled "HIV drug resistance among pre-treatment cases in Thailand: 4 surveys during 2006-2013" in this issue of OSIR demonstrates another obstacle to the ending of AIDS. It reports the HIV drug resistance level to be almost 5% (1 in 20) in newly untreated cases and up to 14% (1 in 7) in cases undergoing treatment. In the pool of the current 500,000 HIV positive persons in Thailand, the people with drug resistance will be at least 25,000 cases. How can this high number of people with HIV be supported by the term "ending AIDS"? Furthermore, this significant level of drug resistance will definitely undermine the efforts to promote PrEP as a main strategy to prevent the spreading of HIV; and the circulating virus in the PrEP era will be mainly the drug resistant strains.

To overcome the challenge, the Thai Health Promotion Foundation together with the Bureau of AIDS, Tuberculosis and Sexually Transmitted Diseases, Department of Disease Control have recommended a set of 10 measures for ending AIDS in Thailand8. They include the following:

1. Strengthening of coordination mechanisms for ending HIV/AIDS

2. Public campaigns on HIV/AIDS, STI and ending discrimination
3. Promoting access to HIV testing and antiretroviral treatment and care (including RRTTR, PrEP and prevention of mother-to-child transmission)
4. Condom promotion
5. Harm reduction among drug users
6. Ending AIDS in health service facilities
7. Prevention and treatment for sexually transmitted infections
8. Integration of HIV programs with other health issues: TB-HIV, teen pregnancy, hepatitis, etc.
9. Innovations, monitoring and evaluation
10. Human resource capacity building

This set of strategies is currently being implemented in three provinces of Thailand: Kampaeng Phet, Ubol Ratchathani and Chonburi. Implementation approaches and experiences which will be gained from the three provinces will be very useful for the nation-wide response, if the end of AIDS is to be ensured.

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Adult Japanese Encephalitis Outbreak Following an Immunization Campaign in Children, Shwe Pyi Tha Village, Sittwe Township, Rakhine State, Myanmar, 2016

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Abstract

On 31 Aug 2016, one Japanese encephalitis (JE) case was notified from Shwe Pyi Tha Village, Sittwe Township, Rakhine State. The case was the first reported JE case from this village after implementing the single-dose JE vaccination in the area during May 2015. An outbreak investigation was conducted during September-October 2016 to confirm the outbreak and identify possible risk factors. The patient and family members were interviewed. Suspected cases were searched in the family and the village, and the medical records were reviewed. Serum samples were collected and sent for JE antibody, and dengue antigen-antibody testing. Environmental investigation, including entomological study, was conducted. The patient was a 46-year-old male worker. He got fever with headache on 14 Aug 2016. He developed convulsion and was admitted to Sittwe General Hospital with final diagnosis of viral meningitis. He was an alcoholic and usually did not sleep in a mosquito net. Active case finding among 1,758 villagers found 12 feverish villagers, including two suspected cases. Out of 11 serum samples tested, one was positive and two were equivocal for JE antibody testing. Environmental investigation revealed domestic animals, poor drainage and houses without mosquito screens. *Culex tritaeniorhynchus* and *Culex quinquefasciatus* were identified in the village as well. Considering the environmental conditions, Rakhine State should be a high priority area for routine JE immunization program.

Keywords: Japanese encephalitis, outbreak, investigation, Rakhine

Introduction

Japanese encephalitis (JE) is a leading cause of viral encephalitis in Asia, including Myanmar.[1-3] The pathogen is a mosquito-borne *flavivirus*, and transmission is occurred through *Culex* mosquitoes, pigs and water birds.[2,4] Clinical manifestations exhibit in one out of 250 infections.[5] However, the case fatality rate of JE infection can be 30%, and permanent neurological or mental sequelae can occur in 20-30% of survivors.[2,4] In endemic regions, JE infection is common in rural and agricultural areas.[1,2,4] Although it usually infects children, people in all ages can be infected, especially among people with no immunity.

In 1974, the first JE outbreak in Myanmar was reported from Tachileik, Shan State. During 1979, JE cases were reported from other states and regions.[3] Thus, the hospital-based surveillance for acute encephalitis syndrome (AES) was initiated in limited places during 2007. The AES surveillance was

incorporated into integrated disease surveillance system in 2016.[6] From January to October 2016, 1,552 AES and 377 JE cases were reported from all states and regions.[7]

In Rakhine State, the first JE case was reported in 1979, and outbreaks were detected in four townships during 2007 and 2008.[8] Eight out of total 17 townships in the state had been affected in 2015. Outbreak occurred in agro-based villages with pig husbandry under or near the houses.[8,9] A single dose of JE vaccination was given to 1-14 years old children in some villages including Shwe Pyi Tha Village in May 2015.[8]

On 31 Aug 2016, the Vector Borne Disease Control (VBDC) Team in Rakhine State was notified of a 46-year-old male from Shwe Pyi Tha Village, Sittwe Township. He was the first reported JE case from that village in 2016 and his clinical manifestation was very severe. Thus, the state VBDC team and township health department conducted an investigation in September-October 2016. The objectives were to confirm the outbreak, verify the diagnosis, describe the epidemiological and environmental characteristics, and identify the risk factors associated with the outbreak.

Methods

The index case and his family were interviewed about the illness, risk behaviors, and travel history and his routines to identify possible source of infection. Medical records of the index case in Sittwe General Hospital were also reviewed. Data on JE and acute encephalitis cases from the affected village and Sittwe Township during January 2010 to October 2016 in Sittwe General Hospital, Special Diseases Control Unit, and Rakhine State VBDC Team were examined as well.

Active Case Finding

Active case finding was conducted among family members of the index case and other villagers in Shwe Pyi Tha Village, Sittwe Township, by allocating the investigation teams into four teams by a door-to-door survey.

A suspected case was a person in Shwe Pyi Tha Village, Sittwe Township, who had acute onset of fever and a change in mental status such as confusion, disorientation, coma or inability to talk, and/or new onset of seizures (excluding simple febrile seizures) during August to October 2016. A confirmed case was a suspected case with laboratory confirmation of JE virus immunoglobulin M (IgM) antibody by enzyme-linked immunosorbent assay (ELISA). Serum samples were taken from suspected cases and sent to the National Health Laboratory for serological analysis of JE virus IgM antibody using an IgM-capture ELISA (JE Detect TM MAC-ELISA test). The serum samples were also tested for dengue NS1 antigen, IgM and immunoglobulin (IgG) testing by the state VBDC.

Environmental Investigation

Environmental surveys were conducted in Shwe Pyi Tha Village to find out possible environmental factors for disease transmission, including vector breeding sites, animal husbandry and disease occurrence in those animals. Entomological study was carried out at the index case's house and surrounding 10 houses by spray sheet collection. Larva collection was done in nearby water collected areas, ponds and farms.

Results

Description of the Index Case

The index case was a 46-year-old male worker, living in Shwe Pyi Tha Village. He was a chronic alcoholic and had no history of similar illness before this episode. He suffered fever with headache on 14 Aug 2016 and took antipyretic drugs by himself. On 15 Aug 2016 evening, he had sensory changes and speech impediments. On the next morning, he got convulsion and became unconscious. The patient was admitted to Sittwe General Hospital on 16 Aug 2016. Although the patient remained unconscious, he was discharged from the hospital on 17 Aug 2016, following the request of the family who thought that he would die.

On 18 Aug 2016, the patient was better and admitted to the hospital again on 19 Aug 2016. During the second admission, he had fever (37.8°C) and remained unconscious with no meningeal signs. Complete blood picture showed hemoglobin 11.3 gm%, white blood cells 15,000/cumm, neutrophil 85%, lymphocyte 10%, monocyte 2%, eosinophil 1% and myelocyte 2%, platelets 90,000 per mcL and erythrocyte sedimentation rate (ESR) 80 mm/hr. Infection screening showed negative for human immunodeficiency virus (HIV) antibody, hepatitis B virus surface antigen (HBsAg), hepatitis C virus (HCV) antibody and malarial parasites, and normal urine on routine examination. Serum of the patient was sent to the National Health Laboratory for detection of JE antibody. The patient was discharged from the hospital on 27 Aug 2016. Although the patient had good conscious level, could not walk well at the time of discharge. The final diagnosis by the physician was viral meningitis.

The patient had no previous history of similar illness or travel to other places before the illness. However, he usually drank alcohol every evening in the village and came back home in the late evening. He did not sleep under a mosquito net while his family members usually did.

Active Case Finding

Active case search was conducted among family contacts and people in Shwe Pyi Tha Village. There were total five family members (one adult male, three adult females and one 8-year-old boy). Only the boy had fever with no neurological features on the same day as the patient. The boy had received one dose of live attenuated JE vaccine in 2015.

Active case search was also conducted among 1,758 persons in the village. Total 12 cases, including the index case, had fever with onset between 1 Aug 2016 and 7 Oct 2016. Of these 11 cases, five cases were from nearby houses. Three were admitted to the hospital and among them, two met the case definition of JE suspected case. One of the two suspected cases was a 9-year-old girl with fever and disorientation, and the other one was a 1-year-old boy with fever and convulsion. Both of them did not get JE vaccination in 2015. The houses of the two suspected cases were close to that of the index case (Figure 1).

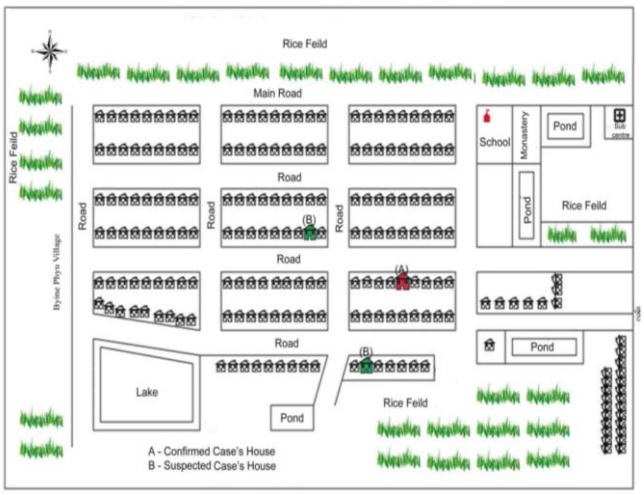

Figure 1. Location of confirmed (in red) and suspected JE cases' houses (in green) in Shwe Pyi Tha Village, Sittwe Township, Rakhine State, Myanmar, 2016

Laboratory Findings

Seven serum samples from the index case, his 8-year-old son, one suspected JE case and four cases with fever were sent to the National Health Laboratory for JE IgM antibody testing by ELISA and also tested for dengue NS1 antigen and IgM/IgG testing at the state VBDC. All samples were negative for dengue infection. Only the index case had positive JE IgM antibody. His 8-year-old son and one from nearby house had equivocal JE IgM antibody. The remaining four (one suspected JE case and three cases with fever) had negative results.

Environmental Study

Shwe Pyi Tha Village is situated in peri-urban area of Sittwe Township. There were 348 households with total population of 1,758 in 2016. The major economy of the village was fishery. Some villagers were farmers and some were workers in Sittwe Township. There were huge rice fields near the village. There were no pig farms. However, domestic breeding of animals such as pigs, cattle and chickens were found in most of the households. During the investigation, there were total 45 pigs, 25 cattle and 321 chickens in the village. No history of diseases in animals was reported in the village. There was a pig in the index case's house and also in the nearby house. Water supply was from four ponds in the village. The water drainage system was not good with a lot of ditches and ground pools near the index case's house and within the village. Most of the houses in the village, including the index case's house, were made of dry leaves or wood without mosquito screens (Figure 2).

Figure 2. Environmental condition in Shwe Pyi Tha Village with JE confirmed case, Sittwe Township, Rakhine State, Myanmar, 2016

Entomological study was carried out at the index case's house and surrounding 10 houses by spray sheet collection. One *Culex tritaeniorhynchus*, eight females and two males of *Culex quinquefasciatus* were detected. Unfortunately, JE virus detection was not carried out in these mosquitoes. Larva collection was done in water collected from swamps, ponds and farms. However, no larva was found.

AES and JE cases in Shwe Pyi Tha Village

One JE case (16 years old boy) was reported from this village in 2010. Two acute encephalitis cases were reported during 2015 and one of whom was JE IgM positive. Both cases were under one year old. One

dose of live attenuated JE vaccine was given to 292 children aged 1-15 years in the village during May 2015 and vaccine coverage was 61.5%. In 2016, there was also one adult JE case reported from a nearby village.

AES and JE cases in Sittwe Township

During 2010, one AES case was confirmed to have JE in Sittwe Township. There were six AES cases in 2014 and 13 AES cases in 2015. Among them, two JE cases were confirmed in each year. As of 10 Oct 2016, there were total 17 AES cases and seven JE confirmed cases (Table 1). Up to 2015, all cases were less than 14 years and in 2016, three out of seven cases were more than 14 years.

Table 1. Number of acute encephalitis syndrome (AES) and Japanese encephalitis (JE) cases in Shwe Pyi Tha Village, Sittwe Township, Rakhine State, Myanmar, 1979-2016

Year	AES	JE	% of JE in AES
1979	1	1	100.0
2007	1	1	100.0
2010	1	1	100.0
2014	6	2	33.3
2015	13	2	15.4
2016	17	7	41.2

Discussion

The index case in this outbreak was an adult male aged 46 years old with history of chronic alcohol use. The patient had fever and neurological manifestations, with laboratory confirmation of JE virus IgM antibody. Alcoholic abuse, which generally weakens the immune system[10] and not using the mosquito net might increase risk of infection in this patient.

His 8-year-old son who had received one dose of live attenuated JE vaccine in the previous year had only fever and equivocal serological analysis for JE IgM antibody. The clinical manifestations of the boy did not meet the JE case definition. Two suspected JE cases were found during active case finding.

JE is predominantly, although not exclusively, a rural disease. JE virus is transmitted primarily by *Culex* mosquitoes, and circulates in an enzootic cycle in pigs and water birds, which serve as amplifying hosts.[2,4] The most important vector is *Culex tritaeniorhynchus* which breeds in flooded rice fields and water pools. Living in close proximity to rice fields and family or neighbor ownership of pigs were significant risk factors for JE.[11] In this outbreak investigation, huge rice fields, ponds and families with pig ownerships found in the area were environmental conditions favorable for JE spread. *Culex tritaeniorhynchus* (main vector of JE transmission) and *Culex quinquefasciatus* (competent vector) were also detected in the affected area.

Due to the animal reservoirs, although JE virus cannot be eliminated, the disease can potentially be controlled by universal human JE vaccination in endemic areas.[1,4,5] Immunization is the most effective JE prevention strategy in reducing JE morbidity and mortality, and had shown to be cost-effective in studies carried by several countries.[12] The World Health Organization recommends that JE vaccine should be incorporated into immunization programs in all areas with high disease burden.[1] The most effective immunization strategy is an one-time campaign in locally defined target population, followed by incorporation of JE vaccine into the routine childhood immunization program.[1,4,5]

JE has been endemic in most townships of Rakhine State and reported cases increase by year.[7,8] There was a big JE outbreak in Rakhine State in 2014 and there were total 23 cases from nine townships.[9] There were also 52 JE cases in 2016 from 10 townships.[8] Environmental conditions are favorable for breeding of vectors and disease transmission. Rakhine State should therefore be a high priority area for routine JE immunization program.

Prevention and Control Measures

Health education about JE cause, clinical manifestations, mode of transmission, importance of early treatment, environmental sanitation and animal husbandry was provided to the villagers. Malathion fogging was done in the index case's house and nearby 31 houses. One dose of live attenuated JE vaccine was given to 253 children aged 1-15 years old who had no previous JE vaccination in September 2016.

Limitations

Cerebrospinal fluid sample of the index case was not tested for JE antibody. One suspected JE patient refused to test JE and thus, the information could not be obtained. JE virus detection was not carried out in the collected vectors or domestic animals. JE immunity level of the community was not measured.

Conclusions and Recommendations

An investigation confirmed JE infection in an adult male with a history of chronic alcohol use and lived in

a village of Sittwe Township, Rakhine State. Two suspected JE cases were found during the active case search. Vectors of JE transmission were also detected in the affected area. Routine JE immunization program is essential and was needed to initiate at earliest. Children should be the priority for JE immunization. However, serological survey of JE immunity level in adults in high-risk areas should be conducted and used for considering JE vaccination in adults.

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Suggested Citation

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HIV Drug Resistance among Pre-treatment Cases in Thailand: Four Rounds of Surveys during 2006-2013

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Abstract

In Thailand, antiretroviral therapy (ART) was initiated to treat human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) cases using the empirical regimen with no prior genotypic test to determine drug resistance. In order to assess prevalence rate of HIV drug resistance (HIVDR) among pre-treatment cases, four rounds of survey were carried out in ART clinics, including six, eight, 33 and four ART clinics in each round during 2006-2013. For which, HIVDR testing results were available in 310, 350, 797, and 413 cases in four rounds. It was revealed that HIVDR rates among naive cases were 2.0%, 2.8%, 4.0% and 4.8%, while in experienced cases, the rates were 0, 3.3%, 11.4% and 13.9%. The rates among all cases were 1.9%, 2.9%, 4.4% and 5.6%. Resistant drugs with the highest rates among all cases in the survey round 4 were nevirapine (3.6%) and efavirenz (3.1%). The results indicated the need to continue surveillance for pre-treatment HIVDR, and posed challenges to implement activities for protecting efficacy and prolong the use of empirical first-line regimen. A strategy to apply genotyping test, in a cost-effective approach, should be considered to prepare for situation when HIVDR increases beyond a critical level.

Keywords: antiretroviral therapy, HIV, resistance, pre-treatment, Thailand

Introduction

The antiretroviral therapy (ART) has been scaled up in Thailand for all eligible human immunodeficiency virus infection (HIV) infected cases and acquired immune deficiency syndrome (AIDS) since 2002.[1] As of September 2014, 271,652 people living with HIV/AIDS (PLHIV) were treated with ART in nearly 1,000 ART clinics nationwide.[2] The first national HIV/AIDS treatment guideline was published in 2002, and the enrollment criteria were revised in 2010 and 2014. Highly active ART, consisting of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI), is recommended as an empirical first-line regimen with no prior genotyping. Criteria for enrollment to ART in earlier guidelines were symptomatic cases with CD4 count at 200 cells/µl or less. However, the recruitment criteria using CD4 level has been shifted to 350 cells/µl or less in 2010.[3] Since 2014, PLHIV are eligible for ART, regardless of CD4 level.[4]

Monitoring of treatment includes regular testing of CD4 and viral load (VL). Cases with good drug adherence and VL of more than 1,000 copies/ml after a year of treatment are tested with genotypic analysis to identify possible antiretroviral drug resistance. Reports of genotyping are used for deciding to switch

to a second-line regimen. All recommended treatment and laboratory testing costs are subsidized by health insurance schemes.

The objective of this study was to assess the prevalence of HIV drug resistance (HIVDR) in ART pre-treatment PLHIV. The Bureaus of AIDS, tuberculosis and sexually transmitted infections, with technical support from the Thailand MOPH – US CDC Collaboration, launched the survey projects among newly enrolled PLHIV initiating ART in selected clinics since 2005. Up through 2013, four rounds of surveys were conducted. Monitoring of HIVDR prevalence rates among ART pre-treatment cases overtime enables the national program to review the efficacy of empirical first-line treatment regimen.

Methods

The survey was designed to describe characteristics of pre-ART cases and assess prevalence of HIVDR. The first round was carried out in six clinics in 2006, and subsequently in eight clinics in 2007, 33 in 2008-2009 and four clinics in 2013. To collect sufficient specimens, duration of each survey ranged between 6-15 months (Table 1).

Table 1. Pre-treatment HIV drug resistant (HIVDR) surveys information in Thailand, 2006-2013

Survey information	Round 1	Round 2	Round 3	Round 4
Survey sites: antiretroviral therapy (ART) clinics	6 (3 regional/provincial and 3 community hospitals in 3 northern provinces)	8 (8 regional/provincial hospitals in 8 provinces)	33 (19 regional/provincial and 14 community hospitals in 12 provinces)	4 (4 regional/provincial hospitals from 4 provinces)
Enrollment period (months)	9 (Feb-Oct 2006)	6 (Jul-Dec 2007)	15 (Jul 2008-Oct 2009)	12 (2013)
HIVDR laboratory	Chiang Mai University	Multi-sites, depending on the existing systems		National Institute of Health
HIVDR test	Commercial	Commercial		In-house
Location of participating clinics				
● Regional/provincial hospital ○ Community hospital				

Sample Size Estimation

Sample size was calculated using the standard normal approximation set for expected proportion of treatment failure and/or observed genotypic mutation between 8-25%. Distance from proportion to limit was ±2-5%. Sample size of each survey was at least 300 naive cases.

Survey Site Selection Criteria

The sites were selected purposively in each round. Selected criteria included ability to provide ART for HIV cases, having on site laboratory facilities or being connected to another laboratory to monitor treatment results, possessing the required data set, and being forecasted to have sufficient cases for the survey.

Population Frame and Data Collection

The study population was PLHIV aged 18 years old or above. Cases eligible for the first-line ART initiation at the sites were those who were naive to ART, or who were experienced to ART and had stopped using ART (ART prophylaxis) or mother to child prevention. Consecutive sampling of every patient presented at the clinic was used until the enrollment period ended.

Data were extracted from the routinely collected data, including demographic data (gender, age, marital status, education and occupation), clinical findings (asymptomatic or symptomatic), history of previous exposure (naive or experienced) and CD4 results.

Specimen Collection and HIV Genotypic Test

Plasma for VL and genotyping were separated on site. Samples were shipped in cold chain using frozen cold packs. Duration from blood drawn to reach the laboratory was warranty processed within 72 hours without temperature monitoring.

The key laboratory tests were HIV VL and genotyping. In all rounds, VL was performed for all cases at the pre-treatment stage in the regular laboratory connected to each ART clinic. Genotypic test was performed in subjects with VL more than 1,000 copies/ml as recommended[3]. In the first round, genotyping was performed at Chiang Mai University using the TRUGENE HIV-1 genotyping Kit. In the second and third rounds, tests were carried out at the regular laboratories using the same commercial kit. In the fourth round, the in-house test was conducted at the National Institute of Health, World Health Organization (WHO) and a designated laboratory for HIVDR testing for surveillance using both reverse transcriptase (RT) and protease inhibitor (PI) primers. The methodology followed as previously described[5,6] and sequences were then interpreted using the Stanford HIV drug resistance database[7].

In this study, major drug resistance mutation interpreted by the genotypic test with the most updated version at the time of each survey was reported as resistance. Resistance to PIs was not analyzed since PI was not used in the first-line regimen and to avoid misleading factors from naturally occurring polymorphism[8].

Data Analysis

Demographic and other collected data were analyzed to observe frequency distribution of each variable. Survey statistics adjusted for clusters and Kruskal-Wallis test were used to test significant differential of each characteristic between the surveys. Likelihood-ratio chi-square for trend was applied to test HIVDR prevalence by rounds.

Trends of HIVDR prevalence rate among naive and experienced cases were determined with the likelihood-ratio chi-square test for trend analysis using Stata statistical software version 13 (College station, Tx stataCorpLP). Frequency of resistance to each drug was also analyzed.

Ethical Consideration

Cases were fully informed of the objectives and benefits of the survey. Data were collected after an informed consent was obtained. Participant's confidentiality was maintained using anonymous testing protocol. For subjects found to have HIVDR, the treatment was switched to second-line regimen according to the national guideline.

The Ethical Review Committee for Research in Human Subjects in the Ministry of Public Health, Thailand, approved Survey 1 as endorsed by document number 60/2007. The ethical approval was extended for Surveys 2 and 3 in the official letter with reference number 0327/2534 dated 11 Dec 2009. Survey 4 was approved by the same committee in document number 6/2013.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the funding agencies.

Results

The number of cases treated with ART for the first time at the sites during the survey rounds 1 to 4 were 311, 362, 969, and 431 respectively. HIV genotyping was conducted on 310, 351, 823 and 415 cases, and results were available in 310, 350, 797 and 413 cases respectively. The distribution of cases by occupation and type of hospital in four rounds showed no significant difference (Table 2). However, other demographic variables, including gender, age, marital status and education, were statistically different. In round 4, 61.5% of cases were male when compared with 48.4-53.2% in rounds 1-3 (p-value 0.006).

Among cases in round 4, 26.9% were less than 30 years old while participants in this age group in the earlier three rounds ranged between 9.7 and 19.4% (p-value 0.002). Proportion of cases with single marital status was higher (31.7%) in round 4 compared to 13.4-17.4% in rounds 1-3 (p-value 0.0002). In rounds 1-3, proportion of cases who held a bachelor degree or higher were 7.3-13.2% while proportion in round 4 (21.6%) was higher (p-value <0.001).

In terms of clinical condition, cases in round 4 tended to be more asymptomatic (59.9%) than in rounds 1-3 (15.7-48.1%, p-value <0.001). Median CD4 count increased from 38 cells/µl in round 1 to 167 cells/µl in round 4 (p-value <0.001). Median VL observed in round 1 was 212,000 copies/ml while it was 158,099 copies/ml in round 4. However, the trend did not reach the significant level (p-value 0.063).

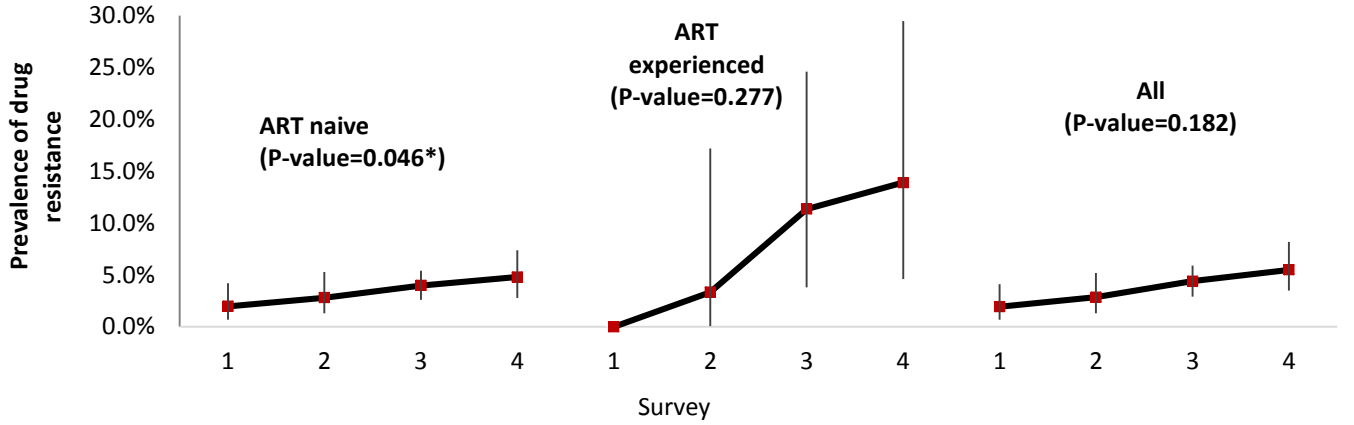
Table 2. Distribution of cases by demographic characteristics, types of hospital, symptoms and laboratory results from 4 rounds of surveys in Thailand, 2006-2013

Variable	Number (Percent)				P-value
	Round 1	Round 2	Round 3	Round 4	
Gender	310	351	823	413	
Male	164 (52.9)	170 (48.4)	438 (53.2)	254 (61.5)	0.006*
Female	146 (47.1)	181 (51.6)	385 (46.8)	159 (38.5)	
Age (year)	308	351	805	413	
Median age (min-max)	38 (21-65)	35 (18-62)	36 (18-67)	37 (18-70)	0.002#
<30	30 (9.7)	68 (19.4)	143 (17.8)	111 (26.9)	<0.001*
30-39	151 (49.0)	183 (52.1)	388 (48.2)	123 (29.8)	
40-49	96 (31.2)	77 (21.9)	204 (25.3)	122 (29.5)	
≥50	31 (10.1)	23 (6.6)	70 (8.7)	57 (13.8)	
Marital Status	310	351	823	401	
Single	54 (17.4)	47 (13.4)	133 (16.2)	127 (31.7)	<0.001*
Married/widowed/ divorced	256 (82.6)	304 (86.6)	690 (83.8)	274 (68.3)	
Education	299	349	799	408	
Grade 6 and below	210 (70.2)	160 (45.8)	480 (60.1)	159 (39)	<0.001*
Grade 7-12	67 (22.4)	143 (41.0)	261 (32.7)	161(39.5)	
Bachelor degree and higher	22 (7.4)	46 (13.2)	58 (7.3)	88 (21.6)	
Occupation	263	338	726	407	
Commercial and business owner	32 (12.2)	60 (17.8)	79 (10.9)	77 (18.9)	0.279*
Government/private sector	6 (2.3)	53 (15.7)	62 (8.5)	51 (12.5)	
Farmer and laborer	185 (70.3)	164 (48.5)	455 (62.7)	167 (41.0)	
Unemployed	40 (15.2)	61 (18.0)	130 (17.9)	91 (22.4)	
Student				21 (5.2)	
Hospital Type	310	351	823	415	
Community	44 (14.2)	0	272 (33.0)	0	0.112*
Regional and provincial	266 (85.8)	351 (100.0)	551 (67.0)	415 (100)	
Symptom	310	351	823	401	
Asymptomatic	149 (48.1)	55 (15.7)	159 (19.3)	240 (59.9)	<0.001*
Symptomatic	161 (51.9)	296 (84.3)	664 (80.7)	161 (40.1)	
CD4	310	350	815	408	
Median (cells/µl) (IQR)	38 (15-96.5)	58 (20-139.5)	55 (20-136)	167 (47-278.7)	<0.001#
Viral Load	310	351	810	400	
Median (copies/ml) (IQR)	212,000 (87,775-494,000)	194,000 (55,200-568,000)	209,767 (75,075-537,500)	158,099 (48,675-455,860)	0.063#

* Survey statistic adjusted for Clusters, # Kruskal-Wallis test

Among cases with HIVDR results, the majority was ART naive. In rounds 1-4, numbers of naive cases were 304, 320, 753 and 377; and experienced cases were seven, 30, 44 and 36. Overall HIVDR prevalence rates among naive cases by rounds using aggregated computing were 2.0%, 2.8%, 4.0% and 4.8% (p-value 0.046), and in experienced cases, the rates were 0, 3.3%, 11.4% and 13.9% (p-value 0.277) (Figure 1). Prevalence rates among total subjects in rounds 1-4 were 1.9%, 2.9%, 4.4% and 5.6% (p-value 0.182).

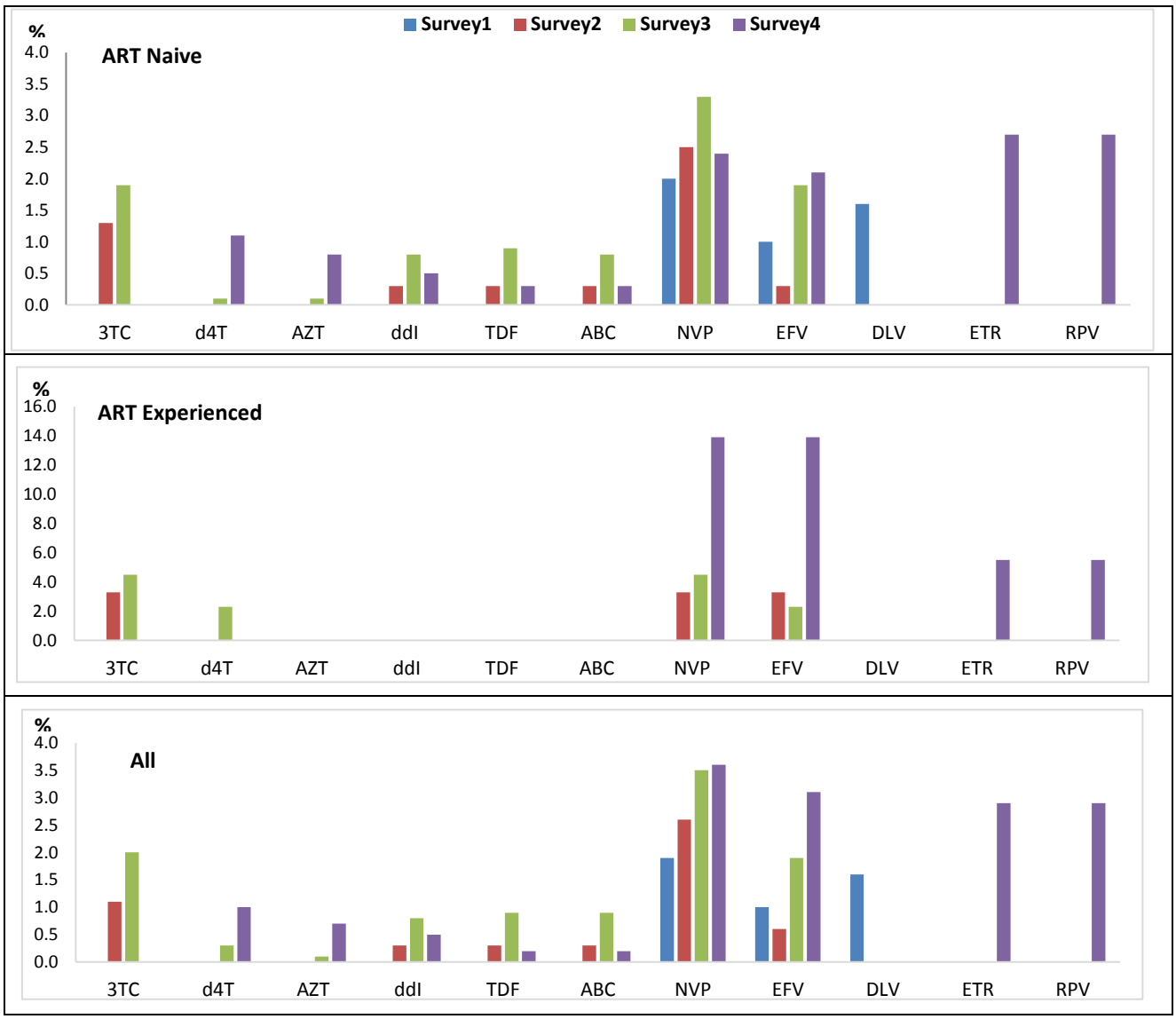
Among naive cases, the highest rate of resistance (3.3%) was observed in nevirapine (NVP) in round 3. Resistance to etravirine (ETR) and rilpivirine (RPV) in round 4 were equal (2.7%). In addition, HIVDR was also found with NRTI group such as lamivudine (3TC) at 1.9% in round 3. In experienced cases, the highest rates of resistance were to NVP and efavirenz (EFV) in round 4, with a rate of 13.9% to each drug. In total, NVP (3.6%) and EFV (3.1%) were the highest in round 4 (Figure 2).



n/N**	6/304	9/320	30/753	18/377	0/6	1/30	5/44	5/36	6/310	10/350	35/797	23/413
Aggregated rate	2.0%	2.8%	4.0%	4.8%	0	3.3%	11.4%	13.9%	1.9%	2.9%	4.4%	5.6%
(95% CI)	(0.7-4.2)	(1.3-5.3)	(2.6-5.4)	(2.8-7.4)	NA	(0. 08-17.2)	(3.8-24.6)	(4.6-29.5)	(0.7-4.1)	(1.3-5.2)	(2.9-5.9)	(3.5-8.2)

* Likelihood-ratio chi-square for trend, statistically significant ** n = HIVDR cases, N = cases with HIVDR test results

Figure 1. Trend of HIV drug resistance prevalence rates among antiretroviral therapy (ART) naive, experienced and all cases from 4 rounds of survey in Thailand, 2006-2013



Abbreviations: antiretroviral therapy (ART), lamivudine (3TC), stavudine (d4T), zidovudine (AZT), didanosine (ddI), tenofovir (TDF), abacavir (ABC), nevirapine (NVP), efavirenz (EFV), delavirdine (DLV), etravirine (ETR), etravirine (RPV)

Figure 2. HIV drug resistance rates in each antiretroviral drug classified by antiretroviral therapy naive, experienced and all cases from 4 rounds of survey in Thailand, 2006-2013

Discussion

In current ART practice in resource-limited countries, empirical regimen is used without prior genotypic testing[4]. This practice is based on the assumption of low HIVDR rates and the genotyping of each PLHIV before initiating ART would not be cost-effective. However, when large number of HIV cases received ART, HIVDR can emerge and be transmitted[9]. Therefore, periodical surveys to monitor the prevalence of HIVDR in pre-ART cases were essential to assess program effectiveness. Such surveys were also recommended by WHO[10,11].

In this article, a series of four consecutive surveys during 2006-2013 to assess HIVDR rates among pre-ART cases was reported. The selected demographic factors and certain laboratory results in survey round 4 were found to be different from rounds 1-3. This difference might be caused by change in enrollment criteria. The eligibility in 2010 was a CD4 of 350 cell/µl or less[3] while the cutoff for initiation in the earlier was a CD4 at 200 cell/µl or less.

Our study found an upward trend of HIVDR prevalence, with the highest rates of 4.8% among ART naive cases and 13.9% among experienced cases in round 4. Among all ART naive cases, the rates were still low, yet rising with significant trend over time. This finding indicated the necessity to continue monitoring HIVDR for evaluating the use of the currently recommended ART regimens without prior individual genotyping. The experienced cases, such as those receiving ART prophylaxis or prevention mother-to-child transmission, or those who have defaulted from previous ART should be closely monitored since the observed rates in these individuals were relatively high.

Resistance was the most common for NNRTIs while resistance to NVP and EFV were observed in round 4 as well. Resistance to other antiretroviral was lower in all rounds.

Other studies in Thailand revealed that HIVDR prevalence rates among pre-treatment cases varied from 2-17.6%[12-16]. However, these surveys aimed to measure single-period prevalence rate and some were performed in tertiary care settings. As participants were enrolled from regional, provincial and community hospital settings in this study, characteristics of participants in the pre-treatment HIVDR prevalence study might be different, which reflected variation of HIVDR rates.

Pre-treatment HIVDR rates from other countries varied widely. The prevalence rate during 2009-2010 in Vietnam was 3.5%[17]. In Zimbabwe, the overall HIVDR rate during 2008-2010 was 6.3%[18], with the prevalence in experienced cases being 12.1% and naive cases 5.7%. During 2013-2014, a survey in South Africa showed a prevalence of 9.0%[19]. Data from Latin America country revealed higher prevalence. In Honduras, the prevalence in 2013-2015 was observed to be 11.5%[20] while the prevalence during 2011-2015 was 13.4% in Nicaragua[21]. An alarming prevalence of 15.5% was reported from Mexico in 2015[22].

In this study, there were three major limitations. Firstly, survey sites were varied, not randomly chosen, and sample sizes differed in each round, effecting data representativeness. Variation existed for reagent kits and interpretation of resistance among laboratories used, noting that genotyping test in the first three rounds was commercial assay based. The other limitation was that small samples in ART experienced cases were included in the study. Therefore, prevalence of HIVDR in this group must be interpreted with caution. To overcome these limitations, the fifth survey following the WHO recommended method[23] has been planned for 2017. Findings from the upcoming survey would be essential to assess HIVDR among pre-treatment cases.

Public Health Actions and Recommendations

Results from this study as well as from the other surveys, locally and globally, indicated a need to continue surveillance for pre-treatment HIVDR and serious challenges to ART programs in resource-limited countries. Activities in developing practical guidelines to protect efficacy and prolong the use of empirical first-line ART regimens, such as HIV treatment literacy and strengthening of adherence to medication, should be implemented. The manager of national ART program together with partners should consider stewardship strategy on the use of empirical ART regimen as well as a strategy to apply genotyping test when HIVDR has increased beyond a critical level. In addition, since pre-exposure prophylaxis for HIV using selected ARV was promoted, particular attention should be given to monitor the circulating HIVDR.

Acknowledgement

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Measles Outbreak among Nomadic Population with Low Herd Immunity in an Eastern District of Bhutan, 2016

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Abstract

During September-November 2016, a number of measles cases were reported from Trashigang District, Bhutan. The outbreak was investigated to determine epidemiological characteristics and risk factors, and recommend control measures. Hospital records of measles cases were reviewed. An active case finding was conducted in the affected communities and schools. Vaccination records were also reviewed. A case-control study was conducted to determine risk factors for measles infection. Tests for measles and rubella immunoglobulin M (IgM) antibodies, viral identification in throat swabs by polymerase chain reaction, and viral genotyping were performed. Total 62 suspected cases were identified, with no reported deaths. The first case developed symptoms on 15 Sep 2016 and 72.2% of the cases occurred in October 2016. The majority (85.0%) were 14 years old and younger (median 8.2 years, interquartile range 6.5-12.0). Cases were from Sakteng (87.1%) and Merak (12.9%) Subdistricts, the latter being a common place where nomads lived. Among 40 cases tested for measles IgM and viral identification, 33 (82.5%) were found to have measles IgM antibodies. All positive samples were genotyped and 11 (33%) were identified as D8 strains which circulated in India during 2016. The measles vaccine efficacy was 82.0%. Significant risk factors were having previous contact with a measles case (OR = 8.46, 95% CI = 2.08-34.41) and not receiving measles vaccination (OR = 6.61, 95% CI = 2.60-16.82). Immunization for outbreak response, case-based investigation and supplementary immunization activities were recommended.

Keywords: measles, outbreak, nomad, vaccine, coverage, Bhutan

Introduction

Measles is an acute and highly communicable viral disease characterized by fever with maculopapular rashes, cough, coryza, conjunctivitis and Koplik's spots. The disease is transmitted by respiratory droplets or direct contact. Normally, infected persons are contagious from four days before eruption of the rash until four days after eruption. The incubation period varies from 8-15 days.

Measles can be prevented readily with immunization. However, 95% of a community needs to be immunized in order to develop herd immunity. Measles remains a significant cause of morbidity and mortality worldwide. In 2012, 43% of global measles deaths occurred in the South-East Asia region. Of which 14% were from India.[1,2]

In Bhutan, measles monovalent vaccinations were introduced into the routine immunization program during 1979. The measles-rubella (MR) containing vaccine was replaced in 2006, and two doses of measles vaccination was added in the routine immunization program in 2010, with the first dose of measles vaccine given at nine months of age and the second dose of MR at 24 months. Hence, the incidence of measles per 100,000 population declined from 254 in 1980 to 24 in 1990, which could also attribute to high measles vaccination coverage (95%) in Bhutan during recent years.[3]

In line with the measles elimination goal of the World Health Organization (WHO) South-East Asia Regional Office, a measles elimination strategy was developed in Bhutan to achieve zero indigenous measles cases by April 2017. Measles elimination is defined as an

interruption of indigenous measles virus transmission in a geographical area for at least 12 months in the presence of a well-performing surveillance system.[4]

In Bhutan, measles is an immediately notifiable disease, which implies that a single suspected measles case has to be reported to the National Early Warning Alert and Response Surveillance (NEWARS) within 12 hours of detection. The last endemic measles case was detected in 2012, and no measles cases were detected in 2013 and 2014. However, sporadic cases of measles (12 laboratory confirmed) were reported from some districts in the last quarter of 2015. These cases were classified as being imported or travel-related since the source of infection was epidemiologically linked to an Indian border town.

During September to November 2016, a number of measles cases were reported in Sakteng Subdistrict, Trashigang District, Bhutan. Following a preliminary investigation conducted by the district rapid response team in early November 2016, a team from the Royal Center for Disease Control (RCDC) joined the investigation on 15 Nov 2016.

The objectives of this report are to confirm the outbreak, determine epidemiological characteristics, identify risk factors of the outbreak, assess the measles vaccine coverage and efficacy, and recommend preventive and control measures.

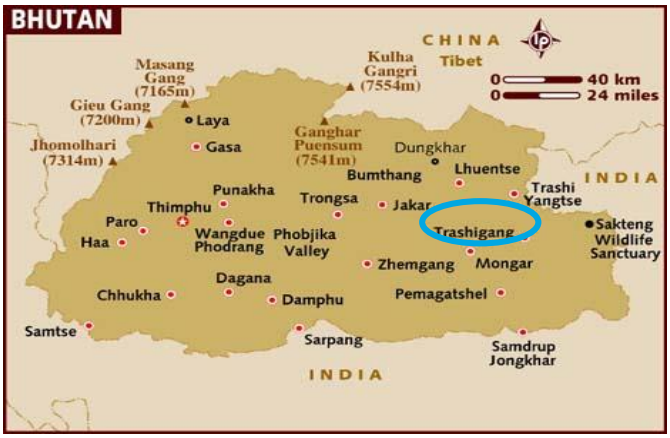
Methods

Trashigang District is located in the eastern part of Bhutan. A population of 3,152 resided in Sakteng Subdistrict which is located at an altitude of about 3,000 meter above the sea level. It borders with the Indian state of Arunachal Pradesh to the northeast and Tibet of China to the north (Figure 1). The majority of the people of Sakteng were nomads whose livelihoods were contingent on their yak herds. They frequently migrat with their yak herds to many areas.

Epidemiological Investigation

Retrospective case finding was carried out by reviewing patient records at local and district hospitals from 15 Sep to 25 Nov 2016 to identify suspected measles cases. Active case finding in the communities and schools was also conducted using a standard measles case-based investigation form.

A suspected case was defined as an individual who lived in Sakteng Subdistrict and had fever with maculopapular (non-vesicular) rash between 15 Sep and 25 Nov 2016. A confirmed case was defined as a suspected case with positive measles IgM antibody or viral identification by real time polymerase chain reaction (RT-PCR).



(a)

(b)

Figure 1. Maps showing (a) Bhutan and (b) Sakteng Subdistrict in Trashigang District

Cases or their guardians were interviewed to obtain information on vaccination status, history of contact with other cases, travel history, and illness among family members.

Mother and child health records in health centers were reviewed to determine the measles vaccination coverage for the period of 2011-2015 as well. In addition, a community survey was conducted to ascertain the immunization coverage for measles and each individual was asked about the vaccination status.

Laboratory Investigation

Specimens of blood serum and throat swabs were collected in cryovials and viral transport mediums from the outbreak areas, and transferred to the national measles reference laboratory at RCDC for laboratory testing[5]. A cold chain transportation system, with temperatures maintained at 2-8°C, was used for the sample shipment. Enzyme-linked immunosorbent assay was performed on serum samples for detecting anti-measles immunoglobulin M (IgM) antibody using Enzygnost® Anti-Measles-Virus/IgM (Siemens). Using the manufacturer's instructions, viral RNA was extracted from the throat swabs (Qiagen extraction kit) and RT-PCR was performed (invitrogen®).

Positive samples were referred to the National Institute of Health (NIH), the WHO measles regional reference laboratory in Thailand, for genotyping under cold chain. All samples were tested for anti-rubella IgM antibody as well.

Analytic Study

A case-control study was conducted using four controls for each case to identify possible risk factors associated with the outbreak. Cases (either suspected or confirmed) were recruited from the descriptive study. Controls were those who did not contract measles during the outbreak and had no history of previous measles infection.

Frequencies, proportions and attack rates were computed to describe the epidemiological characteristics of the outbreak. Odds ratios (OR) and 95% confidence intervals (CI) were used to assess the strength of association of risk factors for measles infection. Multiple logistic regression was used to adjust for potential confounders. Vaccine efficacy was calculated using the standard formula: [ARU-ARV]/ARU, where ARU is attack rate among unvaccinated and ARV is attack rate among vaccinated. All statistical analyses were performed using Epi Info version 7[6].

Results

To confirm the outbreak, we reviewed monthly reported number of measles cases during 2016 in Bhutan and compared to 5-year monthly median. A steep increase in the number of cases in October 2016 was evidenced. Compared to 5-year median, there was a measles outbreak during October and November 2016 (Figure 2).

From 15 Sep to 25 Nov 2016, a total of 62 suspected measles cases were identified in Trashigang District. The majority (61.3%) were males. Out of 40 cases tested, 33 (82.5%) were laboratory confirmed by anti-measles IgM antibody and RT-PCR while the rest were epidemiologically linked and clinically compatible with measles infection.

The first case of this outbreak was a 36-year-old male from Merak Block who had an onset of fever and rash on 15 Sep 2016. A 3-month old child who visited the first case on 16 Sep 2016 was the first laboratory confirmed measles case during the outbreak. A school teacher who was probably infected while in a hospital could have transmitted the disease among the students of Sakteng Lower Secondary School. Onset of the last case was 19 Nov 2016 and the curve also suggested as a propagated outbreak (Figure 3).

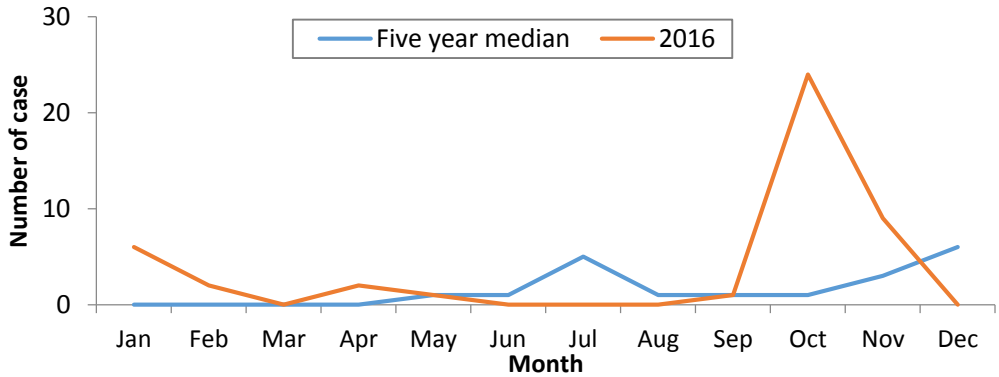


Figure 2. Number of confirmed measles cases in 2016 by month and past 5-year monthly medians (2011-2015), Bhutan

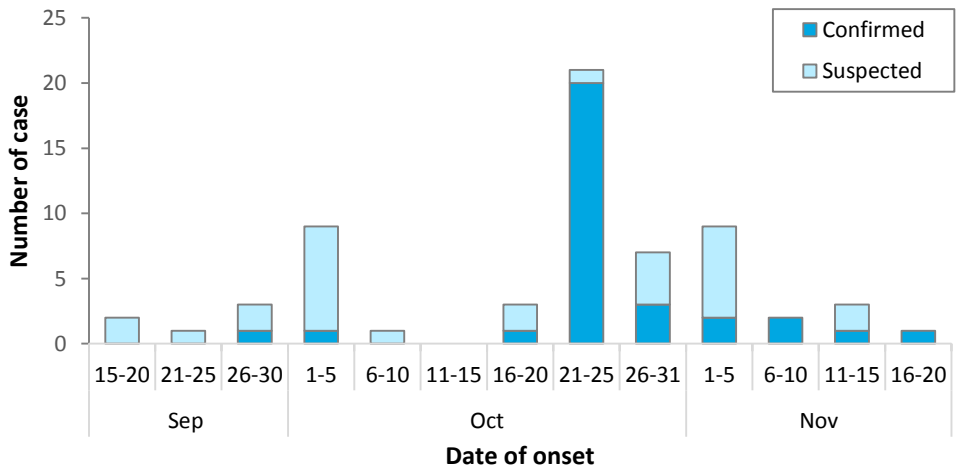


Figure 3. Number of measles cases by date of onset in Sakteng Subdistrict Trashigang District, Bhutan, September-November 2016 (n = 62)

The majority (85.0%) of the 62 cases were 14 years old and younger. The highest attack rate (50.0%) occurred in 5-9 years old (Table 1). The median age was 8.2 years (interquartile range: 6.5-12.0). The youngest was aged three months while the oldest was a 48-year old male. While the majority of cases (71.0%) were students, 9.7% were pre-school children, and the rest were six farmers, five monks or a teacher. Measles cases were only reported from Sakteng and Merak Blocks in Sakteng Subdistrict, and the highest number of cases (87.1%) was observed in Sakteng Block.

All cases had fever with maculopapular rash. Other common symptoms included cough (71.0%), coryza (66.1%), and conjunctivitis (32.3%). Twenty-one (34.0%) cases were admitted at the district hospital in Trashigang for further management. No complications or deaths was identified in this outbreak.

Laboratory Findings

Of 40 cases tested, 33 (82.5%) were positive for measles by IgM antibody and RT-PCR. Molecular sequencing and phylogenetic analysis of all positive specimens isolated the measles virus in 11 (33%) samples as D8 genotype which was similar to the strain reported from India during 2016 (Figure 4). All samples were negative for rubella IgM antibody.

Vaccination Status

Of total 3,152 population in Trashigang District, 388 people from highly affected communities were contacted and interviewed for vaccination status. Among 62 cases, six (10.0%) had documented evidence of measles vaccination, 44 (71.0%) had never been vaccinated, and 12 (19.4%) could not confirm their vaccination status. Age group that had the highest complete proportion of vaccination was among 1-4 years old (64.3%) (Figure 5).

There was a significant difference between the overall MR immunization coverage achieved at the district level (95%) and that achieved in Sakteng Subdistrict (48%) (p-value <0.001). The overall attack rate was found to be 15.0% and the measles vaccine efficacy was 82.0%.

Case-control Study

A total of 388 individuals living in Sakteng Subdistrict were interviewed, regarding vaccination status, contact with measles cases, and whether they lived in the same house with a measles case. Those who did not receive the measles vaccine were 4.78 (95% CI = 1.97-11.60) times more likely to have measles infection compared to those who received the vaccination. Those who had contact with another measles case were 7.90 times more likely to be a case (95% CI = 4.25-14.68), and those who lived in the same household with a case were 5.28 (95% CI = 2.96-9.43) times more likely to be a case (Table 2).

Two factors remained significant in the multivariate analysis were having contact with a measles case (OR = 8.46, 95% CI = 2.08-34.41) and not received measles vaccination (OR = 6.61, 95% CI = 2.60-16.82) (Table 3).

Discussion

The recent measles outbreak in Sakteng Subdistrict posed a great setback to the Department of Public Health for the national goal to achieve measles elimination by April 2017.

Since the last case of measles reported in 2012, there were no measles cases identified in 2013 and 2014. However, the country experienced a resurgence of measles with sporadic cases reported across the country in 2015. However, all measles cases detected in 2015 were classified as imported or travel-related since the sources of infection for all cases were epidemiologically linked to a measles outbreak in an Indian border town. In 2016, sporadic measles cases were reported from most of the districts, including Trashigang District, which was followed by the outbreak notification in Sakteng Subdistrict.

Table 1. Attack rates by age groups and blocks in Sakteng Subdistrict, Trashigang District, Bhutan, September-November 2016

Age group (year)	Total population		Number of case		Attack rate (%)	
	Sakteng	Merak	Sakteng	Merak	Sakteng	Merak
1-4	240	166	4	2	1.7	1.2
5-9	332	148	27	4	8.1	2.7
10-14	259	144	14	1	5.4	0.7
15-19	252	162	3	0	1.2	0.0
≥ 20	959	492	6	1	0.7	0.2
Total	2042	1112	54	8	2.7	0.7

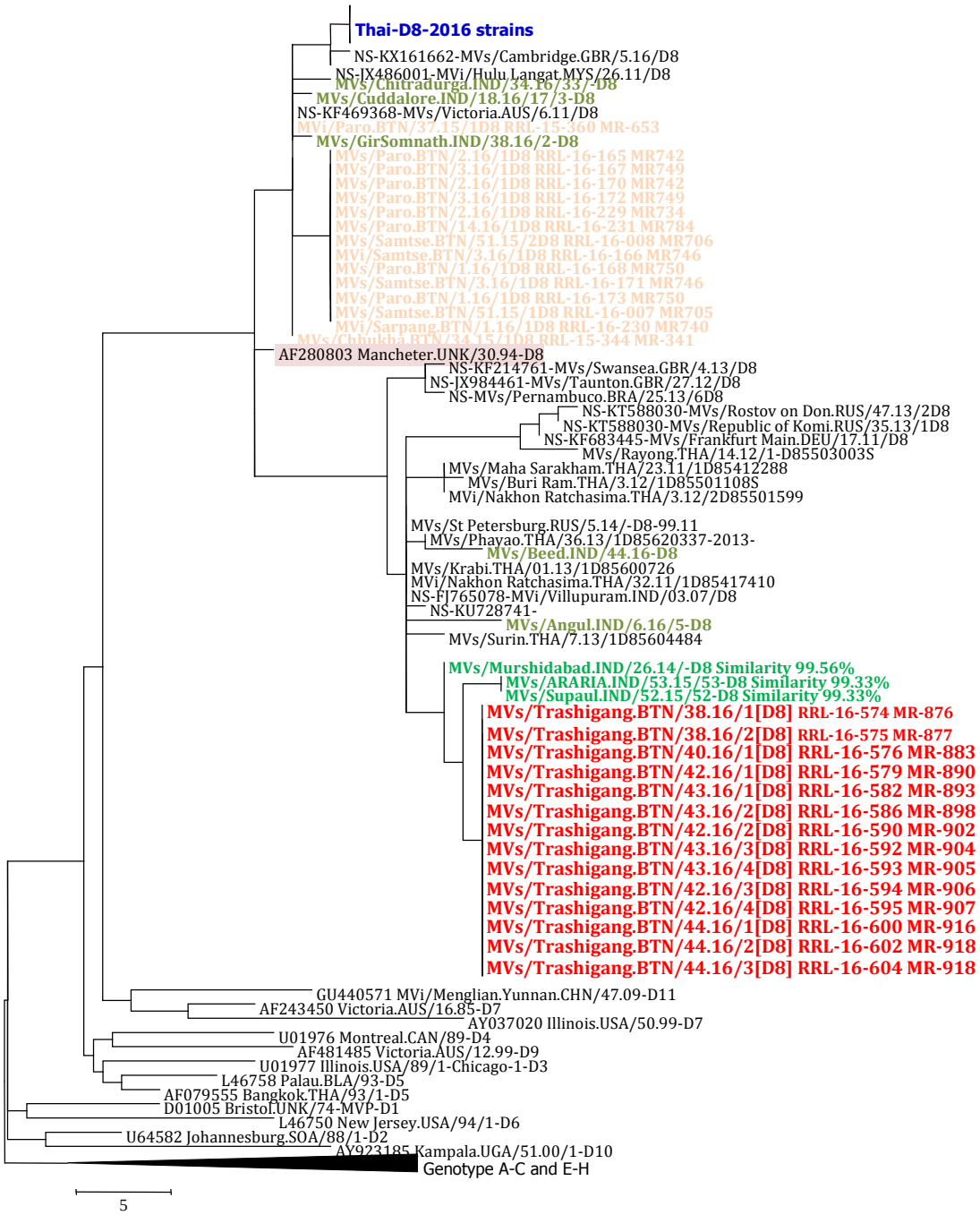


Figure 4. Phylogenetic analysis of measles virus isolated from samples during the outbreak (red color) in Sakteng Subdistrict, Trashigang District, Bhutan, 2016

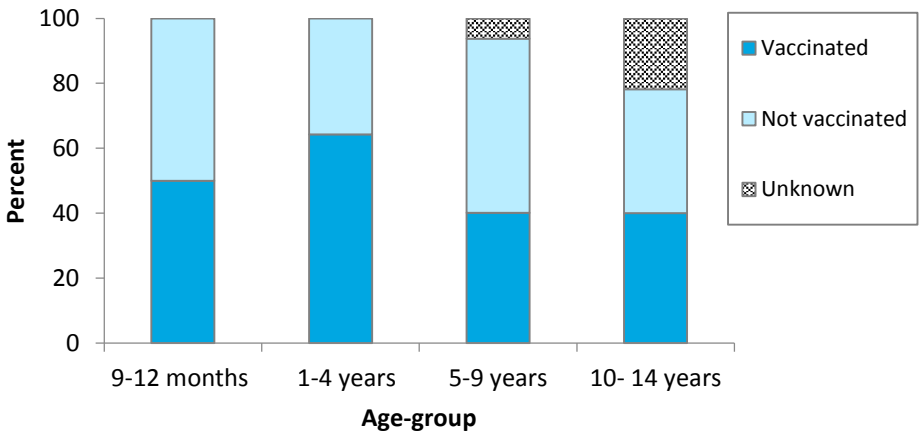


Figure 5. Measles vaccination coverage among children 9 months to 14 years, community survey, Sakteng Subdistrict, Trashigang District, Bhutan, September-November 2016 (n = 266)

Table 2. Potential risk factors for measles infection, Sakteng Subdistrict, Trashigang District, Bhutan, September-November 2016

Risk factor	Case (n=62)		Control (n=326)		Odds ratio	95% CI
	Yes	No	Yes	No		
Contact with a measles case	46	16	87	239	7.90	4.25 - 14.68
Being a boarding school student	41	21	88	238	5.28	2.96-9.43
No previous vaccination*	41	6	176	123	4.78	1.97-11.60
Pre-school children	6	56	134	192	0.15	0.06-0.37

*Excluding those with no data.

Table 3. Association between potential risk factors and measles infection by multiple logistic regression analysis, Sakteng Subdistrict, Trashigang District, Bhutan, September-November 2016

Risk factor	Adjusted odds ratio	95% CI
Sex (male vs female)	1.45	0.73-2.89
Age (years)	0.95	0.92-0.99
Contact with a measles case	8.46	2.08-34.41
Being a boarding school student	0.67	0.17-2.69
No previous vaccination	6.61	2.60-16.82

The measles vaccine coverage of 48% among those aged under 14 years in Sakteng was found to be low compared to the national coverage of 95% and much lower than the minimum vaccination coverage required to protect the population from a measles outbreak[1]. Our study showed that having previous contact with a measles case and being unvaccinated against measles were independent risk factors for contracting measles. The low vaccine coverage could have increased the susceptibility of children and adults for measles infection. Similar findings of low vaccine coverage being associated with measles outbreaks were observed in different parts of India[7,8], Nepal[9], Bangladesh[10,11], Sri Lanka[12,13], Thailand[14] and Lao PDR[14].

The most affected age group was 5-9 years, with 85% of cases being aged 14 years or less. Analysis of our data showed that there was a cohort of susceptible individuals who had perhaps never received measles immunization nor been exposed to natural infection. The migratory habit of residents and low literacy rate of nomadic populations might have further contributed to poor uptake of vaccination programs by the communities living in Sakteng. They might have migrated to temporary posts at the time of vaccination campaigns and might not visit health centers once they returned to the communities. This highlighted the challenge of achieving herd immunity among migrant populations.

Additionally, higher number of cases and delay in public health response could be due to late diagnosis due to low suspicion of measles by clinicians. Our investigation revealed that a substantial number of initial cases were misdiagnosed by the attending physicians, which indicated that health care providers or clinicians in hospitals were no longer familiar with the clinical presentation of measles cases, probably due to high measles vaccine coverage and low incidence of measles in the community. Late diagnosis and confirmation of the primary cases resulted in delayed contact tracing and follow-up. Early action in notifying public health authorities and timely provision of MR vaccination to those at risk are crucial steps in minimizing the risk of secondary cases.

This outbreak also highlighted the importance of molecular epidemiology. The phylogenetic analysis of the measles virus isolated from the outbreak samples revealed D8, a genotype which has a high similarity with the strains reported in India during 2015. This further suggested that acquisition of the infection could have been from across the border in India. The D8 genotype had been reported to be circulating in most regional and neighboring countries such as India, Nepal, Bangladesh and Sri Lanka[5,15-17]. Genetic analysis of measles isolates can aid in identifying the geographic and personal source of the outbreak, confirm possible relationship among cases, and identify routes of transmission. The measles outbreak

among this particular nomadic population in Bhutan during 2016 served as a reminder that imported measles cases could lead to large outbreaks, particularly among unvaccinated populations. If the infection is introduced into areas with pockets of unvaccinated migrants, the population could remain at risk for acquiring and transmitting the disease. The countries that had achieved measles elimination goal such as Australia, United Kingdom and USA had experienced measles outbreaks imported from other endemic countries as well.[18-20]

Conclusions

This reported measles outbreak was predominantly localized in the nomad populations with low vaccine coverage. The consistently low immunization coverage over the past few years had created a large immunity gap among populations in the community and could have increased the susceptibility for measles infection. The introduction of a single measles case, whether from within the country or across the border, consequently triggered the outbreak. The majority of cases were children aged 14 years or less and reflected a significant percentage of non-immune young population. This outbreak caused a setback to the national measles elimination program of the country. As D8 measles genotype had commonly been reported from India, the outbreak could be considered as imported based on findings from epidemiological investigation and molecular characterization. However, whether the D8 genotype was indigenous among the Bhutanese people or imported from another country needed further confirmation.

Public Health Interventions and Recommendations

As an outbreak response, the mop-up MR vaccination were provided to those aged nine months to 40 years within two days after detecting the outbreak. Active case finding was conducted by visiting the communities and school. Isolation of cases and onsite case management, including provision of vitamin A supplement to all cases, were performed. Health education on importance of immunization in preventing diseases, isolation of cases to prevent further spread, and seeking health care during sickness was delivered to the public.

Constant health education campaigns should be provided to raise awareness in the communities about benefits of vaccination and increase acceptance of the vaccine. The district health authorities should assess the vaccination coverage, particularly in hard-to-reach populations. At the same time, a mechanism should be developed to obtain an accurate estimate of the vaccine coverage across the nation. When merely a single laboratory confirmed case was detected, immunization for outbreak response and investigation should be recommended as a policy guidance. Supplementary immunization activities among hard-to-reach and high risk populations were recommended as well. Policy makers should take all possible measures to maintain high level of measles vaccination coverage for disease elimination in the near future. Training courses on clinical management of measles cases for clinicians was also recommended.

Limitations

The incidence of measles in this study might have been under-estimated as people with mild symptoms might not seek medical care at a health facility. Data interpretation might be hampered by recall bias as some subjects could not remember their vaccination or disease history. Finally, the sample size for the genotype study might be small to make concrete conclusions.

Acknowledgements

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What do you "Expect"?

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Since "Statistics 101" course, we all learned that chi-square (χ^2) test is used when analyzing the association between exposure and outcome that are categorical variables, for examples: association between smoking ("smoke" vs. "not smoke") and lung cancer ("yes" or "no"), or association between treatment ("Drug A" vs. "Drug B") and treatment outcomes ("worsen", "stable", "improved"). Many might still remember that there are ***Pearson's chi-square test*** and ***Fisher's exact test***; and we would prefer to use Fisher's exact test rather than Pearson's chi-square when small ***"Expected Counts"*** are presented. So what do you "expect" in Pearson chi-square or Fisher's exact?

Back to Basic of "Probability"

When a teacher starts his/her statistic course, he/she will talk about tossing coins, rolling a dice, drawing cards out of a deck, and then "probability" theory. Many students start getting lost from there. But, in fact, it is not that difficult and it is the basic of most statistical methods. Let's look at some terms.[1-3]

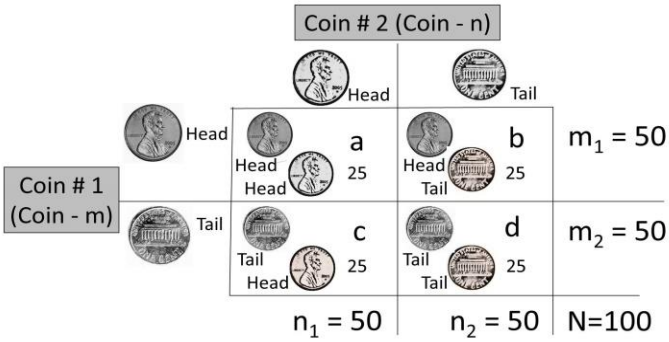
"Probability" or "Probable" derives from Latin "Probabilis" which means plausible or generally approved. "Probability", or another common term "Chance", deals with the stochastic (random) processes which lie behind data or outcomes. It could be considered as a measure of how some events will likely occur; it is usually expressing as the proportion of the number of cases of interest happening among the whole number of cases possible, for example, "the probability that you will get number 3 face landing after rolling a dice is 1 in 6 (or 0.1666..) as each dice has six faces".

Probabilities may be calculated either as marginal, joint or conditional functions. Most statistical methods rely on this concept. ***Marginal probability***, p(A), can be considered as an unconditional probability; that is, an event A that occurs is not conditioned on any other events. As an example in tossing an unbiased coin, the probability that a "Head" side will fall is unconditioned to chance that the "Tail" side will fall; thus p(head) = 1 in 2 (or 0.5). (The two sides of a coin are expressed as "Head" or "Tail" because head and tail has been historically considered as opposite body parts.) ***Joint probability***, p(A and B) or p(A ∩ B), refers to the probability of event A and event B are occurring together; it is the likelihood of two independent events happening at the time frame of interest (of note, it could be the probability of the intersection of two or more events). But wait – there are conditions that we have to take into consideration here: (a) the events A and B must be able to happen within the certain time frame and (b) the events A and B must be independent of each other. As an example, tossing two coins at the same time is independent events as the outcome of tossing one coin has no influence on the outcome of tossing the other coin. With the independent events, we can use the joint probability formula to calculate a chance of getting the jointed outcome of interest by the simple formula: p(A ∩ B = p(A) x p(B). As shown in figure 1, in tossing two unbiased coins, the joint probability to get "Tail" and "Tail" of the two coins will be 0.25.

Chi-square and "Expected Counts"

Historically, Pearson's paper of 1900 introduced what subsequently became known as the chi-square test of goodness of fit. In series of tossing of ten shillings at a time "frequently in the open air", Pearson's analysis of these artificial experiments led to the concept of "deviations from the most probable" or "a criterion of the probability"[4].

Let's look at an example of a simple case of flipping a coin. If the coin is unbiased, meaning that it is fair and balanced, then the "most probable" or "expected" frequency of to get head is 0.5 or 50%. If we toss a coin 100 times and we get 45 or 55 heads, we may be not suspicious as the "deviations from the most probable" seems to be acceptable. But if only 31 heads occur in 100 flips, we would be now skeptical and



Example: If tossing two unbiased coins for 100 times,

- Probability of getting "Tail" of Coin # 1 = (m_2/N) = 50/100 = 0.5
- Probability of getting "Tail" of Coin # 2 = (n_2/N) = 50/100 = 0.5
- Probability of getting "Tail" of Coin # 1 AND "Tail" of Coin # 2 = (m_2/N) x (n_2/N) = 0.5 x 0.5 = 0.25

Thus, in tossing 100 times, one should get "Tail-Tail" for

[(m_2/ N) x (n/N) x N] = 0.25x100 = 25 times

Figure 1. Outcomes of tossing two unbiased coins

suspect that the coin is somehow unfair or weighted to come up with tails. This is the concept of Pearson's chi-square test, the test that compares the observed distribution of counts against the expected distribution from some theoretical baseline which allow us to quantify the probability of such an event[5]. The size of the difference between observed and expected distributions is reflected in the test statistic.

The statistical null hypothesis is that the number of observed counts in each category is equal to that expected or predicted by a probability theory, and the alternative hypothesis is that the observed numbers are different from the expected. Then we will use a mathematical relationship, in this case the chi-square distribution, to estimate the probability of obtaining that value of the test statistic[6-8]. The chi-square test statistic is calculated by using the formula:

$$x^2 = \sum \frac{(O - E)^2}{E}$$

where O represents the observed frequency (counts). E is the expected frequency (counts} under the null hypothesis.

As an example of a study to determine association between exposure (E– vs. E+) and outcome (D– or D+), such as smoking (yes vs. no) and lung cancer (yes or no), we can generate a 2x2 table as shown in figure 2. The observed counts (from data collection in the study) would be: a, b, c, d as shown in each category (cell). Then how we do get the expected counts? Back to our joint probability concept - if "exposure" and "outcome" are independent (not associated), then we can calculate the probability of the joint event in each cell. As shown in figure 2, the probability of "not exposed, E-" and "not having outcome, D-" can be calculated and then compared against its observed count, d. The chi-square test statistic is then based on the combination of Os and Es of all categories in the table.

		Outcome		
		D +	D -	Total
Exposure	E +	a	C	m_1
	E -	c	D	m_2
	Total	n_1	n_2	N

Example:

- Observed value = cases that not being exposed (E-) AND not having outcome (D-) among N people = d
- Expected value = (Prob. of being not exposed, E-) AND (Prob..of not having outcome, D-) of N people = (m_2/N) x (n_2/N) x N

Figure 2. Observed and expected frequencies (counts) in a 2x2 table

The chi-square statistic is a non-parametric (distribution free); that means it is robust with respect to the distribution of the data. Specifically, it does not require equality of variances among the study groups or homoscedasticity in the data[9]. Chi-square test can be used for both dichotomous independent variables (a shown in 2x2 table above) and multiple groups/outcomes. However, the chi-square test does not provide an exact calculation of the p-value but rather an approximation of the p-value. But no need to worry - when the assumptions of the test are met, it is like all probability density functions, the chi-square distribution is a continuous function whose area sums to one[5]. Just a note for the reader who is interested in mathematical foundation, the chi-square distribution is based on the summing of the square values of k standard normal distributions, whereas k is corresponding to the degrees of freedom for the chi-square distribution. Degree of freedom for chi-square is equal to (r-1)x(c-1),

where r is the number of levels of one categorical variable and c is the number of levels of another categorical variable. As shown in figure 3, the observed counts vs. expected counts in the 2x2 table (4 cells) were compared in Pearson's chi-square test statistic and the p-value was calculated basing on chi-square distribution. The degree of freedom as shown next to the chi-square is 1, chi2(1), because we have 2 levels of exposure and 2 levels of outcome. Based on the p-value, we can then conclude that there is statistically significant association between exposure (infection at ICU admission) and outcome (vital status).

Example 1:

infection probable at icu admission	vital status lived	died	Total
no	100 92.8	16 23.2	116 116.0
yes	60 67.2	24 16.8	84 84.0
Total	160 160.0	40 40.0	200 200.0

```
        Pearson chi2(1) =   6.6502   Pr = 0.010
         Fisher's exact =                 0.012
1-sided Fisher's exact =                  0.008
```

Observed = 24 cases with "probable infection" AND "died"
Expected = (40/200) x (84/200) x 200 = 16.8 cases

Figure 3. Person's Chi-square test based in observed and expected frequencies

Karl Pearson vs. Ronald A. Fisher

It is not a strange phenomenon to see a scientific controversy debating on certain issue publicly and privately. In 1935, Karl Pearson and R. A. Fisher exchanged hot letters in Nature, one of the most prestigious scientific journals, on testing statistical hypotheses. The disagreements and rivalry between Ronald A Fisher and Karl Pearson were also noted in history in many other statistical theories; after dying of Karl Pearson, Fisher even continued to argue with Ergon Pearson (Karl Pearson's son) and Jerzy Neyman on this hypothesis testing concept[10,11]. In fact, there has been another debate on philosophy of hypothesis testing from Bayesian approach which is based on stronger assumptions[10]. This is fun to read but it is beyond the purpose of this article.

Fisher argued that in all cases of applying the chi-square test it is mathematically necessary to take account of the number of degrees of freedom of the observations in relation to the expected distribution to which they are compared[12]. Fisher then developed the "Exact" test which means that we can calculate from the marginal totals and get exactly what is the probability of getting an observed result, in the same way that we can work out exactly the chance that we may get 55 heads out of 100 tosses of an unbiased coin. However, the method and formula for Fisher's exact test is not easy to write up; it is based on the "factorial" or successive multiplication by numbers in descending series[13].

It was suggested in literature that the Pearson's chi-square test involves using the chi-square distribution to approximate the underlying exact distribution. The main assumptions for Pearson's chi-square test include: (a) individual observations are independent of each other, and (b) individual cells contain sufficient counts. The approximation becomes better as the expected cell counts grow larger, and may be inappropriate for tables with very small expected cell counts[14]. There are many recommendations about the sufficient counts[5,14,15]. A standard (and conservative) rule of thumb is to avoid using the Pearson's chi-square test statistics for tables with expected cell counts <1, or when more than 20% of the table cells have expected cell counts <5. Another rule of thumb is that if the total number of observations is at least 10, the number categories is at least 3, and the square of the total number of observations is at least 10 times the number of categories, then the Pearson's chi-square approximation should be reasonable. Caution should be made when cell categories are combined (collapsed together) to fix problems of small expected cell frequencies as it may destroy evidence of non-independence[14].

So – when to use Fisher's exact test? According to the common rule of thumb, we should use Fisher's exact test when the Pearson's chi-square test is inappropriate due to small sample sizes and expected counts in the 20% of the table cells are <5 (for the 2x2 table, when the expected value in a cell is <5)[15]. Note that for some statistical software, Fisher's exact test is applied to only 2x2 table; but there are extensions that allow the test to be applied to cases with more than two categories per variable.[5]

As examples shown in figure 4, the decision to report p-value of Pearson's chi-square or Fisher exact test would generally be based on the expected counts in the table cells. In the scenario shown in example 2 representing the association between the exposure (type of ICU admission) and the outcome (vital status), the cell of "elective admission" and "died" contains 2 observed cases but 10.6 expected counts; the p-value of Pearson's chi-square test is thus applicable. In contrast, in the scenario shown in example 3 representing the association between the exposure (CPR prior to ICU admission) and the outcome (vital status), the cell of "having CPR" and "died" contains 7 observed cases but 2.6 expected counts; the p-value of Fisher's exact test is more appropriate.

Example 2:

type of admission	vital status lived	died	Total
elective	51 42.4	2 10.6	53 53.0
emergency	109 117.6	38 29.4	147 147.0
Total	160 160.0	40 40.0	200 200.0

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        Pearson chi2(1) =  11.8663   Pr = 0.001
         Fisher's exact =                 0.000
 1-sided Fisher's exact =                 0.000
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Observed = 2 cases with "elective admission" AND "died"
Expected = (40/200) x (53/200) x 200 = 10.6 cases

Example 3:

cpr prior to icu admission	vital status lived	died	Total
no	154 149.6	33 37.4	187 187.0
yes	6 10.4	7 2.6	13 13.0
Total	160 160.0	40 40.0	200 200.0

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        Pearson chi2(1) =   9.9548   Pr = 0.002
         Fisher's exact =                 0.005
 1-sided Fisher's exact =                 0.005
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Observed = 7 cases with "cpr" AND "died"
Expected = (40/200) x (13/200) x 200 = 2.6 cases

Figure 4. Person's Chi-square test vs. Fisher's Exact Test

It should be noted that the Pearson's chi-square test would be more close to Fisher's exact test as the number of observations increases. As its name implies, Fisher's exact test gives an exact probability for all sample sizes. So, why don't we just use Fisher's exact test for all, and not using Pearson's chi-square at all? This is back to the debatable issue - some statisticians would argue that Fisher's exact test may give the exact answer to the wrong question and the test itself is based on experimental study with the assumption that the row and column totals are fixed, which is not quite fit to many other kinds of study[14].

In fact, there is another controversial idea against Pearson's chi-square test. That is the Yates's correction for continuity (or Yates's chi-square test) which was designed to make the Pearson's chi-square approximation better. However, many argued that it may adjust too far making the p-value too large (too 'conservative') and thus its use is limited. Moreover, with large sample sizes, Yates' correction makes little difference. Again, there were statisticians who agree and disagree on whether to use Yates's correction[16].

Conclusion

The chi-square test is the most well-known statistics used to test the agreement between observed and expected counts while the probability to reject the null hypothesis is calculated based on the theoretical chi-square distribution. The hot arguments regarding the use and misuse of chi-square tests came from different schools of thought in the assumptions and applications of hypothesis testing[10,11,17]. Despite different approaches, there have also been studies suggesting that Fisher's exact and Pearson's chi-square tests are "asymptotically equivalent" (the statistics term meaning that the two tests are eventually becoming "essentially equal") and a formal similarity also exists in small samples[18]. In fact, Pearson's chi-square test even gave an excellent approximation to the actual Bayesian probability approach except for those with extremely disproportionate marginal frequencies[18]. So – the common practice among researchers to use Pearson's chi-square test or Fisher's exact test is still based main assumption – the sufficient "expected" counts!

Suggested Citation

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