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Population Attributable Fraction of Stroke Risk Factors in Thailand: Utilization of Non-communicable Disease Surveillance Systems

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Abstract

In Thailand, stroke is the third leading cause of death. The objective of this study was to measure the impacts of behavioral risk and underlying disease factors on stroke. The study design was a case-cohort study comparing prevalence of demographic characteristics and risk factors between stroke patients and general population. We obtained data of stroke patients and risk factors in general population of 12 provinces from two non-communicable disease surveillance systems to calculate population-attributable fraction: the national health information system for morbidity and mortality surveillance, and the behavioral risk factor surveillance system. Multiple logistic regression, based on weighted data and adjusted for clustering effect in each province, was carried out. It was found that approximately 41.6% of stroke in Thai population was related to hypertension. From an age group-specific model, among those aged 15-34 years, smoking carried the highest population attributable fraction of stroke, 32.7% (95% CI = 3.9-40.4). This study demonstrates that impact measurement of stroke risk factors could help devise stroke prevention and control strategies to tackle influential factors. In Thailand, hypertension control program should be a priority in middle and old age groups whereas smoking prevention should be emphasized in young people.

Keywords: stroke, Thailand, population attributable fraction, risk factor, behavioral impact

Introduction

Stroke is the major consequence of cerebrovascular disease. It is the second most common cause of mortality worldwide.[1] According to the World Health Organization (WHO), stroke is the second leading cause of death for people above 60 years old, and the fifth leading cause in people aged 15 to 59 years old. It is estimated that about 6.2 million people worldwide die from stroke each year, and one in six people worldwide will have a stroke in their lifetime.[2] In Thailand, stroke is the third leading cause of death. During 2014, the country annual morbidity and mortality from stroke were 352.3 and 38.7 per 100,000 population respectively.[3]

Risk factors of stroke include non-modifiable and modifiable factors. Age is the single most important non-modifiable risk factor whereas multiple risk factors can be changed, treated, or controlled such as high blood pressure, diabetes mellitus, dyslipidemia, cigarette smoking, unhealthy diet and physical inactivity.[4-7] Less well-documented risk factors include alcohol consumption, drug abuse and socioeconomic factors.[8-9] Understanding attributions of various risk factors, including behaviors and underlying diseases, is important to help prioritizing stroke prevention and control strategies.

Non-communicable disease (NCD) surveillance systems in Thailand include morbidity and mortality surveillance system for major NCDs (hypertension, diabetes mellitus, renal diseases, ischemic heart diseases and cerebrovascular diseases) and behavioral risk factor surveillance system (BRFSS). The morbidity and mortality surveillance system uses data from the national health information system (NHIS), an electronic database collecting patients' data from all government hospitals to monitor public health indicators, to determine burden of diseases and epidemiological characteristics of NCD patients.[10] The BRFSS is conducted among Thai population aged 15-75 years old every three years in sentinel provinces following the guideline of United States

Centers for Disease Control and Prevention (US CDC)[11] and STEPwise approach to chronic disease risk factor surveillance (STEPS) of WHO.[12]

Although multiple risk factors of stroke have been documented worldwide including Thailand[13-14], to the best of our knowledge, impacts of those known risk factors on stroke in Thailand have never been assessed quantitatively. The objective of this study was to measure the impacts of behavioral risk and underlying disease factors on stroke using the data of patients from NHIS and general population recruited in BRFSS to calculate population attributable fraction (PAF).

Methods

This study was conducted as a case-cohort study based on the secondary data. The first database was information on stroke patients included in surveillance evaluation of NHIS in 2014 conducted by local surveillance team[15]. The second database was the general population from BRFSS conducted by the Bureau of Non-communicable Diseases, Department of Disease Control in 2015, exploring lifetime behavioral risk factors of NCDs. Since the two databases were anonymous, de-identified and unlinked, the ethical approval was not applied.

The study areas were 12 provinces throughout Thailand where BRFSS was conducted (Figure 1). The study populations were Thai residents aged 15-79 years old from the two surveillance systems.

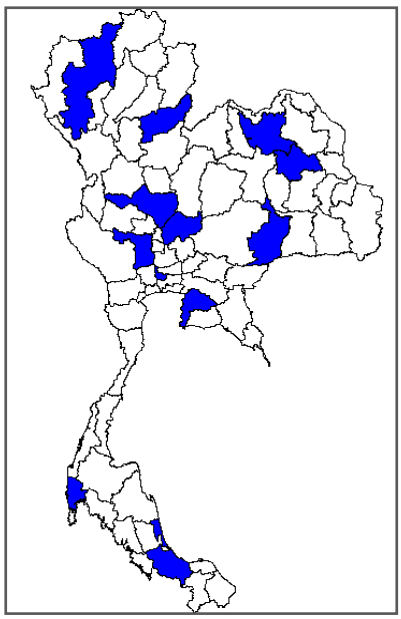

Figure 1. Geographical distribution of 12 provinces in Thailand covered in this study

Stroke patients' data were collected from NHIS by selecting ICD-10 of I60-69. Additional information was also extracted from the findings of surveillance evaluation. Variables included residential province, age, gender, year of stroke diagnosis, history of tobacco consumption and underlying diseases such as hypertension and diabetes mellitus. Information of behavioral risk factors and underlying diseases among general population were obtained from BRFSS. Tobacco smokers were defined as persons who had history of regular smoking documented in NHIS or reported smoking at least 100 cigarettes in their lifetime to BRFSS.[16]

Demographic characteristics and prevalence of risk factors were described and compared between stroke patients and general population. Since complex survey designs with different sampling fractions were used in the original databases of the two surveillance systems, weighted prevalence calculation using size of stroke patients and total population was performed to obtain overall estimates of 12 provinces. In bi-variate analysis, chi-square test was used for comparing patients and population characteristics. In multivariable analysis, multiple logistic regression based on the weighted data and adjusted for clustering effect in each province was carried out to determine exposure odds ratios (OR) and PAFs, including 95% confidence intervals. PAFs were calculated using the following formula:[17]

$$PAF = (\text{Exposure prevalence among cases}) \times (RR - 1)/RR$$

where RR is risk ratio. Since OR from the case-cohort design could be used to estimate RR,[18] RR in the formula was replaced with OR when calculating PAFs in this study.

Overall, age group-specific multivariable models were developed and factors included in the models were known risk factors of stroke based on the previous literature. STATA version 14 was used for all data analyses.

Results

There were 3,041 stroke patients reported in NHIS. About 59.6% were male and 57.2% were 60 years or older. Their demographic characteristics were significantly different from those of general population in BRFSS database, in which 48.3% were male and mostly aged 35-59 years old. Prevalence of hypertension and diabetes mellitus in stroke patients were higher than those among general population while prevalence of hypertension was particularly higher than 50% among stroke patients. Overall, there was no significant difference of tobacco smoking between two groups (Table 1).

Table 1. Characteristics and risk factors of stroke patients and general population in 12 provinces of Thailand, 2014-2015

Characteristic and risk factor	Weighted proportion (Percent)		P-value
	Stroke patients[a] (n = 3,041)	General population[b] (n = 10,752)	
Male	59.6	48.3	< 0.001
Age group (years)			< 0.001
15-34	2.8	35.6	
35-59	40.0	47.8	
60 or more	57.2	16.5	
Underlying diseases			
Hypertension	57.8	16.9	< 0.001
Diabetes mellitus	23.5	8.5	< 0.001
Tobacco smoking	33.2	26.9	0.22

[a] Weighted by number of total stroke patients in the national health information system

[b] Weighted by number of total population 2014, mid-year population statistics 2014, Civil Registration, Ministry of Interior

In case-cohort comparison, multiple logistic regression, which was weighted and adjusted for clustering effects, showed that male and age were baseline risk factors. Males had about 2-fold higher risk of stroke than female. OR of 35-59 years age group and 60 years or more, compared with those 15-34 years old, were 3.4 (95% CI = 2.5-4.6) and 8.5 (95% CI = 6.0-12.1) respectively. For modifiable risk factors, hypertension carried the highest OR, 3.6 (95% CI = 2.6-5.0) whereas tobacco smoking was not identified as a significant risk in the data analysis. The greatest PAF of stroke among Thai population was 41.6% for hypertension (Table 2).

Age group-specific multivariable model estimating PAF of stroke for hypertension, diabetes mellitus and tobacco smoking showed that hypertension carried the highest PAF of stroke among Thai population aged 35-59 years and 60 years or more, 44.0% (95% CI = 39.6-47.1) and 32.4% (95% CI = 17.7-43.1) respectively. While there was no statistically significant association between tobacco smoking and the disease in the overall model (Table 2), different finding was found in the age group-specific model. Among those aged 15-34 years, OR of tobacco smoking were 4.1 (95% CI = 1.1-15.0) (Table 3).

As shown in the figure 2, this significant association led tobacco smoking to being the most influent risk factor with highest PAF of stroke, 32.7% (95% CI = 3.9-40.4) in this youngest age group, instead of hypertension, which PAF was 22.9% (95% CI = 19.8-23.8).

Discussion

Even though risk factors of stroke have been clearly identified in many populations worldwide which help guiding appropriate prevention and control strategies,[19-20] Thailand should determine the impacts of various risk factors on stroke among populations to adapt prevention and control policies that are relevant to the national context especially when

Table 2. Multiple logistic regression, weighted[a] and adjusted for clustering effects[b], of stroke risk factors between patients and general population (case-cohort comparison) and population attributable fraction in 12 provinces of Thailand, 2014-2015

Risk factor	Prevalence among stroke patients (%)	Adjusted OR (95% CI)	PAF percent
Male	59.6	2.04 (1.59-2.65)	-
Age group (years)			
15-34	2.8	reference	-
35-59	40.0	3.38 (2.47-4.61)	-
60 or more	57.2	8.53 (6.01-12.12)	-
Hypertension	57.8	3.56 (2.55-4.97)	41.6
Diabetes mellitus	23.5	1.68 (1.29-2.27)	9.5
Tobacco smoking	33.2	1.26 (0.61-2.60)	6.9

[a] Weighted by number of total stroke patients in the national health information system and number of total population 2014, mid-year population statistics 2014, Civil Registration Section, Ministry of Interior

[b] Adjusted for clustering effect in each province

Table 3. Age group-specific multiple logistic regression, weighted[a] and adjusted for clustering effect[b], of stroke risk factors between patients and general population (case-cohort comparison) and population attributable fraction in 12 provinces of Thailand, 2014-2015

Risk factor	Adjusted odds ratio of age in years (95% CI)		
	15- 34	35- 59	60 or more
Male	0.79 (0.31-2.03)	2.29 (1.62-3.24)	1.82 (1.46-2.29)
Hypertension	16.84 (5.44-52.18)	5.31 (3.73-7.56)	2.13 (1.37-3.29)
Diabetes mellitus	3.77 (0.31-46.23)	1.65 (1.20-2.28)	1.65 (1.15-2.37)
Tobacco smoking	4.14 (1.14-15.04)	1.39 (0.68-2.84)	0.99 (0.42-2.35)

[a] Weighted by number of total stroke patients in the national health information system and number of total population 2014, mid-year population statistics 2014, Civil Registration Section, Ministry of Interior

[b] Adjusted for clustering effect in each province

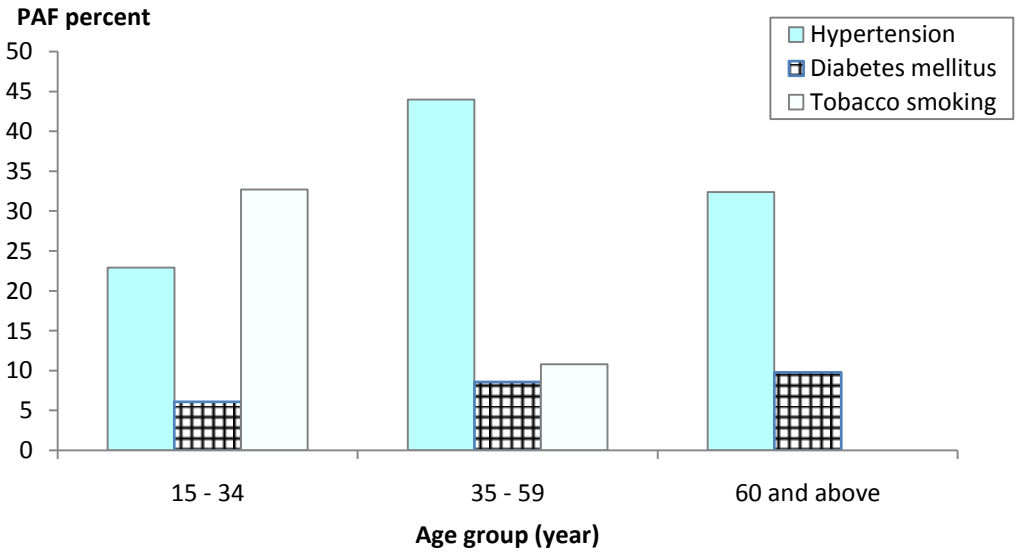

Figure 2. Population-attributable fraction of stroke risk factors by age group in 12 provinces, Thailand, 2014-2015

resources are limited. In our study, male and increasing age were important non-modifiable risk factors. As Thailand is becoming an aging society[21], burden of stroke and its related health care costs would be increasing consequentially. Therefore, stroke primary prevention should be emphasized and directed to appropriate target populations.

Similar to other studies in Asia, Middle East and North Africa,[7,22] hypertension was a major modifiable risk factor of stroke. The prevalence of hypertension among stroke patients was about 3-fold higher than general population. Furthermore, in this case-cohort comparison, hypertension also played a major role to stroke. Approximately 41.6% of stroke in Thai population was related to hypertension and PAF for hypertension was even more prominent in middle-aged group of 35-59 years, 44.0%. Since the prevalence of hypertension in Thailand was 21.4% from the national health examination survey 2009 or more than 13 million of Thai people were living with high blood pressure[23]. Hypertension prevention and control programs should be seriously promoted, particularly in middle-aged group.

While there was no statistically significant association between tobacco smoking and the disease in the overall model, opposite finding was observed in the age group-specific model. Among those aged 15-34 years, smoking carried the highest PAF of stroke, 32.7%. Additionally, the annual incidence of stroke among Thai population aged 15-39 years was about three per 100,000 population.[24] Thus, if Thailand could implement successful smoking prevention and cessation interventions in young people, annual incidence of stroke in the young generation would be significantly reduced.

This study also showed a significant association between diabetes mellitus and stroke. The similar finding was documented in South-East Asia and Western Pacific regions of WHO.[8] However, in age group-specific multivariable model, PAFs were less than 10% for diabetes mellitus in all groups. Effects

of diabetic control could reduce risk of stroke in all age groups similarly.

This study has several limitations. Firstly, since the BRFSS was conducted in purposively selected provinces and we obtained stroke patients data of the same provinces from the NHIS 2014, there might be a concern about statistical representativeness of the national picture. However, 12 selected provinces are geographically distributed throughout Thailand, which could reflect diversity of the disease and its risk factors among regions in the country. Secondly, the use of secondary data from NHIS 2014 could have resulted in selection bias as stroke patients who had not visited any hospitals would not have been captured in the database. Selection bias could also occur among BRFSS samples because the surveillance recruited only people who were alive. Lack of risk factor information among deaths may lead to underestimated prevalence of risk factors in general population. Thirdly, several risk factors such as dyslipidemia, obesity and physical inactivity were not recorded in NHIS properly, so we could not evaluate the prevalence and impact of these factors.

Conclusion

This study demonstrates that measuring the impacts of stroke risk factors can help researchers devise stroke prevention and control strategies to tackle influential factors and pinpoint appropriate populations. In Thailand, hypertension control program should be a priority in middle and old age groups whereas smoking prevention and cessation interventions should be emphasized in young people.

Acknowledgements

The authors thank the staff from Center of Information and Communication Technology, Ministry of Public Health, for providing NHIS data to serve the national NCD surveillance system. We also thank the staff from Bureau of Non-communicable Diseases, Department of Disease Control for supporting BRFSS data. Lastly, we thank public health officers in the Regional Offices of Disease Prevention and Control and the Bureau of Epidemiology, Department of Disease Control for conducting NCD surveillance evaluation to improve quality of NCD surveillance data.

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Prevalence of Blood Parasites Infestation among Buffaloes with Abortion in Nakhon Phanom Province, Thailand

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Abstract

From 2007 to 2011, the buffalo population in Nakhon Phanom Province decreased 34% with approximately 150-300 aborted buffaloes each year. However, the etiology was not systematically examined. Our objectives were to determine prevalence of blood parasites in buffaloes with abortion, and identify causes and possible factors related to abortion. We conducted a case control study in Nakhon Phanom from April 2011 to March 2012. Cases were buffaloes that reported abortion and controls were female buffaloes that previously calved without history of abortion in the same sub-district with the case. Farmers were interviewed, and placenta, aborted fetus, blood, serum, and fecal specimens were collected from both cases and controls. The specimens were tested for blood parasites, brucellosis, and gastrointestinal parasites and hematocrit using Giemsa staining of blood smear and hematocrit centrifugation technique by Woo's method, and identified for blood parasites by microscopy. *Trypanosoma* spp. was the only blood parasite found with the true prevalence of 16% (95% CI =2.6-30.0). We identified risk factors for abortion using logistic regression. Significant risk factors for abortion were high density of tabanus (adjusted OR=12.9, 95% CI=1.2-135.7) and blood parasite (adjusted OR=13.1, 95% CI=1.2-142.9). Reducing the density of the vector such as tabanus might reduce the risk of abortion in buffaloes. In addition, a surveillance system on abortion etiology should be established.

Keywords: blood parasites, buffalo, abortion, Nakhon Phanom

Introduction

Nakhon Phanom is one of the provinces with the highest number of buffaloes in Thailand apart from Ubon Ratchathani, Surin, Buriram, Sisaket and Sakon Nakhon Provinces. From 2007 to 2011, the number of buffalo decreased from 95,331 to 32,529 heads while the overall buffalo population across the country reduced from 1,577,798 to 1,234,179 during the same period.[1] This posed a crucial problem for Thailand where livestock is one of the most important farming commodities.

Buffalo productivity in Thailand is limited due to low pregnancy rate. Estrus detection in Thai buffalo is difficult to observe and thus, effective insemination remains challenging. Furthermore, farmers' preference for female buffaloes resulted in low number of male buffalo. Other factors such as inbreeding, low pregnancy and production rates of calves, including weak or small calves, contribute to the low buffalo production.[2] In addition, abortion is one of the major factors for production loss, leading to low birth rate and less productivity.[3] Economic loss could arise from diagnosis and treatment expense of sick animals, replacing animals, and low milk and meat productivity from animal loss.[4] Due to the facts mentioned above, factors contributing to abortion in buffaloes should be assessed and the etiology should be investigated systematically, especially when the abortion rate was higher than 3% or more animals in a herd aborted when compared with that of the same period from the previous year.[5]

Blood parasites, such as *Trypanosoma evansi*, are one of the factors causing abortion among cattle and buffaloes in Thailand.[6,7] Other causes include bacteria, virus, fungus, chemicals, protozoa such as *Neospora caninum*, *Babesia* spp., *Anaplasma* spp., *Theileria* spp., microfilaria, *Tritrichomonas* spp., and *Toxoplasma* spp.[5,8,9] Other risk factors associated with abortion in cattle and buffaloes were season, parity, farm size, age at pregnancy, malnutrition and parasitic infection during pregnancy.[6,10-12]

Abortion in buffaloes has been a common problem in Nakhon Phanom Province. There were a total of 249 buffaloes with abortion in 2008 while 258 and 149 buffaloes aborted in 2009 and 2010 respectively. Nevertheless, etiology of buffalo abortion was never systematically studied before in Nakhon Phanom. This study aimed to determine the prevalence of blood parasites in buffaloes with a history of abortion, and identify causes and possible factors related to abortion in Nakhon Phanom Province during April 2011 to March 2012.

Methods

A case-control study was conducted in Nakhon Phanom Province from 1 Apr 2011 through 31 Mar 2012. Among 13,341 farmers who raised 64,751 buffaloes in Nakhon Phanom during 2012, 69% were female buffaloes with 30% had history of calving (more than first parity).[1]

A case was a buffalo that reported abortion while a control was a buffalo from other farms in the same village with normal calving and within two months before the matching case abortion date. Controls were selected by simple random sampling. If such buffaloes were not found, buffaloes in an adjacent village in the same sub-district with similar criteria were selected by simple random sampling.

Sample size was calculated by Epi Info version 3.5.1. The case-to-control ratio was 1:2, 312 samples (104 cases and 208 controls) is required to achieve 95% confidence interval (CI) and 80% power. Expected prevalence of blood parasites infection among controls and the studied population was set at 15.5% and 30.0%.[13]

Owners that reported buffalo abortion were interviewed using a questionnaire, including information of farmers, characteristics of farms and potential risk factors for abortion in buffaloes such as rearing and production context of buffaloes, parasitic control, source of feed/pasture and water, history of vaccination, disease outbreak in the farm and history of buffalo abortion in the village.

Age of aborted fetus was estimated through farmer interviewed including size measurement of the fetus[14]. We collected and submitted the fetus and placenta for laboratory testing. Blood, blood smear slide, serum and fecal specimens from both cases and controls were collected, and submitted to the Veterinary Research and Development Center VRDC (Upper Northeastern Region) in Khon Kaen Province for testing blood parasites, brucellosis, gastrointestinal (GI) parasites and hematocrit (Hct). Blood parasites were tested by two methods which included Giemsa staining of blood smear slide and hematocrit centrifugation technique by Woo's method[15]. Blood parasites (*Anaplasma* spp., *Theileria* spp., *Babesia* spp. and *Trypanosoma* spp.) were identified by direct microscopic observation. If any of the methods tested positive, it was considered to have that infection. Rose Bengal test was examined for brucellosis diagnosis while floatation technique and simple sedimentation were carried for GI parasites infestation.

A descriptive analysis was performed using frequency, percent, mean and apparent prevalence. Abortion rate (number of aborted buffaloes/number of pregnant buffaloes) and proportion of each aborted fetus age (number of aborted in each fetus age/total of aborted buffaloes) were calculated. Apparent prevalence was produced from method of the survey data analysis (cluster analysis), accounting for clustering effect of some buffaloes being from the same village. True prevalence was calculated using the following formula to adjust for errors from imperfect sensitivity and specificity of the diagnostic tests.[16]

$$TP = \frac{(AP + Sp - 1)}{(Se + Sp - 1)}$$

$$CI. = TP \pm Z_{\alpha/2} \frac{1}{(Se + Sp - 1)} \sqrt{\frac{AP(1 - AP)}{n}}$$

where TP = true prevalence, AP = apparent prevalence, Se = sensitivity, Sp = specificity, CI = confidence interval (Direct microscopic examination: Se = 50.0[17], Sp = 100[18,19]). For CI wider than 0-1, 0 or 1 was applied as the prevalence will not go beyond 0-1.

Due to hierarchical nature of the data, i.e. buffaloes in the same farm possibly shared several characteristics and therefore, not independent to each other, potential risk factors for abortion were examined using multiple logistic regression. Farm and animal-level factors included as random effects and model intercepts were included. The model also accounted for the matching effect from the way cases and controls were selected. All factors from the univariate analysis with p-value equal to or less than 0.2 with complete information were included in

multivariate analysis. The results of Hct were also compared by t-test.

Results

Epidemiological Characteristics of Buffaloes

Twenty two out of 1,123 villages from nine sub-districts (99 sub-districts in total) in seven districts (12 districts in total) reported buffalo abortion. Phi Man Sub-district in Na Kae District reported the highest abortion rate of 8.4% compared with the number of buffalo that had the first parity and above (Figure 1).

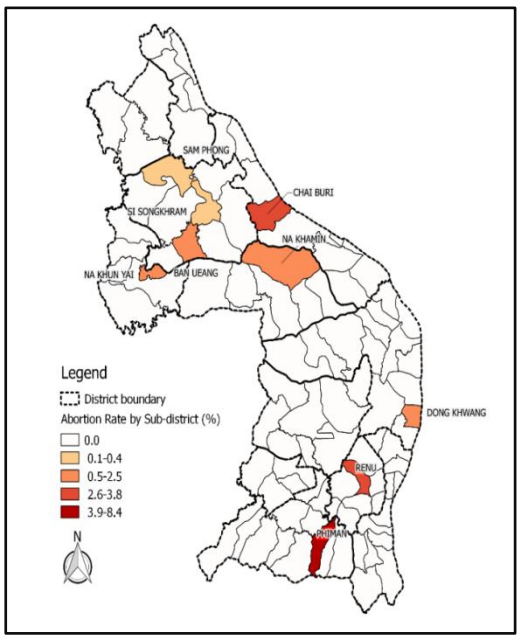

Figure 1. Distribution of buffaloes with abortion reported in sub-districts of Nakhon Phanom Province, Thailand, April 2011 to March 2012

Of total 13,341 farms in Nakhon Phanom Province, 123 farms participated in this study, there were 42 farms with the cases and 81 farms with the controls (Figure 2). Of 42 case farmers, 78.0% were male. Most of them raised the buffalo in a stall outside the house (75.6%) and did not clean the stall regularly (63.4%). In general, farmers did not examine buffaloes for pregnancy and noticed pregnancy by visual observation (Table 1). If a buffalo did not show heat behavior after breeding and was observed with gravid abdomen, it was considered to be pregnant.

A total of 758 buffaloes were raised in the studied farms with the median of four buffaloes per household (range 1-14). Out of 567 (74.8%) female buffaloes, 229 (40.4%) had history of pregnancy. Among them, 88 buffaloes with recent abortion were reported. However, we were able to collect samples from 62 buffalo cases. Median age of the 62 cases and the 118 controls were six years old (range 2-15 years). Ages of buffaloes that bred for the first time were three years (range 2-6 years) for the cases and four years (range 2-5 years) for the controls. Pregnancy rate of the cases and the controls was 33% for first-calf heifers. The majority of female buffaloes (96.7%) were bred naturally. The buffaloes were raised mostly for tracking in the field (58.1%) or were free-range (37.1%) (Table 2).

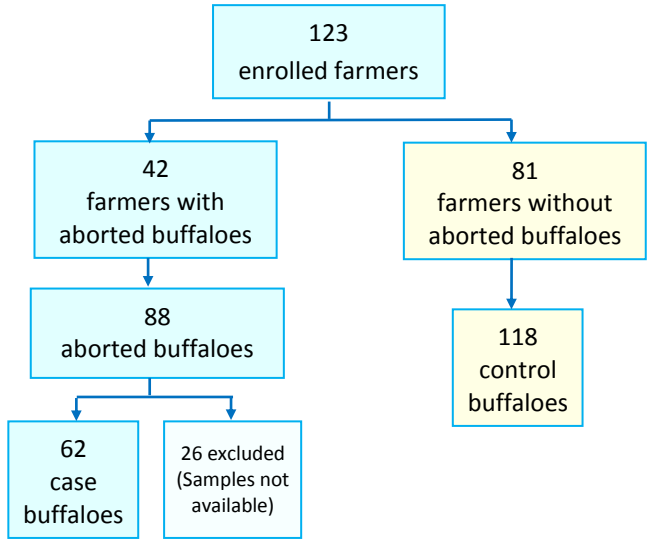

Figure 2. Number of samples collected in this study

Table 1. Characteristics of farmers and buffalo management in Nakhon Phanom Province, Thailand, April 2011 to March 2012 (n=42)

Characteristic	Number of farmer	Percent
Male	32	78.0
Primary school	28	68.3
Raising type		
Tying in the stall	1	2.4
Tying under the long-legged house	2	4.9
Tracking in the field	21	51.2
Free-range	17	41.5
Stall		
None	2	4.9
Outside the house	31	75.6
Under the long-legged house	8	19.5
Clean stall	15	36.6
Food for buffaloes		
Grass	41	100.0
Thatch	38	92.7
Rice bran	2	4.9
Other crops	3	7.3
Supplement foods	0	0
Mixed food	0	0
Deworming in buffalo herd	7	17.1
Vaccination in buffalo herd	15	36.6
Breeding		
Natural	40	97.6
Insemination	1	2.4
Pregnancy examination	1	2.4

Abortion rate of buffalo population in this study was 38.4% (88/229). About 93.5% (58/62) of them were not reported to have other symptoms beyond the observed abortion. Estimated age of the aborted fetus (the maternal gestational age) ranged from 3-10 months. Proportions of abortion in each age group (3-10 months) were 6.9%, 13.8%, 15.5%, 31.1%, 20.7%, 5.2%, 5.2% and 1.7%. Abortion was reported every month, except March 2012, with the highest number of abortion in July 2011 (Figure 3). No samples were collected from September to December as the livestock personnel were occupied by an emergency mission.

Laboratory Results

Blood and fecal specimens were collected from 62 out

Table 2. Characteristics of buffaloes with abortion (cases) and normal delivery (controls) included in this study

Characteristic	Case			Control		
	Median	Range	Percent	Median	Range	Percent
Age in year (n=180)	6	2-15	-	6	2-15	-
Age of the first breeding in year (n=173)	3	2-6	-	4	2-5	-
Chest length in cm (n=62)	186	160-213	-	183.5	173-210	-
Herd size (number of animal)	4	1-14	-	5	2-36	-
Parity	2	1-11	-	2	1-11	-
Gestational age of aborted buffalo in month (n=59)	6	3-10	-	-	-	-
First parity (n=173)	-	-	33.3	-	-	33.9
Age ≥ 10years (n=172)	-	-	11.7	-	-	9.8
Abortion history (n=180)						
Individual buffalo	-	-	6.5	-	-	11.0
Herd	-	-	0.0	-	-	16.1
Adjacent household	-	-	12.9	-	-	10.2
Village	-	-	43.5	-	-	73.7
Natural breeding (n=178)	-	-	96.7	-	-	93.2
Deworming history (n=178)	-	-	5.0	-	-	58.5
Vaccination history (n=176)	-	-	35.0	-	-	81.0
Raising type (n=180)						
Tying in the stall	-	-	1.6	-	-	1.7
Free-range	-	-	37.1	-	-	50.0
Tracking in the field	-	-	58.1	-	-	43.2
Tying under the long-legged house	-	-	3.2	-	-	5.1
Stall						
None (n=179)	-	-	3.2	-	-	0.9
Outside the house (n=179)	-	-	80.6	-	-	82.1
Under the long-legged house (n=177)	-	-	33.9	-	-	49.6

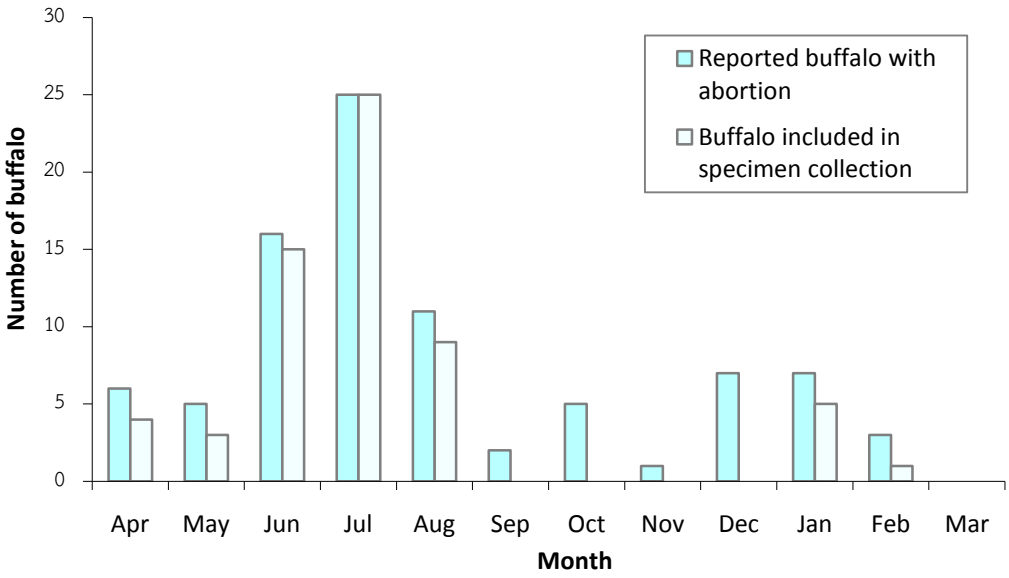

Figure 3. Number of buffaloes reported and included in this study, Nakhon Phanom Province, Thailand, April 2011 to March 2012 (n=175)

of 88 cases and all 118 control buffaloes. Blood parasites were identified in seven out of total 180 specimens tested, with 8% (5/62) from the cases and 2% (2/118) from the controls. No brucellosis and other pathogenic bacteria were detected. Fecal examination revealed GI parasite coccidia and rumen flukes (Table 3). Thirteen specimens of fetus and placenta were collected, and laboratory results were all negative.

Only 155 out of 180 specimens could be tested for Hct as the other specimens were clotted. Cases average Hct was 30.6 (SD = 5.5) and control was 30.9 (SD = 6.0). The difference was not statistically significant. There was no significant difference between four cases and nine controls with low Hct (<24, p-value = 0.8).

Prevalence of Blood Parasites

Two types of blood parasites, *Trypanosoma evansi* and *Trypanosoma theileria*, were identified in seven buffaloes (3.9%, 95% CI = 1.9-7.8). The case apparent prevalence was 8.1% (5/62, 95% CI = 1.1-15.0) and the control was 1.7% (2/118, 95% CI = 0.7-4.1). True prevalence for the cases and the controls were 16.2% (95% CI = 2.0-30.4) and 3.4% (95% CI = 0.0-8.1) respectively.

Risk Factors Related to Abortion of Buffaloes

Out of 10 risk factors considered in the analysis, infection with blood parasites, free-range raising, and prevalence of tabanus and stable flies were included in the multivariate analysis. The risk factors that were likely to be associated with abortion in buffalo included prevalence of tabanus (adjusted OR=12.9, 95% CI=1.2-135.7) and infection with blood parasites (adjusted OR=13.1, 95% CI=1.2-142.9) (Table 4).

Discussion

This study confirmed *T. evansi* infection among buffaloes in Nakhon Phanom Province as it was detected in buffaloes with and without history of abortion. *T. evansi* infection could be detected in many areas of Thailand. The report of VRDC (Upper Northeastern Region) revealed that the infection was identified in every province of the upper northeastern region during 2008-2012, with the highest rate in Nakhon Phanom Province. Beef cattle were infected most, followed by buffalo, dairy cattle and domestic pigs. In Nakhon Phanom, the most common type of infected animal was buffalo.[17] In 2010, the VRDC (Northern Region) reported that 3.9% of buffaloes in the northern Thailand were infected with *T. evansi*.[20] In the previous studies, the seroprevalence of *T. evansi* in beef cattle in Phayao Province was as high as 82.7% and that of dairy cattle in the northern region was higher than 15%.[21] In2007, the prevalence among dairy cattle in the central region was 8.1% while the seroprevalence of *T. evansi* was 15% or more[22]. The report from the southern region in 2003-2005, 0.3% of *T. evansi* infection was found in Nakhon Sri Thammarat Province.[23] These different results might be affected by various laboratory methods used in each study.

Blood parasites infection can cause less efficient animal production, and blood parasites could affect the reproductive system by causing ovarian cyst, ovarian inactivity, delayed puberty and uterine inflammation. In addition, it can interfere the level of progesterone, iron, zinc and selenium. These could be main causes for less reproductive activity[24] as well as reduced fertility rate in buffaloes[25].

Moreover, *T. evansi* infection could suppress the immune system in lymph nodes, spleen and bone marrow,[26] and also have an effect on immune response after vaccination against hemorrhagic septicemia[27,28] and foot and mouth disease[29]. All these facts stated above can reduce the efficiency of production.

Table 3. Laboratory results and prevalence of buffaloes with abortion (cases) and normal delivery (controls) in Nakhon Phanom Province, Thailand, April 2011 to March 2012

Type of infection	Case (n=62)			Control (n=118)		
	Number positive	Apparent prevalence (95% CI)	True prevalence (95% CI)	Number positive	Apparent prevalence (95% CI)	True prevalence (95% CI)
Blood parasites	5	8.1 (1.1-15.0)	16.2 (2.0-30.4)	2	1.7 (0.7-4.1)	3.4 (0-8.1)
T. evansi	4	6.5 (0.2-12.7)	13.0 (0.3-25.7)	2	1.7 (0.7-4.1)	3.4 (0-8.1)
T. theileria	1	1.6 (-1.6-4.8)	3.2 (0-9.5)	0	0	0
Gastrointestional nematodes	1	1.6 (-1.6-4.8)	-	0	0	-
Rumen Fluke	34	54.8 (42.1-67.6)	-	29	24.6 (13.9-35.2)	-
Coccidia	0	0	-	1	0.9 (0.2-4.6)	-

Remark: All the specimens were tested negative for *Fasiola hepatica* and brucellosis.

Table 4. Multi-level logistic regression on possible risk factors for abortion in buffaloes, Nakhon Phanom Province, Thailand, April 2011 to March 2012 (n=175)

Factor	Case (n=59)		Control (n=116)		Adjusted odds ratio		
	Yes	No	Yes	No	Point	95% CI	P-value
Blood parasites	5	54	2	114	13.1	(1.2-142.9)	0.04
Free-range	23	36	59	57	0.4	(0.05-3.04)	0.37
High *Stomoxys* density	35	24	57	59	0.3	(0.03-2.28)	0.24
High tabanus density	44	15	63	53	12.9	(1.2-135.7)	0.03

The causes of abortion in buffaloes apart from brucellosis are blood and GI parasites, including *Toxoplasma* spp., *Neospora* spp. and bovine viral diarrhea virus[30-32]. Detection of blood parasite was possibly a co-infestation. The actual cause of infection could be confirmed by testing the implicated specimens such as the aborted bovine fetus or placenta.[7] Since this study collected mainly blood and serum, the results may not reflect other causes of abortion. Nevertheless, if there was no infectious agent identified, other non-infectious causes and predisposing risk factors for abortion in buffaloes should be explored.

This study revealed that having vectors such as tabanus or multiple stable flies and parasitic infection in the GI tract could increase the risk of abortion in buffaloes[33]. Furthermore, animals with malnutrition and low immunity could be prone to infections. The gestational complications, not only due to parasitic infection in the GI tract, but also by increasing stress-related hormones in the blood stream.[34] We hypothesized that Hct could be used as an indicator in preliminary screening of animals for the surveillance system. However, different Hct between the cases and the controls in this study was not statistically significant. Moreover, due to the nature of livestock farming and subject selection in this study, we calculated the blood parasites prevalence by survey data analysis (cluster analysis) and used multilevel analysis method to identify risk factors of the abortion. Although we could not conclude that abortion among buffaloes in the study area was caused by blood parasites, the buffaloes with blood parasites had higher risk of abortion than those without blood parasites (13.5 times).

Most specimens from aborted fetus, placenta or blood of the fetus were not available due to delayed reporting from the farmers. The blood specimens with ethylenediaminetetraacetic acid (EDTA) did not reach the laboratory within 24 hours after collection which might detect less parasites in some specimens with low parasite count as storage of specimens in high temperature could increase glucose utilization by parasites. A study suggested that preparing blood smear slides just after blood collection could provide higher yield for parasite detection.[17]

Specimen collection was not able to conduct from September to December 2012 due to limited human resources even though the abortion in buffaloes was reported during that period. This might decrease the sample size and affect the analysis in some aspects.

The other limitation of this study was that diagnosis of blood parasites was carried out by microscopy. Though the method is easy, convenient and inexpensive, it takes some time to conduct the testing which can affect the sensitivity of the testing method. As a result, the studied prevalence might be lower than the reality. Despite that, when occurrence of abortion in buffaloes is higher than usual or if there are sick animals suspected of infection by blood parasites, the method could be used as a preliminary testing, along with calculating true prevalence, to overcome the limitation of the testing and assess the actual situation in the affected area. This could foster better planning on finance, resource, equipment and medical supplies for effective prevention and control. In case of aiming to study the true prevalence, the testing should be carried out together with other methods with high sensitivity such as *T. evansi* antibody and antigen detection by enzyme-linked immunosorbent assay (ELISA)[35], indirect hemagglutination test, complement fixation test, indirect fluorescent antibody test and polymerase chain reaction[36-38].

Recommendations

Findings from this study along with the results from other researches indicated that blood parasitic infection in buffaloes from Nakhon Phanom Province was higher than other provinces in the upper northeastern region. Hence, surveillance on blood parasites among buffaloes must be established. Surveillance and reporting systems on abortion in buffaloes and other animals such as cattle and domestic pigs should be improved to be able to determine the situation rapidly and collect the

appropriate specimens in time for better diagnosis. The testing of blood parasites in buffaloes with abortion should be performed by laboratory methods with higher sensitivity and specificity than the parasitological methods.

In order to reduce abortion risk among buffaloes, the sick buffalo and others in the herd and the nearby herd should be treated with diminazene aceturate (berenil)[17] or quinapyramine group (anticide R) once it is reported[39]. Normally, the drug could be administered for single dose as 3.5-5mg/kg body weight by deep intramuscular injection in neck or hip to reduce the swelling. However, the drug could get rid of all parasites completely in buffaloes that required 5-7 mg/kg body weight. Since the infection in most animals is mild, the animals recover rapidly after single dose treatment.

Furthermore, preventive measures for vectors control such as tabanus and stable flies should be recommended to farmers, and deworming of parasites in the GI tract should be carried out consistently.

Acknowledgement

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Acute Gastroenteritis Outbreak Associated with a Sports Event in Bumthang, Bhutan, 2012

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Abstract

On 6 Sep 2012, a medical officer from Bumthang Hospital notified the Royal Center for Disease Control, Ministry of Health in Thimphu, Bhutan, of an outbreak of gastroenteritis among students following an annual sport event. We investigated this outbreak to verify the diagnosis, identify risk factors and recommend control measures. A case control study was used to assess risk factors and determine the cause of the outbreak. We identified 202 cases among 587 participants, giving an overall attack rate of 34.4%. Cases were three times more likely to have eaten beef curry (odds ratio = 2.9, 95% CI = 1.89-4.31) and twice as likely to have drunk pond water (odds ratio = 2.1, 95% CI = 1.39-3.14) than controls. Four of 32 stool samples were positive for *Campylobacter jejuni*, and one was positive for *Shigella sonnei*. This was the first gastroenteritis outbreak related to a sport event in Bhutan possibly caused by *Campylobacter jejuni* which was associated with the consumption of beef curry.

Keywords: gastroenteritis, risk factor, outbreak, Bhutan

Introduction

Outbreaks related to foodborne gastroenteritis occur in relation to social gatherings of people such as those in institutions, schools, restaurants and military units.[1-2] Most foodborne illnesses are undetected as the illness is self-limiting or the cases are sporadic. Bacteria, parasites and viruses as well as chemical agents may cause these outbreaks.[3,4] Of these agents, *Campylobacter* is recognized as a major cause of bacterial foodborne gastroenteritis among people of all age groups in both developed and developing countries.[3-6]

The most recently reported outbreaks of gastroenteritis in Bhutan were linked to poor sanitation and contaminated water.[7-8] In all these instances, the causative organism was from *Shigella* species. Data on foodborne gastroenteritis outbreaks are limited in this country and only scant information is available on *Campylobacter* infections. As per the surveillance on diarrheal disease for children below five years of age, the Royal Center for Disease Control (RCDC) has identified 1.4% and 1.1% of *Campylobacter* infection in 2015 and 2016 respectively.[9] The RCDC under the Ministry of Health in Thimphu is the only laboratory facility available to identify *Campylobacter*. All stool specimens have to be shipped from distant sentinel hospitals to RCDC to identify enteric pathogens. Here, we report on the investigation of the first institutional outbreak of gastroenteritis among participants of a three-day sport event held in Ura Middle Secondary School (UMSS), Bumthang, Bhutan. An investigation was carried out on 7-15 Sep 2012 to verify and describe the characteristics of the outbreak, identify risk factors, and recommend appropriate prevention and control measures.

Methods

Epidemiologic Investigation

A case-control study was used for this outbreak investigation. A suspected case was defined as a person who participated in the three-day sport event at UMSS, and developed diarrhea or abdominal pain with any of the following symptoms: fever, headache, vomiting or joint pain at any time from 31 Aug to 9

Sep 2012. Controls were students or staff who participated in the sport event at UMSS, yet did not develop the gastroenteritis symptoms. Controls were selected from the same school as the cases by convenient sampling, without using any matching variable to have one control for each case.

Active case findings were obtained by visiting all seven schools in Bumthang District that participated in the sport event. Active cases were sought through personal interview with respective school principal and teachers as well as by visiting every class and asking students about their health status relating to acute gastroenteritis. We interviewed both cases and controls, and collected information on demographic characteristics, clinical information and exposure to various foods and drinks during the sport event.

We asked food handlers about the history of diarrheal disease before the sport event as well as procurement of food, storage and food preparation process prior to and during the event. A list of food and drinks served during the event was obtained from the mess in-charge as well as from the participating students.

Environmental Investigation

Environmental hygiene was assessed by inspecting the hygienic conditions of kitchen, water sources and latrines. Water samples from the piped water and pond in the school were tested for fecal coliform bacteria by Millipore membrane filtration method. All specimens were tested in the Bumthang Hospital by the investigating team from the RCDC, Thimphu.

Laboratory Investigation

Total 70 stool specimens were collected from students, teachers and food handlers during the time of outbreak and when patients visited the hospital. Specimens were processed on modified charcoal cefoperazone deoxycholate agar (mCCDA; HiMedia Laboratories Pvt. Ltd, India), Hektoen enteric agar (HEA; Becton, Dickinson and Company, USA) and Mac-conkey agar (Becton, Dickinson and Company, USA). Except for mCCDA which was incubated in microaerophilic atmosphere, all other media were incubated aerobically at 37^0C. The isolated organisms were identified biochemically using triple sugar iron, indole, lysine decarboxylase and mannitol motility media. Antisera were used for serological typing of *Shigella* species.

Statistical Analysis

Epi Info version 7.2[10] was used for statistical analysis. Descriptive analysis was presented in terms of percentage, mean, standard deviation and graphs. Odds ratios (OR) and 95% confidence intervals (CI) were computed for various food items consumed by cases and controls. Attributable risks for the suspected food items were calculated by subtracting the attack rate of unexposed subjects from the attack rate of exposed subjects.

Results

Descriptive Study

The Ministry of Education, Royal Government of Bhutan organized an annual school-based sport event in UMSS, Bumthang from 31 Aug to 2 Sep 2012. Students from seven different schools in Bumthang District participated in the event. All participants were provided with three meals a day, including hostel facilities during the sports event. Of 587 participants, 202 suspected cases were identified, with an attack rate of 34.4%. The attack rate for boys was higher than that for girls (46.1% vs. 25.4%, p-value <0.01). The attack rate for staff was 10.8%. The overall attack rate was the highest with Wangdichoeling Lower Secondary School (WLSS) (77.8%) followed by UMSS (33.8%) and Geytsa Lower Secondary School (GLSS) (32.7%) (p-value <0.01) (Table 1). Most cases (56.4%) were 10-15 years old. The median age was 16 years for males (range 11-49 years) and 15 years for females (range 12-21 years).

Table 1. Attack rate (AR) by schools of participants who attended a 3-day sport event in Bumthang, Bhutan, 2012 (n=587)

School	Male student			Female student			Staff		
	Total	Sick	AR (%)	Total	Sick	AR (%)	Total	Sick	AR (%)
UMSS	80	39	48.8	50	10	20	15	0	0
GLSS	22	14	63.6	24	1	4.2	3	1	33.3
SKHSS	22	10	45.5	24	2	8.3	1	0	0
CMSS	58	12	20.7	62	11	17.7	5	0	0
WLSS	33	29	87.9	35	26	74.3	4	1	25
JHSS	25	11	44.0	30	7	23.3	4	0	0
THSS	42	15	35.7	43	11	25.6	5	2	40
Total	282	130	46.1	268	68	25.4	37	4	10.8

UMSS = Ura Middle Secondary School
GlSS = Geytsa Lower Secondary School
SKHSS = Sonam Kuenphen Higher Secondary School
CMSS = Chumey Middle Secondary School
WLSS = Wangdichoeling Lower Secondary School
JHSS = Jakar Higher Secondary School
TLSS = Tang Lower Secondary School

No cook or food handler reported symptoms of gastroenteritis before or after the event.

A total of 238 participants consumed beef curry, resulting in 143 cases (60.1%). Out of 181 participants who drank the pond water, 108 (59.7%) of them were cases. These two items also revealed the highest attributable risk, with 25.6% for beef curry and 18.4% for pond water (Table 2).

Onset of the first case was on 31 Aug 2012 (Figure 1). The number of cases increased sharply by 2 Sep and peaked on 3 Sep 2012. The shape of the epidemic curve was also compatible with a common-point source outbreak. The most common symptoms were diarrhea (90%) and abdominal pain (87%), and 35% of the cases reported passing blood in stools. All cases have recovered, without any deaths. The mean duration of illness was 3.6 ± 1.4 days.

Analysis of Suspected Risk Factors

Of 587 participants, 409 persons were included in the case-control study (202 cases and 207 controls). Univariate analysis calculated statistically significant values for consumption of beef curry (OR = 2.9, 95% CI = 1.89-4.31) and for drinking pond-water (OR = 2.1, 95% CI = 1.39-3.14) (Table 3).

Table 1. Attack rate and attributable risk for different food items among participants who attended a 3-day sport event in Bumthang, Bhutan, 2012

Food item	Ate			Did not eat			Attributable risk (%)
	Total	Case	Attack rate (%)	Total	Case	Attack rate (%)	
Beef	238	143	60.1	171	59	34.5	25.6
Pond water	181	108	59.7	228	94	41.2	18.4
Salad	354	182	51.4	55	20	36.4	15.0
Pork	244	125	51.2	165	77	46.7	4.6
Boiled egg	267	140	52.4	142	62	43.7	8.8

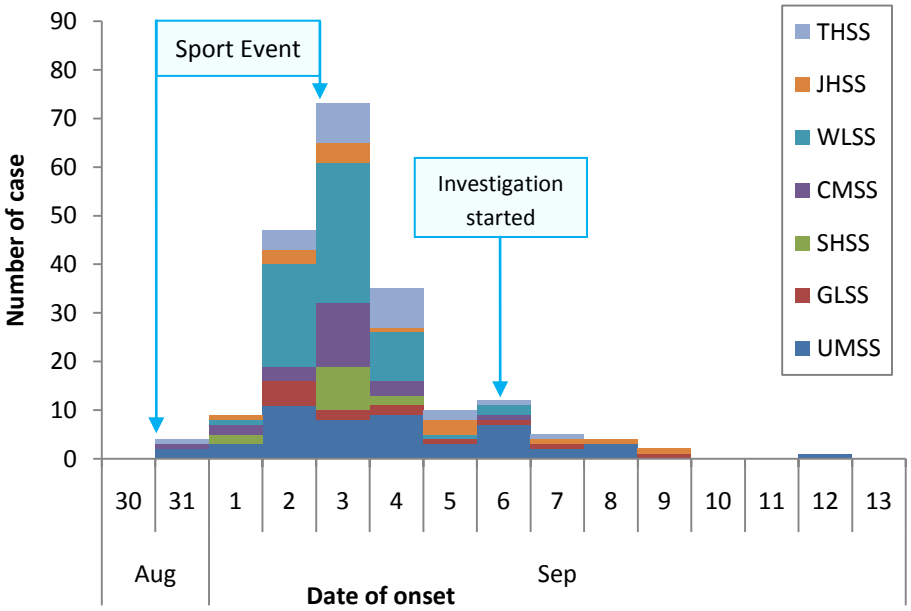

Figure 1. School-wise epidemic curve of acute gastroenteritis outbreak among participants who attended a 3-day sport event in Bumthang, Bhutan, 2012 (n=202)

Table 3. Univariate analysis of suspected risk factors for gastroenteritis among participants in a 3-day sport event in Bumthang, Bhutan, 2012

Food item	Exposed		Non-exposed		Odds ratio	95% CI
	Case	Total	Case	Total		
Beef	143	238	59	171	2.9	1.89-4.31
Pond water	108	181	94	228	2.1	1.39-3.14
Salad	182	354	20	55	1.8	0.90-3.34
Pokora*	164	314	38	95	1.6	0.96-2.61
Pork	125	244	77	165	1.2	0.81-1.82
Boiled egg	140	267	62	142	1.4	0.93-2.15

*Pokora is a popular fast-food item. Cabbage, onion, potato, chilies, salt, wheat powder are mixed and deep-fried in oil.

Environmental Study

Fresh food, such as meat, cheese, vegetables and chilies, were brought in from a bordering Indian town and kept for up to one week on the cement shelves adjacent to the kitchen (Figure 2). Refrigeration was not available, and daily temperatures varied between approximately 12.2-22.3^{0}C. Meals were prepared on a daily basis and were not kept overnight. None of the participants reported bringing foods from outside the school.

Figure 2. Vegetables stored in the kitchen on the cement-shelves at the sport event venue in Bumthang, Bhutan, 2012

The school was supplied with water from an unprotected ground well about half kilometer from the school campus. Domestic animals were grazing around the well. There was intermittent rain during the investigation. Participants drank untreated piped water as well as pond water, which was neither chlorinated nor boiled before consumption.

Availability and accessibility of pond water made students more convenient to drink water directly from the pond rather than drinking boiled water from the kitchen.

Laboratory Study

Laboratory analysis indicated that both piped and pond water were contaminated with fecal coliform bacteria with more than 30 colony-forming units per 100 ml in piped water sample and over 50 colony-forming units per 100 ml of pond water sample.

Out of 70 stool specimen containers distributed to the students, 32 cases provided the specimens for culture. Of those, four (12.5%) were positive for *Campylobacter jejuni* and one (3.1%) was positive for *Shigella sonnei*. One of the positive specimens was obtained from a teacher that tested positive for *Campylobacter jejun*i. Four cooks in the school refused to provide stool samples.

Discussion

The clinical symptoms and abrupt onset of illness among young healthy students suggested that the infection was transmitted through food and water or both, and probably caused by *Campylobacter jejuni*, if it was indeed caused by a single organism[1,3]. However, *Shigella* also causes similar signs and symptoms as *Campylobacter* like diarrhea, abdominal cramps, fever and sometimes bloody diarrhea, and these symptoms may last for a week. The difference is observed with respect to incubation period and mode of transmission of these two organisms: shigellosis is usually manifested after 1-2 days of infection, while campylobacteriosis is manifested within 2-5 days of infection. *Shigella* is transmitted through a direct exposure to fecal matter mainly via contaminated water and contaminated hands, while *Campylobacter* is transmitted by consuming raw or undercooked meat, dairy products and contaminated water[11-12]. *Shigella* species was the only enteric pathogen recovered from outbreaks of gastroenteritis reported in Bhutan[7-8]. Outbreaks with similar symptoms, incubation time and epidemiological curves were reported in Thailand, Taiwan, China and Switzerland, which showed the causative organism was *Shigella sonnei*[13-16]. The clinical symptoms reported by those studies were also consistent with this outbreak. Hence, it was possible to have a mixed infection of *Campylobacter* and *Shigella* in this outbreak.

None of the cases took antibiotics prior to specimen collection, yet delays in specimen processing of the laboratory might have contributed to low recovery of pathogens. Some stool specimens might have been stored overnight in a school at room temperature before submitting for laboratory analysis in the hospital.

The epidemic curve suggested a common-point source outbreak, possibly associated with the consumption of beef. The absence of a cold box or refrigerator for storing the meat could have increased the chances of bacterial growth on beef. The study participants also reported that the beef had not been properly cooked and was difficult to eat. Under-cooked beef had been found to cause gastroenteritis outbreaks during mass feedings in India and Greece.[17] Consumption of under-cooked beef was reported to be associated with an increased risk of campylobacteriosis in France as well.[18] We could not rule out the possibility that contamination of the food was introduced by an infected food handler as their stool specimens were unable to obtain for laboratory testing. No food items were available for testing as every food item was discarded on the day of the event.

Another possible risk factor could have been the pond located near the girls' hostel at the school. Although this pond water was grossly contaminated with fecal coliform bacteria, it was generally believed to be holy

water. This could have encouraged many visiting students to drink the pond water, and some might have opted to drink the pond water in order to avoid the crowds around tap water outlets.

The presence of coliform bacteria in the water supply indicated that the piped water could also have been a source of infection. Gastroenteritis outbreaks related to unchlorinated drinking water had been reported by other studies conducted in Taiwan, China, Switzerland, Sweden and Finland.[14,15,18-20]

Campylobacter were found in the intestines of many domestic and wild animals, including rodents and a variety of birds, and fecal droppings from such infected animals could introduce *Campylobacter* into water supplies.[21,22] In addition, continuous rainfall could have washed away human or animal feces which contaminated the water. The failure to isolate *Campylobacter* from the water supply might be explained by the technical difficulties in isolating these organisms from water even though the organism has been reported to survive in water at low temperatures for long duration[23]. Therefore, the possibility of transmission of these organisms by drinking water directly from outlet water taps as well as through handling of water could not be overlooked, especially in a setting with poor hygiene and sanitation.

Health education was given to every student, teacher and cook by visiting all schools in Bumthang District about importance of food hygiene and sanitation. The affected students were managed with antibiotics and asked to increase fluid intake in addition to oral rehydration solution provided in the health center. The team also recommended the school management to be more vigilant when food was served at mass gatherings and ensure that foods were cooked properly.

Conclusion

This was the first documented gastroenteritis outbreak related to a sport event at seven schools in Bumthang, Bhutan. This large outbreak which took place in a mass gathering highlighted the importance of food hygiene and water quality in preventing foodborne outbreaks during such events.

Acknowledgements

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Suggested Citation

Tsheten, Darnal JB, Dorji T, Wangchuk S, Zangpo T. Acute gastroenteritis outbreak associated with a sports event in Bumthang, Bhutan, 2012. OSIR. 2017 Mar;10(1):16-21.

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The Grammar of Science: Are You Confident to Say So?

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The Grammar of Science

The Grammar of Science is a book written by Karl Pearson and was first published in 1892.[1] It is the book that was read and had impact on young Albert Einstein in creating many greatest scientific theories. In the first chapter, Pearson wrote about definitions of science while explaining about requirements and inquiries to be scientific in nature. I like one of the Pearson's definitions regarding distinctive features of scientific method - discovery of scientific laws by aid of the "creative imagination" and "self-criticism".[1] Later on, Pearson had a classic quote "Statistics is the grammar of science." What does he mean by the word "Grammar"? I opened up an online Oxford dictionary and one of the definitions of "grammar" is "the basic elements of an area of knowledge or skill"[2]. Thus, this has become the name of this column.

We will take a look at basic elements in doing research covering research methodology, epidemiology and statistics. There are times that we take it for grant, thinking that we know this and that, and then explain it the way that we think it is or should be. But we sometimes forget the origin or even the true definition or meaning of the terms that we use. Several authors will take turn writing up in this column with the expectation to reflect "back to basics" of what have been commonly used among researchers.

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Are you confident to say so?

I would like to start the column with the concept of "confidence" in statistics. I just bought a new book, "A Field Guide to Lies and Statistics"[1] and enjoyed reading it a lot. The author started his chapter one that – because it is about numbers so statistics seems to represent hard facts given to us by nature. But - is it so? The argument is that – it is people who decide what to count, how to go about counting, how to group or analyze the numbers, and how to describe, present and interpret them. So statistics are not facts – they are interpretations! I agree with the author. Back to my first question – how do you interpret the numbers that you see in your study results? In the other word – how confident you are to claim that numbers are the facts in nature?

First of all - Back to basics

When we conduct a research, we do not have to collect data from the entire "population". We simply collect data from "samples" with expectation that they are good representatives of our population of interest and we have enough sample size to estimate the value that could be in that population. We hope that we can generalize or infer the value from samples, so-called "statistics", to the value in the population, so-called "parameter". That is why the statistics that we learned is called "Inferential Statistics". (Additional note: We usually use Greek symbol for "parameter" like μ σ ρ π to represent value that we never know (because we hardly or never collect data from the whole population) and we use English symbol for "statistics" like μ σ ρ π to represent the value that we know (because it comes from the samples that we collect by ourselves)[2-5].

What is "parameter estimation"?

When the researchers want to estimate the value in population from the value that they get from the

samples, this is called "parameter estimation". For example, researchers want to estimate the mean score of quality of life among the patients with cancer stage 3 (μ), they do not have to collect data from all cancer stage 3 patients in the whole world or from all patients in the hospital, but simply collect data from the random or representative samples of the patients at that stage and get the sample statistics as ($\bar{X}$ and SD). Then they can estimate μ from that $\bar{X}$ and SD.

What we usually see as the estimate of the parameter is not only a single value, so-called "point estimate" but also the "interval estimate", also-called the "confidence intervals" (CI) around the value[2-5]. For example, say when analyzing an estimate of mean score for quality of life in a sample of 100 patients with cancer stage 3 we produce a mean result of 30 and SD of 5. From these statistics we can calculate a 95% confidence interval of +/- 1.96 (SE) for the population mean estimate. Our point estimate is 30 and interval estimates presenting as confidence interval is (30-1.96x0.5) to (30+1.96x0.5), or we can say that the confidence interval is (29.02 to 30.98)[6-8].

So - What is a "confidence interval"?

A confidence interval or CI is defined as a range of values that describes the uncertainty surrounding an estimate[6-8]. In the "Biostatistics for Dummies"[9] defines it in simple words informally that a CI indicates a range of values that's likely to encompass the true value in population; and a more formally as a specified chance of surrounding (or "containing") the value of the corresponding population parameter. The interval represents by two numbers as lower and upper bounds or limits of the confidence interval; sometimes they are written as CI_L and CI_U, respectively.

It should be noted that the confidence interval itself is also an estimate from the samples in our study as it depends on how we do sampling, measuring, and modeling the numbers that we collected. It could be said that confidence interval is the uncertainty between the true value of what we are estimating and our estimate of that value[6].

How do we calculate confidence interval?

The most commonly used term in research report is "95% Confidence Interval" or "95% CI". In fact, you can see that 95% CI is reported along with different parameter estimates, say 95% CI for mean, proportion, relative risk (RR), odds ratio (OR) and several others. (Note that there might be some studies reporting other level of CI such as 90% CI or 99% CI.) In general, we can interpret 95% CI around any estimate somewhat the same way. But let's look into basic concept from the 95% CI of mean as an example.

When we conduct a study to estimate mean in population (μ), we draw a sample and calculate $\bar{X}$ and SD. What we get are only values from that sample. The question is - will the value that we get from that sample be the value in population? It may or may be not, and most likely maybe not. Now assume that if we can repeat the study again and again, we will get several samples from the same population and get several $\bar{X}$s and SDs. The distribution of different $\bar{X}$s is called sampling distribution as the scatter of $\bar{X}$s is due to sampling that we keep repeatedly doing it. Thus, we can calculate the "mean of the means" (mean of $\bar{X}$s = $\bar{\bar{x}}$). The $\bar{\bar{x}}$ could be said as the estimate of μ. The distribution of $\bar{X}$s around the μ (or $\bar{\bar{x}}$) is thus called "standard error" (SE). But in real life, we never conduct the study again and again, so we simply say that the estimated μ is the $\bar{X}$ that we get from our one time sample. And the SE is also estimated from the "standard deviation" (SD) that we get from that sample as relative to the sample size (n). The simple formula in this case is: $SE_{\bar{X}} = \frac{SD}{\sqrt{n}}$. Based on the concept of area under normal cure, the cut offs for the middle 95% area under curve is +1.96 (we may revisit this concept of area under curve at some other time). Thus, the 95% CI of the mean estimate is usually reported around $\bar{X}$ + 1.96*SE. As shown in Figure 1 - an example of the estimate of mean[2-5].

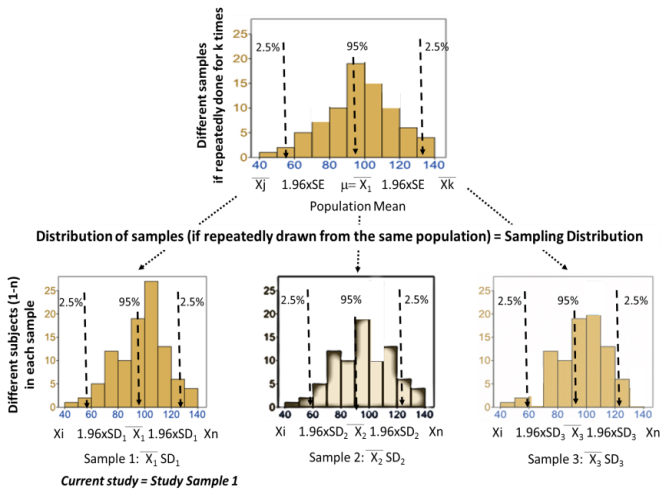

Figure 1. Estimation of μ in population from the $\bar{X}$ and SD of a sample

Similarly, we can calculate SE for different other statistics. For example, to estimate proportion of HIV infection among teenagers (π), the researchers collect data among a sample and get a proportion (p). Then estimate π from p; and they will have to estimate SE of p from the formula $SE_{(P)} = \sqrt{p(1-p)/n}$ and then report the 95% CI of the proportion estimate around p ± 1.96*SE[10].

Estimation of other statistics which is not a single parameter estimate also follows the same algorithm. For example in the estimate of confidence interval for the difference in means (μ1-μ2) from two independent samples, the CI of the difference could be $(\bar{x}_1-\bar{x}_2) \pm z\, S_p \sqrt{\frac{1}{n_1}+\frac{1}{n_2}}$ where z is the confidence level desired (it does not have to be fixed at 95% or 1.96) and Sp is the pooled estimate of the common standard deviation, $S_p=\sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}}$. Another example, in estimating a risk ratio (RR) or prevalence ratio (PR) from two independent samples, RR = p_1/p_2, the CI for RR could be calculated as $Ln\left(\widehat{RR}\right) \pm z\sqrt{\frac{(n_1-x_1)/x_1}{n_1}+\frac{(n_2-x_2)/x_2}{n_2}}$ and then antilog or take exp[lower limit of Ln (RR)] and exp[upper limit of Ln (RR)] to get the CI_L and CI_U for RR. Similarly, the CI for an odds ratio (OR) can be calculated from $Ln(OR) \pm z\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$. Note that these are formulas for larger samples[11-12].

How do we interpret a confidence interval?

The true value for the population does exist and it is a fixed number, but we just do not know exactly what it is. Although we may conduct a perfect study collecting data from the samples that are well (or even perfect) representatives of the population; the very good estimate of the value in the population that we get from our sample may not be the exact value of the population parameter[11-13]. However, we want to be somewhat certain about the value that we get from our sample so that we can say or make inference about the population value. That is, CI allows us to say what the true value in population could be[13]. In other words, we may simply explain that if we can repeat the studies many times, 95% percent of the CIs would contain the true population mean[14-16]. As shown in figure 2, the true value in population, μ does exist but we do not know; however, if we repeated the studies in the same population again and again 100 (or 20 in figure 2) times, our 95% confidence interval generated from each sample will cover μ in 95 studies (95/100 or 19/20) but we may miss that true value for about 5 times (5/100 or 1/20)[11,15,16].

Back to the example of the estimation of mean score for quality of life in patients with cancer stage 3, suppose the true μ is 29.67; and from a sample of 100 patients with cancer stage 3 we have got a mean result of 30 and SD of 5. For these estimates we can calculate a 95% CI as: (30-1.96*0.5) to (30+1.96*0.5), or we can say that the 95% CI is (29.01 to 30.99).That would mean this range of 95%CI does cover the true μ of 29.67.And if we repeat the studies again 100 times, 95% of the times the ranges would still cover 29.67. The interpretation of a 95% CI as indicating a range within which we can be 95% certain that the true population parameter lies is a loose interpretation, but is useful as a rough guide[17]. The strictly-correct interpretation of a CI is based on the hypothetical notion of considering the results that would be obtained if the study were repeated many times; and if a study were repeated infinitely often, and on each occasion a 95% CI calculated, then 95% of these intervals would contain the true value in population [8,14,17].

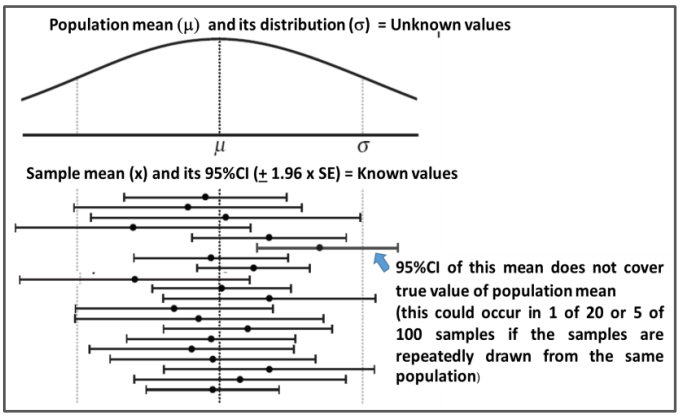

Figure 2. Estimation of population mean with 95% confidence

Confidence interval and p-value

When the study compares outcomes of different groups, the report could be presented with an estimate of the difference (say mean difference, risk difference, risk ratio, odds ratio, hazard ratio) and its CI along with p-value. Some studies, however, skip CI or p-value. In fact, there is logical correspondence between the CI and the p-value. In general, the 95% CI for the estimate will exclude the null value (i.e., null for RR, OR or HR is 1.0; and null for mean difference or risk difference is 0) if and only if the test of significance yields a p-value < 0.05; and either the upper or lower limit of the 95% CI will be at the null value if the p-value is exactly 0.05[15,17,18], given that the 95% CI and p-value are both calculated from the same method.

Back to our example in an estimation of risk ratio between teenagers and adults in getting infection with HIV, suppose the RR=3.2 and the 95% CI is shown as (0.8 to 5.4); that would mean the true risk ratio between the populations of teenagers vs. adults might not be 3.2 but could be somewhere in this range of 0.8 to 5.4. Since 95% CI includes 1, we will also see that p-value >0.05; thus we cannot conclude that there is a statistically significant risk difference between the two groups. If you want to interpret from the 95% CI without looking at the p-value (which the researcher may decide not to present), we could still say that the risk ratio is not absolute and not

significant. In our sample we found that the teenagers have higher risk than adults (3.2 vs. 1) but the estimates of the true risk ratio in population could be that the teenagers have lower risk (0.8 vs. 1) or they may have even higher risk (5.4 vs. 1). In contrast, suppose the results from the same study show the estimate of RR=3.2 and 95% CI (1.9 to 4.5). Since 95% CIexcludes 1, we will also see that p-value <0.05; thus we can conclude that there is a statistically significant risk difference between the two groups. If you want to interpret from the 95% CI without looking at the p-value, we could say that the risk ratio is absolutely shown in one direction. In our sample we found that the teenagers have higher risk than adults (3.2 vs.1) and the estimates of the true risk ratio in populations of the two groups would always be that the teenagers have higher risk which might be not at (3.2 vs. 1) but could be as low as (1.9 vs. 1) or as high as (4.5 vs. 1).

What is "good" or "not good" CI estimates?

CI could technically tell us how "good" an estimate is; it is an important reminder of the limitations of the estimates such that the larger a CI for a particular estimate, the more caution is required when using the estimate.[6,7,19] As CI represents margin of error (or the width of the interval), a larger margin of error (wider interval) is indicative of a less precise estimate[12,15,19]. As an example, in an estimation of risk ratio between teenagers and adults in getting infection with HIV, suppose the RR=3.2 (i.e., teenagers are more likely to get infected 3.2 times than adults) and the 95% CI is shown as (1.5 to 60.7); that would mean the true risk ratio in the populations of teenagers and adults might not be 3.2 but could be somewhere in this range which is so wide.

The width of the CI of a study is usually related to the sample size; study with large sample size tends to give more precise estimates (or narrow CI)[13,17,19]. For the estimate of continuous variable, the CI might depend on the variability (or SD); but for the estimate of dichotomous variable, it depends on the chance (or proportion) of the event that could occur; and for the estimate of time-to-event outcome, it depends on the number of events observed[17].When the CI is wide, there are a number of methods we can use to reduce it. In attempt to improve the precision of our results (having narrower CI), we could increase our sample size (if possible),[5,8,11,13]. However, as larger sample sizes would result in narrow CI, but if you increase the sample size to a certain number then it won't help that much anymore. As shown in one reference, increasing the sample size from 100 to 500 reduces the CI from 9.8 to 4.3, but when sample size is 1,000, the CI will reduce down to only to 3.1 which may not worth doing it, comparing to what you have to collect the data from 1,000 rather than 500 subjects[13].

In the study that compares the outcomes between groups, when the estimates come with a wide CI, it may not be that the sample size is too small but it may indicate that the underlying data are disparate, including too few events occurring in one group or another or both, or too many outliers and oddball data points[20]. For example, in an estimation of risk ratio between teenagers and adults in getting infection with HIV, suppose the RR=3.2 and the 95% CI is shown as (1.5 to 132.6); that would mean the true risk ratio between the populations of the two groups could be somewhere in this wide range. If this is the case, the researcher should not emphasize this statistically significant result that much even though we may have a large enough sample size in total but it might be that we have too few subjects in one group or another, or there might be too few infection incidences relative to the sample sizes of one of both groups. In fact, when this wide range is shown, the researcher should look back at the descriptive information about the two groups. It may help explain why so.

So, the question then is - how wide is too wide? As a rule of thumb, the researcher should be cautioned to oneself and to the readers of that study results if a CI is wider than the magnitude of the estimate[20]. For example, when you see a RR=3.2 and the 95% CI (1.5-132.6), the width of the CI thus is 131.1 which is too much higher than the size of the RR. But when you have narrower CI, say RR=3.2 and 95% CI(1.9 to 4.5), thus the width of the CI is 2.6 which is a fraction of the size of the RR; then one can be quite confident in the population estimate.

Final words – how confident you are to interpret your estimate(s)?

The confidence interval tells you more than just the possible range around the estimate but it also tells you about how stable the estimate is[21]. A stable estimate means that the value that you claim in your study result section is one that would be close to the true value in population that we never know. Wider CI in relation to the estimate itself indicates instability and less precision of your estimate. One of the nice things about presenting the estimate with 95% CI is that you never have to commit yourself 100% on anything in statistics. Claiming 100% confidence is impossible anyway since we do not conduct the study in the whole population. A classic quote (or joke?) about statistics is that "Statistics mean never having to say you are certain". This is

quite right as you can always claim “I am under the 95% confidence limit”.

Suggested Citation

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