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Use of risk scores for screening of hepatitis C of blood donors in remote areas

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Hepatitis C virus (HCV) infection is considered as an emerging health problem in Myanmar. Seropositivity rates vary from 3% in blood donors at major blood banks in Yangon to over 10% in northeast and northwest border areas. Determination of HCV is limited in remote areas as it is expensive and some test systems need special equipment. To assist in the control of hepatitis C in Myanmar, we developed a simple system for screening of HCV infection using risk scores. The scores were based on the data obtained from HCV surveys carried out at the northeast and northwest border areas of Myanmar. The database consisted of 652 subjects (250 males, 402 females), aged 18 years to 60 years. Multivariate analyses revealed the following factors to be related to HCV infection in the subjects: more than 30 years of age, Odd Ratios (OR)=2.41 (p=0.001); a history of tattooing, OR= 1.78 (p=0.035); a history of hepatitis in the family, OR=1.58 (p=0.049). The screening scores for HCV infection were developed using risk scores. Validity was analyzed using the Receiver Operating Characteristics curve. The sensitivity of the system was 80% and the specificity 37% when a cut-off score of $\geq$2.5 was used. By increasing the cut-off score, higher specificity (up to 80%) could be achieved at the cost of decreasing sensitivity. The developed risk scores could be applied for screening of blood donors for HCV infection in areas where laboratory HCV testing could not be performed.

INTRODUCTION

Hepatitis C virus (HCV) infection is an important health problem in many countries [1]. HCV was shown to be the major causative agent of non-A, non-B hepatitis and that it is associated with blood transfusion. Although the acute infection is usually asymptomatic and may not be recognized clinically, the subsequent chronic infection is usually life-long and may lead to chronic liver disease leading to liver cirrhosis and hepatocellular carcinoma. In fact it had been proven in Japan as a single most important aetiological factor for the development of hepatocellular carcinoma, where the incidence of hepatitis B virus infection is low [2].

HCV is regarded as an emerging health problem in Myanmar. Reports in early 90's demonstrate HCV infection in one third of patients with hepatocellular carcinoma [3], and in 2.5% of apparently healthy subjects [4]. In recent years, the Myanmar-Japan cooperation study group observed that 2 cases were positive for anti-HCV (5.9% positivity rate) among 34 voluntary blood donors [5]. During May 2000 to Oct 2002, a total of 102,632 donors were screened and the overall anti-HCV positivity rate was

found to be 2.84% [6]. The prevalence of antibody to hepatitis C virus (anti-HCV) was found to be 2.8% among 569 subjects (246 males, 323 females), aged 3 months to 74 years, residing at Mayangone Township, Yangon Division [7]. A recent study showed a higher prevalence of HCV among the population in a northeast border town than blood donors and community in Yangon [8].

For the control of HCV infection in Myanmar, screening for HCV infection is essential. We carried out this study to devise a risk screening form for HCV infection for use in remote border areas where HCV testing is not easily available.

MATERIALS AND METHODS

Cross-sectional, community-based studies were carried out during 2002 and 2003 in northeast and northwest border townships of Myanmar. The northeast border (Muse Township) study population comprised of 349 subjects (137 males, 212 females) aged 12 months to 70 years. Tamu Township (northwestern border) survey included 502 persons (aged 1year to 65 years). During the surveys, consecutive samples were collected from subjects residing at the above mentioned areas. Those who refused consent were excluded from the study. A standardized proforma was used to collect biological and sociodemographic data. Clinical and family histories were carefully asked and recorded. Special emphasis on history of jaundice, history of jaundice in family members, history of dental and surgical operations, and blood transfusion history were noted. From each subject, two milliliters of blood was collected under aseptic measures and sera separated. Sera were transported back to the National Blood Research Centre of the Department of Medical Research (LM) and stored at -80ºC till further analyses. Ortho-HCV Ab PA test II (Orth-Clinical Diagnostics, Fujiredbio Inc., Tokyo, Japan) was used for determination of seropositivity to HCV. These two data bases were combined and those with ages qualified for blood donation were further selected as the data base for the present study. The data base consists of 652 subjects (250 males, 402 females), aged 18 years to 60 years residing at Muse and Tamu Townships.

Statistical methods

Data analysis was performed with SPSS (Statistical Package for Social Scientists Ver 10.1; SPSS Corporation, Chicago, IL, U.S.A.) on a IBM computer. Univariate and bivariate tests were carried out to determine differences between groups. Differences were considered significant if $p < 0.05$. The associated factors were further analysed using odds ratio and multiple logistic regression analysis [9].

RESULTS

General characteristics

Of the 652 subjects, 250 were males and 402 were females. Their ages ranged from 18 years to 60 years with a mean (SD) age of 34.53 (10.9) years. Twenty- five percent of the study population (45%) has achieved high school and university status. Bamars constituted 60% of the study population and the remainder was ethnic groups including Shans, Chins, and Kachins.

HCV antibody prevalence

One hundred and four subjects were found to be seropositive to hepatitis C infection (15.9%). Males had a higher prevalence of anti-HCV seropositivity than females (18.8% vs 14.2%; Student's 't' test, $p=0.12$). No significant difference in age was found between males and females (35.36 ± 11.5 vs 34.02 ± 10.4, $p=0.13$). The anti-HCV seropositivity rate significantly increased with increasing age group. It was found to be the lowest (9.4%) in 18-20 years age group and highest (24.1%) in the 51-60 years age group. Anti HCV seropositivity in 21-30 years, 31-40 years and 41-50 year age groups were found to be 10%, 18.3% and 21.1% respectively.

Associated risk factors for anti-HCV seropositivity

After univariate analysis, it was found that the significant associated factors among the population were: (a) age 30 years and above, (b) presence of tattoos, (c) history of jaundice in the family.

Table 1. Associated factors for anti -HCV sero-positivity by univariate analysis

Associated factors	Anti-HCV seropositivity			Remarks
	No. of tested	No. of positive	Percentage	
Gender				
Male	250	47	18.6	p=0.117
Female	402	57	14.2	
Education group				
Primary & below	271	49	18.1	p=0.418
Secondary & above	327	51	15.6	
Marital status				
Never	265	38	14.3	p=0.339
Ever	367	63	17.2	
Family size				
6 members & below	432	67	15.5	p=0.614
More than 6 members	217	37	17.1	
History of liver diseases				
Yes	139	26	18.7	p=0.317
No	513	78	15.2	
History of transfusion				
Yes	52	8	15.4	p=0.907
No	600	96	16	
History of tooth extraction				
Yes	271	41	15.3	p=0.619
No	380	63	16.6	
History of surgery				
Yes	210	33	15.7	p=0.891
No	440	71	16.1	
History of ear piercing				
Yes	302	45	14.9	p=0.486
No	349	59	16.9	
History of tattooing				
Yes	101	23	22.7	p=0.043
No	550	81	14.7	
History of hepatitis in the family				
Yes	176	35	19.9	p=0.047
No	475	69	14.5	
Age 30 years & above				
Yes	388	78	20.1	p=0.001
No	264	26	9.8	

There was no significant association with gender, education, marital status, and size of the family, history of liver diseases, and history of transfusion, history of tooth extraction, history of surgery, and history of ear piercing (Table 1).

Multiple logistic regression was applied for controlling confounders and for evaluating the effects of associated factors on HCV infection in the studied group. After analysis, 3 variables: age of more than 30 years, Odd Ratios (OR) =2.41 (p=0.001); a history of tattooing, OR= 1.78 (p=0.035); a history of hepatitis in the family, OR=1.58 (p=0.049) were found to have effect on HCV infection (Table 2).

Table 2. Associated factors for anti-HCV sero-positivity among the studied population by multivariate analysis

Associated factors	Adjusted OR	95% CI of OR	p value
Age 30 years & above			
Yes	2.41	1.49 - 3.90	p=0.001
No	1		
History of tattooing			
Yes	1.78	1.05 - 3.03	p=0.033
No	1		
History of hepatitis in the family			
Yes	1.58	1.00 – 2.51	p=0.049
No	1		

Development of a simple risk screening form for screening of HCV infection using risk scores

The risk screening form for HCV infection was developed by using scores from Table 2 as follows: risk score = scores of age 30 years and above plus a history of tattooing plus history of hepatitis in the family. Score of age 30 years and above = 2.5, score of a history of tattooing = 2, score of family history of hepatitis = 1.5. The calculation of risk scores was analyzed and the validity of this model for predicting the risk of HCV infection was calculated by Receiver Operating Characteristics curve (ROC curve). The

sensitivity of this model was 82% and the specificity was 39% when the cut-off score of ≥ 2. If the cut-off score was increased to ≥ 4, the specificity increased to 80% but the sensitivity was greatly reduced to 40%. The optimal cut-off score was determined by the ROC curve (Fig. 1) and was found to be ≥ 2.5, with a sensitivity of 80% and a specificity of 37%. A risk screening form for HCV infection in population aged 18 to 60 years is proposed in Fig. 2.

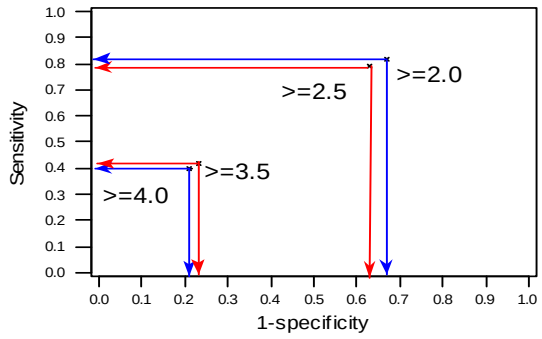

Fig. 1. ROC curve for prediction of HCV infection

Risk screening form for HCV infection among blood donors in remote areas (*where HCV screening is not possible*)			
Name		Age	Years
Gender Male/Female		Marital status	
Residence			
Risk factors	Status	Full scores	Check list scores
Age 30 years & above	Yes	2.5	
	No	0	
History of tattooing	Yes	2	
	No	0	
History of hepatitis in the family	Yes	1.5	
	No	0	
	Total	6	
Interpretation	Total check list scores ≥ 2.5 indicates the risk for HCV infection at the sensitivity of 80%. Please do not allow blood transfusion unless confirmed to be negative by HCV serological test.		

Fig. 2. Risk screening form for HCV screening among blood donors in remote areas

DISCUSSION

The problem of hepatitis C infection in Myanmar is well recognized and efforts to control it in the blood donor population have been initiated since the year 2000. With the support of Japan International Cooperation Agency under the Control of hepatitis C in Myanmar project, hepatitis C screening of 154161 blood donors during May 2000 to April 2004 had demonstrated the prevalence of HCV infection to be 2.6% in Myanmar blood donors [10]. Data from Europe demonstrated lower than 1% HCV seropositivity in blood donors [2], and 1-2% of blood donors were found to be HCV seropositive in the Far East [11]. However, high rates of HCV infection had been reported with 4% of the blood donors being positive for anti-HCV antibodies in Egypt with higher prevalence rate of 15% in the rural areas of the country [12].

We have studied the HCV prevalence among 349 subjects (137 males, 212 females) aged 12 months to 70 years residing at the Muse Township, Northern Shan State. The overall prevalence of anti-HCV postivity was found to be 13.5% [13]. In northwestern border towns, Tamu and Kalay, a field survey carried out during 2003 revealed that 12.7% of the study population of 502 persons (aged 1 year to 65 years) to be anti-HCV seropositive [14]. Although HCV prevalence surveys could not be considered to be representative of the population at large, it could be concluded that significant higher rates of HCV infection exists among apparently healthy populations residing in border areas.

A study carried out in Yangon blood donors had also outlined similar associated factors. Higher prevalence of anti-HCV positivity was found in those with history of surgical operation, tooth extraction, ear piercing and tattooing. The seropositivity increases with age. In addition, education levels and previous history of blood transfusion had been regarded as possible associated factors [7, 15].

HCV screening tests are expensive and are not easily available in all parts of Myanmar, especially remote areas. The developed risk

screening form will be very helpful in such situations. The screening form is cost free and could be easily used by a medical person, in charge of blood collection. The facts are also in consistent with those mentioned in the donor self deferral form but the proposed form is very simple and could be handled by a person with a little medical knowledge.

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

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

INTRODUCTION

Nowadays, a lot of attention has been directed towards avian influenza. However, we should keep in mind that outbreaks caused by the human influenza subtypes can occur and are occurring. Human influenza can cause repeated infections throughout the life of an individual. It occurs worldwide and outbreaks happen every year; pandemics are usually followed by annual epidemics or sporadic outbreaks. Influenza is usually a seasonal winter illness in temperate climates, but it is less seasonal in tropics. Since 1977, there have been three families of influenza circulating (and are varying by antigenic drift) in the human population. They are influenza A (H1N1), influenza A (H3N2) and influenza B. Avian influenza, at present wreaking havoc among birds and humans, is caused by influenza type A, subtype H5N1.

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

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

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

MATERIALS AND METHODS

Area of outbreak

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

Clinical cases

Before the arrival of the investigation team, a medical officer screened the children from the military family quarters and admitted 13 children who had fever and or respiratory symptoms to the 300-bedded No. 3 Military Hospital, Kyaingtone. Clinical cases admitted were examined clinically. Blood samples were collected from all 13 patients and nasopharyngeal swabs were taken from 5 children with respiratory symptoms. A lumbar puncture was done on one child showing neurological symptoms. Nasopharyngeal swabs were also collected from 4 Wanyan Village children who were neighbors of two clinical cases and were showing respiratory symptoms. One of these children from the Wanyan Village was also admitted to the Eastern Shan State General Hospital and a blood sample was also collected from him. Clinical examination and collection of clinical samples were performed by the clinicians and the microbiologist using protective personnel equipment (PPE) like, caps, goggles, masks, gown, apron and boots followed by appropriate disposal of PPE and proper hand washing. The case charts of the three fatal cases were reviewed and discussed with the attending medical officers.

Animals

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

Laboratory investigations

Malaria Rapid Test

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

Dengue Rapid Test

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

Japanese encephalitis (JE) IgM EIA

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

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

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

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

Influenza A Rapid Test

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

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

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

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

Nasopharyngeal swab samples positive for influenza A by the rapid test were tested for influenza A (H1), (H3) and (H5) antigen by the indirect immunofluorescent test utilizing subtype-specific monoclonal antibodies [5]. Briefly, cell deposits from centrifuged nasopharyngeal samples were spotted onto glass slides and fixed with chilled acetone. The fixed cells were incubated with influenza A (H1), (H3) and (H5) subtype-specific monoclonal antibodies. The slides were then washed and anti-mouse antibody conjugated to fluorescene isothiocyanate was added and incubated. The slides were washed, dried and viewed under a fluorescent microscope. Observation of intracellular fluorescence denotes the presence of the corresponding subtype-specific influenza A antigen in the clinical samples.

Rapid Latex Agglutination Test for meningitis

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

Confirmation at WHO National Influenza Centre

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

Epidemiological investigations

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

Control measures

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

Health education

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

RESULTS

Fatal cases (Table 1)

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

Of the 13 children that were later admitted to the hospital, a seven-year-old girl died after the arrival of the investigation team. She had fever and respiratory symptoms and then she became hypotensive and gradually went into stupor and coma. Despite intensive management, she expired on the next day of admission. Blood and nasopharyngeal samples were collected from her and a postmortem examination was also performed. The blood sample was negative for malaria, dengue and JE. The nasopharyngeal sample was positive for influenza A by the rapid near-patient test and confirmed to be influenza A (H1N1) by the WHO National Influenza Centre. Post mortem revealed massive cerebral oedema with normal meninges.

Recovered cases (Table 2)

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

Wanyan Village children

Two of the 4 nasopharyngeal samples collected from Wanyan Village children

Table 1. Case summaries of fatal cases

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

ND = not done Pos = positive Neg = negative

Table 2. Case summaries of recovered cases

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

ND = not done Pos = positive Neg = negative

Table 3. Case summaries from Wan Yan Village children

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

ND = not done Pos = positive Neg = negative

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

Attack rates

The age specific attack rates were:

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

Epidemiological investigation

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

DISCUSSION

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

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

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

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

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

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

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Molecular detection of primary dapsone resistant *Mycobacterium leprae* in Myanmar

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Currently recommended control measures for treating leprosy with multi-drug therapy (MDT) should control the spread of drug resistant strains; however, dapsone (DDS) resistance continues to be reported. Comprehensive estimates of drug-resistant leprosy are difficult to obtain due to the cumbersome nature of the conventional drug susceptibility testing method using mouse footpad inoculation, which requires at least 6 months to obtain results. Recently, it has been determined that DDS resistant strain contains missing mutations in codon 53 and 55 of the *fol* P1 gene of *Mycobacterium leprae*, and definitive evidence linking these mutations with DDS resistance in *M. leprae* has been obtained. Based on these mutations, *fol* P1 gene contained these mutation points (hot spots) was amplified by polymerase chain reaction (PCR) and followed by sequencing to detect mutations. A total of 50 multibacillry leprosy cases before MDT treatment attending Central Special Skin Clinic, Yangon General Hospital were determined *fol* P1 gene mutations. DDS resistance was detected in four cases (8%). This study is part of the detection of multi-drug resistant leprosy in Myanmar.

INTRODUCTION

Primary dapsone (DDS) resistance arises by the infection of new persons with resistant bacteria often shed by a person with acquired resistance to DDS. Primary DDS resistance is very prevalent in many leprosy endemic areas of the world and represents a serious potential threat to the leprosy control programs in these regions [1, 2].

During the late 1960 and 1970, there were alarming reports of increasing secondary and primary DDS resistance at the end of DDS mono-therapy era, which prompted the World Health Organization (WHO) to recommend the introduction of MDT, using DDS, rifampicin and clofazimine [3], which should control the spread of drug resistant strains. DDS remains the first line drug for the treatment of leprosy as well as key component in the chemotherapeutic regimens of MDT. However, DDS resistant strains of *M. leprae* continue to be reported even in areas of world with successful implementation of MDT [4, 5, 6]. Although DDS has a weaker bactericidal action than rifampicin, we need to prevent the development of resistance to any component drug in MDT schedules. One could image a dangerous scenario developing in which high level primary DDS resistance and poor clofazimine compliance leads effectively to rifampicin monotherapy, a condition in which it is already known that rifampicin resistance in leprosy can develop [7].

In Myanmar, DDS mono-therapy was introduced in 1952 and MDT was introduced in 1986, although the coverage of MDT reached 100% a decade later (1996). Since 1980, Mar Mar Nyein *et al.* have been testing DDS resistance using the mouse footpad model [8]. DDS resistance

prevalence survey was done in Myingyan area in 1980 and 1983. Out of 779 lepromatous patients who had been treated with DDS mono-therapy for more than 5 years (90% of them had been treated for more than 10 years), 301 out of 771 (38.6%) were found to have DDS resistant leprosy and annual incidence in the 2 subsequent years was 40 to 45 per 1000 lepromatous patients or 3.8% per year [8].

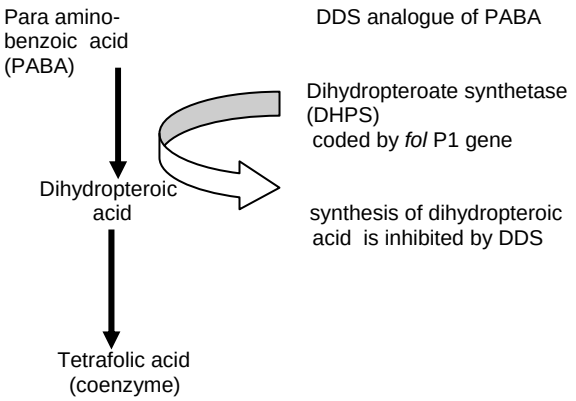
The genetic basis of DDS resistance has been described [9]. Although genetic detection of drug resistance is technically sophisticated, the application of technology in selected leprosy endemic areas will allow a more rapid and widespread assessment of the problem. The gene method was first validated by paralleled mouse footpad experiments in order to be sure what level of DDS resistance was being detected. The results of recent studies on the molecular mechanism of DDS resistance in *M. leprae* demonstrated that the target of DDS is the enzyme dihydropteroate synthetase (DHPS). DDS resistance appears to be the results of mutations in the *fol* P1 gene encoding for DHPS in *M. leprae* [6]. Although many DDS resistant cases have been detected in Myanmar [8], no study has examined the molecular characteristics of the DDS resistant strains or the status of DDS resistance in the country. The aim of the present study, based on PCR and a direct DNA sequencing assay, was to determine the prevalence of *fol*P1 mutations in *M. leprae* isolates from clinical cases of DDS resistant leprosy in Myanmar.

Molecular biological detection of dapsone resistant Mycobacterium leprae

M. leprae has not been cultivated on artificial media, therefore, to identify drug susceptibility pattern, bacteria are tested using mouse footpad technique. This *in vivo* method requires long time and relatively large number of bacteria. Nowadays, molecular biological techniques for DDS resistant *M. leprae* can be done by detection of mutations in the *fol* P1 gene.

The *fol*P1 gene of *M. leprae*, which encodes dihydropteroate synthetase (DHPS) was studied for the presence of mutations associated with resistance to dapsone. When the *fol*P1 genes of several DDS resistant clinical isolates of *M. leprae* were sequenced, two missense mutations were identified. One mutation occurred at codon 53, substituting isolucine for threonine in DHPS-1, and a second mutation occurred at codon 55 substituting arginine for proline [9]. DHPS is a key enzyme involved in de novo synthesis of folate catalyzing synthesis of 7,8 dihydropteroate from 7,8 dihydroprotein-pyrophosphate and para-aminobenzoic acid (PABA). The mode of action of the DDS is to inhibit the synthesis of 7,8 dihydropteroate by the competitive incorporation of sulphonamide as the structural analogue of PABA.

Bacteriostatic mechanism of DDS



Dihydropteroate synthetase of DDS resistant bacteria does not bind DDS.

MATERIALS AND METHODS

Sample collection

After taking informed consent, skin scrap samples were collected from leprosy patients attending the Central Special Skin Center (CSSC), Yangon General Hospital. Fifty MB patients with clinically and bacteriologically documented disease agreed to participate in the study. Leprosy patients were classified clinically and microscopically according to WHO classification [10] which consists of two

categories, paucibacillary (PB) and multi-bacillary (MB). PB leprosy is defined as five or fewer skin lesions with no bacilli in skin smears, and MB leprosy cases have six or more lesions and may be skin smear positive. The project was approved by Institutional Ethical Review Committee, Department of Medical Research (LM).

Specimen preparation

The collected skin scrapings were dipped in 1.5 ml tubes containing 0.5 ml of 70% ethanol and stored at room temperature until prepared for DNA template by Klatser's Method [11].

DNA template preparation (DNA extraction)

DNA was prepared from skin scraps according to the method of Klatser *et al*. Briefly, under sterile condition, the blade was scratched by wood prick and centrifuged at 14,000 rpm for 10 minutes. After discarding the supernatant, the precipitate was washed with PBS and centrifuged again at 14,000 rpm for 10 minutes to remove remaining alcohol. The washed precipitate was suspended in 50 µl of lysis buffer containing proteinase K 10 mg/ml in 1M Tris-HCL, pH 8.5 and 0.5% Tween 20 and incubated at 60°C for 18 hours. Five microlitre of mineral oil was over-layed to prevent evaporation of water from the mixture. After heating at 97°C for 10 minutes, the suspended solution was treated with freezing and thawing twice to inactivate proteinase K, which inhibits Taq polymerase during PCR.

The DNA polymerase chain reaction (PCR)

A set of primers (*fol*P F and *fol*P RS) was used for amplification of the specific region of *M. leprae fol*P1 gene. Chromosomal *M. leprae* DNA was kindly supplied by Dr. M. Matsuoka, Leprosy Research Center, NIID, Japan. This DNA served as a positive control in all PCR experiments. The 50 µl reaction mixture contained 10 µl of template solution, 0.2 µl of *Ex Taq* DNA polymerase (Takara Shuzo Co., Shiga, Japan), 1 µM of each primer, 5 µl of 10x DNA PCR buffer, 8 µl of dNTP solution and 25.8 µl of water. The reaction mixture was overlayed with 5 µl of mineral oil. The reaction was performed with programmable thermal Mastercycler (eppendorf USA).

The reaction mixture was heated 94°C for 1 min, rounds of amplification consisted of a 30 second denaturation step at 94°C, a 2 min annealing step at 55°C and a 3 min elongation step at 72°C for 45 cycles. The amplified DNA fragments were analyzed by electrophoresis on 1.5% (wt/vol) agarose gels in Tris-Borate EDTA (TBE) buffer.

Following primers were used in PCR

Genes	Primers	Sequence
fol P 1	*Fol* P F	GCTTCTCGTGCCGAAGCGCTC
	Fol P RS	GCAAGTTCTTTTACGACAGG

DNA sequencing

First PCR products were electrophoresed in 1.5% agarose gel. The gel was cut and DNA recovery was done by Easy Trap Kit (TaKaRa Shuzo). DNA sequencing was done by BigDye Terminator Cycle Sequencing, FS Ready Reaction kit (Perkin-Elmer applied Bio system, Norwalk, Conn.) and ABI Prism 310 genetic analyzer (Perkin-Elmer). The nucleotide sequences obtained were analyzed by the DNASIS computer program (Hitachi Software Engineering, Yokohama, Japan).

RESULTS

A total of 50 new MB leprosy patients attending the Central Special Skin Center (CSSC), YGH were studied to detect *M. leprae* from skin scrap by PCR using the *fol*P1 gene amplification and following sequencing to detect mutations. In 50 MB patients including both BI positive and negative cases, 4 patients (8%) showed mutations. Three cases revealed mutations

Homology region [All regions]

```
                20                30                40                50                60
M-12. Seq       GAGAGTTTGGCGCCAGTGCAGGTTTTGGGGTTTTGAACGTACTGACAATTCGTTCTCA
Fol P -5'       GAGAGTTTGGCGCCAGTGCAGGTTTTGGGGTTTTGAACGTACTGACAATTCGTTCTCA
                10                20                30                40                50

                70                80                90               100               110
M-12.Seq        GATTGGCGGACGTTGACTCCTGACGATGCTGCCCAGCGGCCTGGCAATGGTCGCG
Fol P-5'        GATTGGCGGACGTTGACTCCTGACGATGCTGCCCAGCGGCCTGGCAATGGTCGCG
                60                70                80                90               100

               120               130               140               150               160
M-12.Seq        GAAGGCGCGGCGATTGTCGACGTCGGTGGCGAATCGGCCCGGCCCGGTGCCATTAG
Fol P- 5'       GAAGGCGCGGCGATTGTCGACGTCGGTGGCGAATCGACCCGGCCCGGTGCCATTAG
                110               120               130               140               150

                170               180               190               200
M-12.Seq        GATCCTCGAGTTGAACTCTCTCGTATCGTTCCTGTCGTAAAAGAACTTGC
Fol P-5'        GATCCTCGAGTTGAACTCTCTCGTATCGTTCCTGTCGTAAAAGAACTTGC
                160               170               180               190
```

Fig. 1. Nucleotide sequence of the target region of the *fol*P1 gene from Myanmar sample (resistant strain) and Thai 53 (sensitive strain)
Mutation occurred at codon 53, substituting alanine (GCC) for threonine (ACC) and no mutation at codon 55 (CCC) in Myanmar sample No. 12 (M-12).

Homology region [All regions]

```
                20                30                40                50                60
M-11. Seq       GTGAGTTTGGCGCCAGTGCAGGTTATTGGGGTTTTGAACGTCACTGACAATTCGTTCT
Fol P -5'       GTGAGTTTGGCGCCAGTGCAGGTTATTGGGGTTTTGAACGTCACTGACAATTCGTTCT
                10                20                30                40                50

                70                80                90               100               110
M-11.Seq        GATGGCGGACGTTACCTTGATCCTGACGATGCTGTCCAGCACGGCCTGGAATGGTCG
Fol P-5'        GATGGCGGACGTTACCTTGATCCTGACGATGCTGTCCAGCACGGCCTGGAATGGTCG
                60                70                80                90               100

               120               130               140               150               160
M-11.Seq        GAAGGCGCGGCGATTGTCGACGTCGGTGGCGAATCGACCCGGCCCGGTGCCATTAG
Fol P- 5'       GAAGGCGCGGCGATTGTCGACGTCGGTGGCGAATCGACCCGGCCCGGTGCCATTAG
                110               120               130               140               150

                170               180               190               200
M-11.Seq        GATCCTCGAGTTGAACTCTCTCGTATCGTTCCTGTCGTAAAAGAACTTGC
Fol P-5'        GATCCTCGAGTTGAACTCTCTCGTATCGTTCCTGTCGTAAAAGAACTTGC
                160               170               180               190
```

Fig. 2. Nucleotide sequence of the target region of the *fol*P1 gene from Myanmar sample (sensitive strain) and Thai 53 (sensitive strain)
No mutation occurred at codon 53 and codon 55 from Myanmar sample No.11 (M-11).

Table 1. Mutations in the *fol*P1 genes of *M..leprae* from leprosy patients

Case No.	Age	Sex	Diagnosis & treatment	*fol* P1 condon	Mutations	Mutant amino/a in DHPS
4	17	F	MB-MDT	53	ACC - ATC	Isoleucine
9	52	M	MB-MDT	53	ACC - AGA	Arginine
12	47	M	MB-MDT	53	ACC - GCC	Alanine
34	60	F	MB-MDT	55	CCC - CAC	Histidine

DHPS = Dihydropteroate synthetase
MB-MDT = Multibacillary – Multidrug Treatment

at condon 53 and one case detected mutation at codon 55 (Table 1). Fig.1 shows mutation at codon 53 substituting alanine (GCC) for theonine (ACC). Fig. 2 shows no mutation at codon 53 and 55.

DISCUSSION

Dapsone is still one of the most important of anti-leprosy drug used today. It is stable, inexpensive, relatively nontoxic and one of the mainstays of the WHO-MDT for both MB and PB leprosy. Unfortunately, development of acquired or secondary resistance in patients treated with DDS mono-therapy for many years has provided a source of infection resulting in the appearance of primary DDS resistance among new patients diagnosed with the disease.

This study is part of the research on detection of multi-drug resistant leprosy in Myanmar. Multidrug resistant leprosy means resistance to both DDS and rifampicin. We performed detection of *fol*P1 mutation for DDS resistance and *rpo*B gene mutation for rifampicin resistance simultaneously. Fortunately, these 4 cases of DDS resistance were susceptible to rifampicin, which is an exceptionally potent bactericidal agent against *M. leprae*. A single dose of 600 mg is capable of killing more than 99.9% of viable organisms [3]. It is a key drug in MDT. These 4 primary DDS resistant cases were given anti-leprosy drugs of rifampicin and clofazimine for 12 months as WHO regime and will be taken regular follow-up for clinical examination and slit skin smear to check *M. leprae*.

In the present study, relatively simple and rapid molecular techniques (PCR and direct sequencing) were applied in an effort to determine *fol*P1 mutations in *M. leprae* isolates. The mutations indicate that mutations in codons 53 or 55 of *M. leprae fol*P1 are responsible for DDS resistance in Myanmar. This information should lead to a better understanding of the status of DDS resistant leprosy in Myanmar and assist in the diagnosis of DDS resistant *M. leprae*.

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Seasonal variation in biological and biochemical properties of Russell's viper (*Daboia russelli siamensis*) venom

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Biological, biochemical and electrophoretic properties of venom of four captive Russell's vipers (*Daboia russelli siamensis)* milked monthly for 11-24 months were studied. In general, all biological activities such as lethal, coagulant, haemorrhagic, necrotic, defibrinogenating and capillary permeability increasing remained fairly stable within the first 3 months in captivity. Variation in activity (except haemorrhage and necrosis) was observed in captivity. Marked reduction in activity; (4-5 times) of lethal and defibrinogenating in July (raining) and November through January (winter) and coagulant (35 - 42 times) in April/May (summer) was observed. However, 4-7 times reduction in capillary permeability increasing activity (CPI) was recorded in July through September (raining). It is noteworthy that marked decrease in capillary permeability increasing activity was observed in two out of four Russell's vipers from 3rd month onward following captivity. There was a variation in protein content, L-amino acid oxidase, esterolytic and phospholipase A_2 activities in between seasons. However, no variation in venom yield was observed. Electrophoretic studies of venoms showed quantitative and qualitative variation in protein bands.

INTRODUCTION

Russell's viper bite is endemic in Myanmar. Myanmar Pharmaceutical Factory is the sole manufacturer of antivenom in Myanmar. Live Russell's vipers are bought every year for venom extraction. Snakes were milked, venom pooled, desiccated and stored at 4˚C. Venom is used for raising antivenom and the latter is used for treating snake bite cases. Batch to batch variation of Russell's viper (*Daboia russelii siamensis)* venom used for raising antivenom has been reported [1]. Venoms used for raising anti-venom are pooled from different milkings. Because of variation in venom property, possibility of seasonal variation of venom is considered. Studies on toxicity and yield of venom following repeated milking of venom from single snake or groups of snakes and of varying ages have been reported [2-10]. However, there were few reports on study of seasonal variation in composition and toxicity of snake venoms [11-13]. In this communication, biological and biochemical properties of four Russell's vipers milked at monthly intervals for 11 to 24 months were studied.

MATERIALS AND METHODS

Snakes

The snakes measuring 84cm (A), 88cm (C), caught in November 1993, snake E (89cm) in May 1994 and snake B (99cm) in July 1994 were housed in separate wooden cages at the Myanmar Pharmaceutical snake farm, Yangon where the environmental temperature ranged from 20º-32ºC. These snakes originated in the Kokekogwa, Taungdwingyi district of Magway Division.

The snakes were fed on 1 to 2 mice per week with water *ad libitum*. During winter, straw was provided for insulation.

Venom

Individual snake was milked and venom was lyophilised at monthly intervals following capture up to the time of death. The total length of the snake and volume of venom yielded (dry weight) were also measured at each extraction. Primary milking of two wild caught Russell's vipers measuring 84 and 88 cm in length, caught in November 1993 and another two measuring 88 and 89 cm in total length, caught in May 1994 from the same locality were also available for the study.

Assays

Biological properties (lethal, coagulant, haemorrhagic, necrotic, defibrinogenating and capillary permeability increasing activities) of venom were determined according to the WHO recommended techniques [14]. Biochemical tests such as L-amino acid oxidase, phospholipase, esterolytic activities, protein concentration (1 mg/ml of dry venom) and SDS-PAGE electrophoresis were also determined by the methods previously described [8].

Statistics

Values expressed were means ± 1 SD. Statistical analysis of the samples was carried out by comparison of means by Analysis of Variance and Student's t test. Level of significance was taken at $p < 0.05$ in the former and $p < 0.001$ in the latter.

RESULTS

Biological activities of the venoms

Results of mean biological activities of two Russell's viper venoms are shown in Table 1. In general, the pattern of individual biological activity of the four venoms was comparable except in the capillary permeability increasing activity (CPI) which waned from 3rd month onward following captivity in 2/4 snakes. All activities remained fairly stable within the first

Table 1. Biological activities of Russell's viper venoms in three seasons

Biological activity	Code	Winter (Nov-Feb)	Summer (Mar-Jun)	Raining (July-Oct)
L aminoacid oxidase (ug/mg/min. venom)	A	164 ±3.57	168 ± 2.37	220.09 ± 3.04
	C	215 ±3.88	215.75 ± 5.36	234.5 ± 6.61
Proteolytic (ug/mg/ min. venom)	A	289.6 ±5.12	339.83 ±7.42	267 ± 9.44
	C	185.53 ±3.16	185.8 ±5.56	214.8 ± 11.66
Phospholipase A_2 (ug/mg/ min. venom)	A	1237.1 ±35.22	1179.3 ±25.64	1265.18 ± 9.38
	C	1258.7 ± 6.11	1135 ±8.06	1247.7 ± 16.2
Protein (1mg/ml)	A	821.7 ±18	850 ±25.2	823.6 ± 32.02
	C	805.5 ±15	845 ±15.66	815.2 ± 36.43
Venom yield dry (mg)	A	53.75 ±11	40 ±13.5	73.3 ± 30.5
	C	82.5 ±3.53	113.75 ±12.5	113.3 ± 25.6

Table 2. Comparison of biological properties of venoms of wild and captive Russell's vipers

Month	Status	Length (cm)	No.	LD_{50} µg / m	MHD µg / r	MND µg / r	MDD µg / m	MCD µg / ml	MCPID µg / ml
Nov	W	84 / 88	2	2.59 ±2.4	22.9	33.5	1.0	0.158	0.0024
	C	84	1	7.3 ±2.9	57	47.3	7.0	0.040	0.0018
May	W	88 / 89	2	8.1 ± 2.2	36.3	27.0	2.5	3.236	0.0017
	C	92	1	9.1 ± 2.3	84	50.0	6.0	0.263	0.0044

W = Wild C =Captive m = mouse r = rat

MHD = Minimum haemorrhagic dose
MND = Minimum necrotic dose
MDD = Minimum defibrinogenating dose
MCD = Minimum coagulant dose
MCPID = Minimum capillary permeability increasing dose
LD_{50} = Lethality

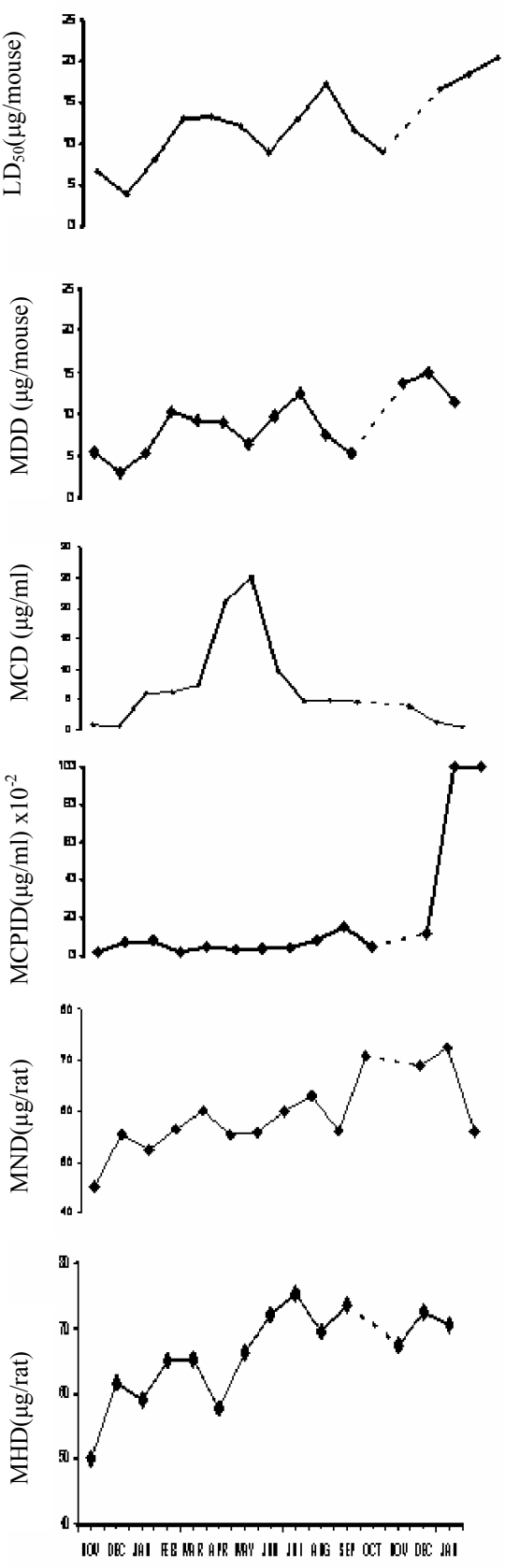

Fig. 1. Biological properties of Russell's viper venom* in captivity

*Each point represents a pooled venom of 4 snakes.

3 months in captivity. Variation in biological activities was observed during captivity except haemorrhage and necrosis. Mark reduction in lethal and defibrinogenating (4-5 times) in July (raining) and November through January (winter) and coagulant activity (35-42 times) in April-May (summer) was observed. About 4-7 times reduction in CPI activity was recorded in July through September (raining) and more than 50 folds reduction in the activity after a year in captivity was observed (Figure 1).

The pattern of biological activities of the venom collected from the wild caught Russell's vipers in November and May also showed a similar pattern of variation as in the venom collected over the same month of the year in captivity. However, the venoms from the wild caught snakes were far more potent than the latter except in coagulant activity (Table 2).

SDS PAGE electrophoresis

SDS-PAGE electrophoresis of monthly collected venoms of snake A is shown in Figure 2. Qualitative and quantitative differences in protein bands were observed among venoms. SDS–PAGE electrophoresis of monthly collected venoms of snakes B and C also showed similar qualitative and quantitative differences in protein bands (Figure not shown). The venoms collected from the wild in November 1993 and 1994 showed similar protein bands (Figures not shown).

Snake's length

While in captivity snake A grew from 84 cm to 106 cm (22 cm) in 24 months, snake E grew from 89 cm to 100 cm (11 cm) in 18 months, snake C grew from 88 cm to 108 cm (20 cm) and snake B, from 99 cm to 103 cm (4 cm) in 11 months. In general, growth in length of the snakes was observed in the first 4 to 6 months in two snakes (up to one year in one) following captivity and then it flattens out.

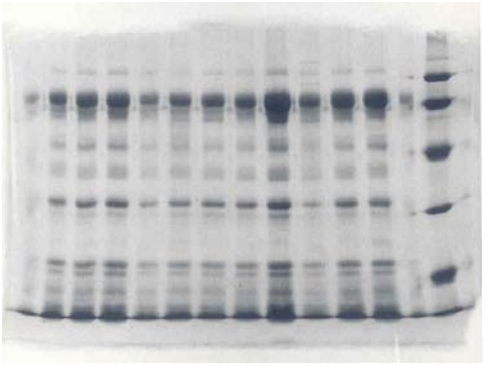

Fig. 2. SDS–PAGE electrophoroesis of venoms of Russell's viper A in captivity.

Lane 1-11 represent monthly collected venom samples of snake A
Lane 12 represent molecular weight marker

Venom yield

Mean volume of venom yield per milking of snake ranged from 0.2 ml to 0.7 ml. The average venom yield of the snakes in winter was 0.35 ml (n=10), summer 0.39 ml (n=14) and raining seasons 0.42 ml (n=11). No significant difference in venom yield of Russell's viper (*D.r.siamensis*) per milking in captivity was observed. However, seasonal variation in venom dry weight was observed in venoms A and C; (winter C 82.5±3.53 mg and summer C 113.75±12.5 mg ($P<0.01$), winter A 53.75 ±11 mg and summer A 40 ±13.5 mg ($P<0.01$), winter A 53.75 ±11 mg and raining A 73.3± 30.5 mg ($P<0.01$) and winter C 82.5± 3.53 and raining C 113.3± 25.6 mg ($P<0.01$). Venom yield was maximal in summer and raining in venom C and raining in venom A.

Biochemical activities of venom

Biochemical properties of venom were studied in two of four snakes (A and C). Seasonal variation in L-amino acid oxidase, esterolytic and PLA_2 activities of the venoms was observed. In venom A, a significant difference in L amino acid oxidase activity in between winter (164±3.57 ug/mg /min venom) and raining (220.09±3.04 ug/mg/min venom)($P<0.003$),PLA_2 between winter (1237.1±35.22ug/mg/min venom)and summer (1179.3 ± 25.64 ug/mg/min venom) ($P<0.2$), summer (1179.3±25.64 ug/mg/min venom) and rainy (1265.18±9.38 ug/mg/min venom) ($P<0.2$) was observed. The venom A has maximal L amino acid oxidase (220.09± 3.04 U/mg/min venom) and PLA_2 (1265.18± 9.38 U/mg/min venom) in raining and esterolytic activities (339.83 ±7.42U/mg/min venom) in summer.

Protein content of the venom (1 mg/ml=850±25 ug/ml) showed variation between winter (821.7 ±18 ug and summer (850±25.2 ug)($P<0.01$) in venom A and 805.5 ±15 ug and 845 ±15.66 ug respectively ($P<0.01$) in venom C.

DISCUSSION

Variation in yield of venom has been reported [2, 4, 9]. However, no significant variation in venom yield of Russell's viper (*Daboia russelii siamensis*) per milking was observed during captivity as snakes were kept in well-cared and feeding readily. Individual seasonal variation in venom yield was observed. Snake C yielded more venom in summer and rainy season compared to winter. Although the venom yield increased with increases in temperature, the maximum yield being obtained in the hottest summer months [15], it was unlikely that the difference seen in the two venoms was attributed to a change in environmental temperature since both were kept in the same environment. Because of small sample size, individual variation in yield could not be excluded. No seasonal variation in venom yield was observed in one C*rotalus atrox* milked monthly for 19 months [10]. It was reported that neither venom yield nor toxicity decreased following repeated venom extraction (16 times) over a period of two years in a group of water moccasins (*Agkistrodon piscivorus*) [3].

Maximum yield of *Naja naja oxiana* was observed in autumn and winter, that of *Vipera lebetina* and *Agkistrodon halys* in summer. However, no significant seasonal variation in venom yield was observed in venoms of *Pseudocerastes persicus*, *Vipera*

latifii, *Vipera xanthina* ssp. and *Echis carinatus* from Iran [9]. Marked variations in venom yield on successive milking for 2-7 months were observed in Australian elapids. Average secondary yield for all species was lower than the average primary yield with exception among the individual snakes [16].

Individual variation in growth in length was unlikely to be due to captive condition since all were kept in the same environment, probably genetic factor could not be excluded.

A significant decrease in PLA_2 activity in summer compared to winter or rainy season was observed in both venoms. There were no significant differences in PLA_2 activity between winter and raining and L amino acid oxidase activity between winter and summer of venom C. Protein content of the venoms showed variation between winter and summer ($P<0.01$). Variation in the composition of the venom from a single specimen of *Pseudonaja texilis* (common brown snake) over a year [12] and seasonal variation (winter and summer) in composition of venom of V*ipera ammodytes* based on comparison of different specimens of snake [11] have been observed. Intraspecific variation in venom colour (L amino acid oxidase), enzyme activity and lethality of V*ipera ammodytes* has been reported [17].

Biological properties of the venom remained fairly stable throughout captivity except marked decrease in coagulant activity in summer and capillary permeability increasing activity in a year end of captivity. It was noteworthy that CPI activity waned in 2/4 snakes from 3rd month onwards of captivity. However, toxicity of Notechis venom was approximately the same between primary and secondary yield provided the snakes were not diseased or starved [16]. Toxicity of the venom of a *Crotalus atrox* milked for 19 months tended to decrease during captivity and no seasonal variation in toxicity was observed [10]. Variation in biological properties of venom according to age in *Crotalus atrox* venom [5, 7] and Russell's viper (*D. r . siamensis*) [8] has been reported. Negligible seasonal intraspecific variability especially in coagulant activity of venom of carpet viper (*Echis carinatus*) has been observed [18].

SDS – PAGE electrophoresis of the venom B in captivity and venoms collected from the wild in November 1993 and 1994 (Figures not shown) showed similar results. Unlike venoms of *Vipera ammodytes* [11], there was no definite loss of protein band attributable to seasonal variation as in habu (*Trimeresurus flavoviridis*) venom [19] and in venom of *Bitis* sp. [20,21] following frequent milking or in snakes milked over a period of 20 months [13].

It has been reported that toxicity and the yield of venom depend not only on the age, size, forced feeding, fasted conditions of milking, stress, period of daylight, temperature and captive care [6] but also on the season and geographical origin of the snake [9].These seasonal variations in composition and biological properties of venom should be taken into consideration when collecting venoms for raising antivenoms.

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Larvivorous potential of dragonfly nymphs

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Predatory rates of naturally occurring dragonfly nymphs-*Bradinopyga geminata* (Rambur) (tank variety) and *Crocothemis servilia* (Drury) (pond variety) collected from local tanks and ponds were studied under laboratory condition in Health and Disease Control Unit, Mingaladon from January 2002 to 2003. The series of experiment included two to five replicates for different sizes of nymphs. The results showed that medium-sized *B. geminata* consumed 62 ± 22 *Aedes aegypti* larvae (third and fourth instars) per nymph per 24 hours and the same size *C. servilia* 53 ± 21 per 24 hours. When all instars and pupae (equal number of 40 each) were introduced, the predatory rates rose to 137 ± 14 per 24 hours and 128 ± 9 per 24 hours for these varieties respectively. Predatory rates of both varieties were found not to be statistically different ($p > 0.05$). Medium-sized nymphs consumed most *Aedes* larvae followed by large and small-sized ($p<0.005$). The predatory rates increased with increasing number of larvae and pupae introduced and they were well correlated ($r = 0.97 – 1.0$, $p< 0.05$). The nymphs consumed most first instar of *Aedes* larvae and progressively fewer of each of successive stage. The nymphs were also found to be superior to other predators on feeding rate. The study highlighted that due to their advantages and the facts of being locally available, harmless to human and high in predation rate, dragonfly nymphs should be used as bio-control agents in the field by augmentative release, monthly or as necessary, into major water-storage containers especially heavy and irremovable containers such as concrete tanks, concrete and metal drums and earthen glazed jars to suppress *Aedes* mosquito, thereby controlling dengue haemorrhagic fever effectively.

INTRODUCTION

Dragonfly is the common name for large, winged, sun-seeking insect belonging to order Odonata which is widely distributed in the world and the world fauna probably does not greatly exceed the 5,000 species now known. They have been in existence for at least 200 million years as members of the global ecosystem. Dragonflies are predatory insects. Their life cycle consists of three stages - egg, larva or nymph and imago or adult. The former two are aquatic in nature and their habitats are mainly ponds, rivers, streams, tanks and tree holes [1, 2]. During the development from eggs, the dragonfly nymphs molt approximately 8 to 15 times and they feed actively on crustacea and protozoa, midge larvae, aquatic beetles, snails, small fishes, tadpoles and culicid larvae and even nymph of their own kind and other species of Odonata being called cannibalism. Therefore, the nymphs are opportunistic and euryphagous predators in nature and they are very quick in catching preys with the labial strike less than 25 miliseconds [2, 3]. The present study was done with the objectives of: (a) to determine the predatory rates of naturally occurring dragonfly nymphs on *Aedes aegypti* (L.) mosquito larvae within 24 hours, (b) to compare their predatory

rates between tank and pond varieties and among different sizes of the predator and (c) to correlate number of mosquito larvae and pupae introduced with predatory rates.

MATERIALS AND METHOD

Study design was a prospective controlled laboratory trial conducted in Health and Disease Control Unit (HDCU), Mingaladon from January, 2002 to 2003. Dragonfly nymph collection areas were Mingaladon, Dagon and Thaketa Townships, Yangon, Myanmar.

Dragonfly nymphs were obtained from domestic water containers like concrete tanks, concrete and metal drums in residential areas of Mingaladon and Thaketa Townships and from the garden pond near Department of Medical Research (Lower Myanmar) in Dagon Township. The dry specimens of nymphs of both tank and pond varieties were sent to Professor PS Corbet, Biologist, Ecologist and Medical Entomologist of United Kingdom and Associate Professor Dr Joan Bryan, Australia Centre for International Tropical Health and Nutrition, the University of Queensland, Australia for species identification and they were all identified as *Bradinopyga geminata* (Rambur) (tank variety) and *Crocothemis servilia* (Drury) (pond variety). Their sizes were small (1.0 ± 0.1cm), medium (1.5 ± 0.2 cm) and large (1.9 ± 0.1cm). *Aedes aegypti* larvae for feeding were collected from domestic water containers in Thaketa Township. Glass bottles were purchased from local markets.

Dragonfly nymph – *B. geminata* collected were kept, for one day for adaptation purposes without any feed, in plastic trays (34.5 cm x 24.5 cm x 6.0 cm) containing rain water admixed with that of larval habitat and some weeds in the laboratory of HDCU. *Ae. aegypti* larvae collected were also kept in the laboratory similarly. Five active medium-sized nymphs were selected and each was placed into each of five clean glass bottles (diameter 7.5 cm and height 17.5 cm) containing 800 ml of rain water. Next, one hundred *Ae. aegypti* larvae (3^{rd} and 4^{th} instars, 50 each) were introduced into each bottle. Larvae in same number in control bottle were monitored to assess the natural mortality. The number of larvae consumed by nymphs was recorded after 24 hours. Each set of experiment was replicated five times.

Similarly medium-sized *C. servilia* nymphs collected were studied for five replicates. Then the small and large-sized of both varieties were tested for predatory rates on third and fourth instars of larvae for two replicates. Then all sizes of both varieties were tested with different number of larvae (all instars) and pupae introduced (50, 100,150 and 200 larvae containing equal number of instars and pupae) for two replicates.

During the experiment period, temperature (25 ± 7 °C) and relative humidity (78 ± 8 %) were recorded. Data analysis was done by Student's t test, the Kruskal-Wallis one-way analysis of variance and Pearson's correlation.

RESULTS

The study results indicated that the predatory rates were satisfactory. The predatory rate of medium-sized *B. geminata* was 62 ± 22 *Aedes* larvae (3^{rd} and 4^{th} instars) per nymph per 24 hours and that of *C. servilia* was 53 ± 21 larvae per 24 hours. The difference between these two predatory rates was found not to be statistically significant ($p > 0.05$). In the control bottle all *Aedes* larvae were alive till 24 hours. Among different sizes of the nymphs, the medium-sized consumed most *Aedes* larvae, followed by the large-and the small-sized. It was statistically significant ($p<0.005$) (Table 1).

Table 1. One-way analysis of variance: differences in mean *Aedes* larvae (third and fourth instars) consumed by *B. geminata* and *C. servilia* of different sizes

Size	No. of nymph tested	Mean larvae consumed		Mean rank		X^2		p value	
		B. geminata	*C. servilia*	*B.geminata*	*C. servilia*	*B.geminata*	*C. servilia*	*B. geminata*	*C. servilia*
Small	10	29.1 ±10.22	21.1± 14.62	7.7	9.5	13.52	12.55	<0.005	<0.005
Medium	10	62.1 ±21.55	53.0± 20.73	22.0	23.2				
Large	10	45.3 ± 9.91	29.2± 10.05	16.8	13.9				

Table 2. Predatory rates of *B. geminata* by size and number of *Aedes* larvae and pupae introduced and larval preference (mean %)

Size	Predatory rates (mean ± SD) by number of *Aedes* larvae (all instars and pupae) introduced				Larval preference by stage (mean %)					r	p
	50	100	150	200	1st	2nd	3rd	4th	pupa		
Small	25.0±5.0	53.5±1.5	73.5±8.5	73.5 ± 3.5	32	25	22	18	3	0.93	>0.05
Medium	39.5±2.5	82.0±5	101.0±15	137.0±14	25	23	22	18	12	0.99	0.01
Large	30.5±1.5	51.0±2	86.5±0.5	93.0± 3	30	25.50	22.50	19	3	0.97	<0.05

Table 3. Predatory rates of *C. servilia* by size and number of *Aedes* larvae and pupae introduced and larval preference (mean %)

Size	Predatory rates (mean ± SD) by number of *Aedes* larvae (all instars and pupae) introduced				Larval preference by stage (mean %)					r	p
	50	100	150	200	1st	2nd	3rd	4th	Pupa		
Small	30.5± 4.5	49.0± 3	66.5±1.5	80.5±7.5	31	28	17	22	2	1	-
Medium	26.5±11.5	57.5± 0.5	93.5±3.5	128.0± 9	27	25	20	15	13	1	-
Large	31.0± 1	33.5± 8.5	57.0±4	96.5±1.5	27.25	25.25	22.25	23	2.25	0.94	>0.05

Another interesting finding was predatory rates associated with number of larvae (all instars) and pupae introduced. It increased with increasing number of prey larvae introduced and these two variables were well-correlated and it was statistically significant (r = 0.97–1.0, p <0.05) except small-sized *B. geminata* (r = 0.93, p>0.05) and large-sized *C. servilia* (r = 0.94, p>0.05) (Table 2 & 3). Predatory rates of the medium-sized on all instars of larvae and pupae of *Aedes* were 137±14 and 128 ± 9 per 24 hours for *B. geminata* and *C. servilia* respectively. Out of different instars and pupae, both varieties of nymphs consumed most first instar (25–32%) and progressively fewer of each successive stage.

DISCUSSION

Dragonflies are popularly supposed to sting, but in reality they are harmless insects and economically they are also of great importance in destroying noxious flies and mosquitoes [3]. Dragonfly nymphs live in aquatic habitats for one to three months or even one to five years in low temperature areas before emergence. They are poly-phagous and can live without food for more than four months. The ability to survive for prolonged period without food may make them touse as valuable biocontrol agents because it allows them to remain viable as predators as the prey is intermittently eliminated. In a comparative study, larval

and adult density of *Aedes* mosquito became much more reduced in treated area in Yangon in 1979. In that study medium-sized *C. servilia* nymphs (three weeks old) were used at the rate of four nymphs per a major container every four weeks. Mosquito larvae were reduced more than 85% within 2 weeks and almost eliminated during following 3 months. There was a marked and unequivocal reduction of adult as well. The nymphs are generalized obligate predators and they consume a large number of mosquito larvae and pupae. The nymphs have many advantages for use. They can cling to the side of container and so unlike other predators, are unlikely to be accidentally flushed or scooped out by the householders; their development is relatively slow; and they do not leave the container until emergence [4, 5].

Larval feeding rate in nature varies *inter alia* with predator size and time of year, and is normally less than maximum possible feeding rate determined in captivity. Experiments showed two-half grown dragonfly nymphs (libellulid) could kill all mosquitoes (range 87-780) in 4 to 9 days depending on the number of mosquito larvae initially present. The predatory rate was about 32 mosquito larvae per dragonfly nymph per 24 hours. On the other hand, medium-sized *B. geminata* nymphs consumed on average 133 ± 21 mosquito larvae (all instars) and pupae per 24 hours [5,6]. In the present study, feeding rates of both varieties were found to be satisfactory. Predatory rates of both varieties are not different statistically ($p>0.05$) but the rate of *B. geminata* (tank variety) is a little higher than that of *C. servilia* (pond variety). The reason may be that pond variety was satiated when they live in the pond where food was relatively more abundant than in the tank.

Being the highest predation rate, the medium-sized dragonfly larvae is the most suitable size to use as a biocontrol agent. The large-sized consumed less than the medium because feeding rate decreases with successive stadia. Predatory rates were also well-correlated with the number of prey larvae introduced. This was consistent with the finding that maximum feeding rate of a nymph is capable when food is supplied *ad libitum* [2]. The nymphs consumed most first instar with decreasing number at successive stage. This finding was also compatible with that in one of the studies [6].

Naturally *B. geminata* nymphs are found to be established in a large number in major sources, sometimes attaining about 200 in a concrete tank of 1.9 m^3 volume or 10 per metal drum containing domestic water [4]. *C. servilia* which are naturally found in local ponds in abundance throughout the year and other species like *Orthetrum sabina* (Drury) may be used as predators. The dragonfly adult is also useful because they eat some pests in paddy fields where they occur and about 80% of farmers use no insecticide. A notion persisted in some quarters in North America that the presence of a large anisoptera in flight will repell mosquitoes [2]. Dragonfly nymphs do not produce bacteria when water in which they live was tested bacteriologically on day one, two, three and seven [7].

Table 4. Predatory rates of different predators on *Ae. aegypti* larvae

Predators	Predatory rate (Larvae/predator /24 hr)	References
Dragonfly nymph *B .geminata*	133 ± 2.1	[6]
Larvivorous fish *T. trichopterus* (1.27cm)	30 ± 1.4	[8]
Larvivorous fish *Xi. helleri* (2.5 cm)	228 ± 21	[9]
Larvivorous fish *A. panchax* (2.5 cm)	55.30± 5.5	[10]
Mosquito *Tx. splendens* (full grown)	40±6	[11]
Cyclopoid *Me. pehpeiensis*	28.50 ± 5.9*	[12]
Cyclopoid *Me. thermocyclopoides*	25.45 ± 6.0*	[12]
Dragonfly nymph *B. geminata* (1.5cm)	137 ± 14	present study
Dragonfly nymph *C. servilia* (1.5cm)	128 ± 9	present study

*only 1st and 2nd instars of *Ae. aegypti* larvae

There are also other larvivorous predators (Table 4). By reviewing the nature and predatory rates of different predators, the dragonfly nymphs were found to be superior to other predators. This is the strong supportive point to recommend the dragonfly nymphs to be used as bio-control agents in the field.

Routine vector control method currently used is effective and cheap. But some people do not follow to carry out these methods due to various reasons like being too busy, difficult to empty heavy and irremovable drums and tanks and lack of cover or lid for water containers. So dragonfly nymphs should be used in the major category containers like metal drum, earthen glazed jars, concrete tanks and concrete drums if there is no proper lid arrangement and regular emptying of water, or in the conditions that whirl formation does not occur in non-circular containers when cotton net sweeper is applied and regular treatment of water with temephos is too expensive and disliked by people due to its particular taste [4].

If an adequate source of supply of nymphs could be assured, for example, by obtaining them from local tanks and ponds by householders, this biological control of *Aedes* mosquito is feasible by using augmentative release of nymphs into domestic water containers monthly or as necessary. Moreover, community should be given health education and motivated and larval control by biocontrol method should be supported by administrative bodies.

It is summarized that dragonfly nymphs which are locally available, harmless to human beings and high in predation rate on *Aedes* larvae should be used in the treatment of domestic water containers especially heavy and irremovable concrete drums, concrete tanks, metal drums and earthen glazed jars to suppress *Aedes* mosquito, the vector of dengue haemorrhagic fever which attacks thousands of children under 15 years in our country annually.

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Antibiotic resistance pattern of enteric bacterial pathogens among childhood diarrhoea

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Antibiotic susceptibility tests on bacterial pathogens isolated from cases of childhood dysentery and diarrhea admitted to some hospitals in Yangon were carried out from 1980 to date. Yearly changing of antibiotic susceptibility pattern was found on these isolates which include enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), *Shigella* species and *Salmonella typhi.* Changing drug resistance pattern of EPEC and ETEC isolates to ampicillin, chloramphenicol and tetracycline was recorded. From this study, it is shown that the drug resistance of *Shigella* species was increased in adults than children. Susceptibility pattern was distinct in newly introduced drugs compared to conventional drugs.

INTRODUCTION

Diarrhoeal diseases constitute one of the causes of morbidity and mortality on a global scale [1]. To an increasingly recognised extent, they are caused by an expanding array of microbial products or "toxins". In Myanmar, diarrhoea and dysentery still play a major health problem and it stood in the fourth position as reported in the National Health Plan (1997-2001) [2]. Acute infectious diarrhoea is caused when a critical number of microorganisms are ingested. They can withstand and survive along the digestive tract even in acidic environment till they reach the intestinal tract. There, they either [i] colonize and multiply in the intestinal lumen or [ii] adhere, penetrate and damage mucosal cells to cause non-inflammatory or inflammatory diarrhoea. Guerrant [3] stated that either in the secretory or in invasive type of diarrhoea, microbial toxins may play critical role in causing diarrhoeal diseases. The magnitude of the global problem of acute diarrhoeal diseases was also reported [4]. Enteric infections caused by *Esch. coli* has been shown since 1971 [5,6]. In 1996, the outbreak caused by *Esch. coli* O111 was reported [7]. Changes in incidence and causal bacteria in hospital practice were well documented [8, 9, 10]. Antimicrobial resistance of *Salmonella*, *Klebsiella* and *Shigella* was demonstrated and it was quite alarming [11, 12, 13]. Thus, this study was carried out to determine the distribution of enteric pathogens and to test the antibiotic susceptibility pattern on the isolated pathogens in our community.

MATERIALS AND METHODS

The enteric bacterial pathogens were isolated according to the method of WHO [14] and Lennette *et al.* [15] using the products of Difco, Nissui and Oxoid Company Limited.

Isolation of bacterial pathogens were conducted only in the stool samples of children under five years. The collecting sites included Yangon Children's Hospital (YCH), North Okkalapa area and Intakaw village. The stool samples were collected from children with diarrhoea and non-diarrhoea (control) cases in all study sites.

Stool sample collections were conducted throughout the year in YCH but in North Okkalapa area and Intakaw village, collections were made only in the cool-dry and hot-wet seasons as diarrhoea cases occur mainly in these two seasons.

Serotyping of bacterial pathogens was done according to the method of Ewing [16] .To determine labile toxin, verotoxin, adhesive and invasive properties, heat labile test (*in vitro*), verocytotoxic, adhesive, invasive; BIKEN and ELISA tests were done as described by Scotland *et al.* [17]. Antibiotic susceptibility test was done by Kirby-Bauer method [18].

RESULTS

It was observed that ETEC and EPEC were the most common pathogens isolated. Isolated serogroups were O6, O26, O27, O119, O125, O126, O127, O128, O146 and O159 and seemed to be increased from 1996 to 2003.

The antibiotic resistance pattern of enterotoxigenic *Esch. coli* (ETEC) and enteropathogenic *Esch. coli* (EPEC) was changed. In 1980, 25 % of ETEC were resistant to ampicillin and elevated up to 100 % in 1995. Chloramphenicol resistance rose from 26 % in 1980 to 53 % in 1995. Similarly, tetracycline resistance increased from 32 % to 79 %. Regarding EPEC, ampicillin resistance increased from 18 % in 1980 to 89 % in 1996, chloramphenicol from 17 % in 1980 to 62 % in 1996, and tetracycline from 26 % in 1980 to 87 % in 1996 (Table 1).

Antibiotic resistance pattern of *Shigella* species and *Salmonella typhi* isolated from children is shown in Table 2. *Shigella* spp was mostly resistant to ampicillin, chloramphenicol, septrin and streptomycin. Out of 47 strains of *S. typhi*, 88% were resistant to ampicillin, 74% to carbenicillin, 79 % to chloramphenicol and septrin, and 70 % to tetracycline .

Table 1. Changing resistance pattern of enterotoxigenic *Esch. coli* (ETEC) and enteropathogenic *Esch. coli* (EPEC) to antibiotics

Antibiotics/ Antimicrobial agents	Resistance (%)			
	ETEC		EPEC	
	1980 n=99	1995 n= 46	1980 n=82	1996 n=61
Ampicillin AM-10	25	100	18	89
Amikacin AN-30	NT	37	NT	2
Carbenicillin CB-100	NT	NT	NT	80
Cephalothin CF-30	3	0	4	10
Chloramphenicol C-30	26	53	17	62
Colistin CL-10	0	0	12	NT
Furazolidone Fx-100	NT	0	NT	15
Gentamicin GM-10	NT	0	NT	12
Kanamycin K-30	0	NT	6	13
Minocycline MNO-30	NT	NT	NT	16
Nalidixic acid NA-30	NT	0	NT	17
Neomycin N-30	NT	0	NT	NT
Norfloxacin N-10	NT	0	NT	16
Penicillin P-10	93	NT	94	NT
Trimethoprim/sulfame-thoxazole(Septrin) SXT	NT	53	NT	80
Sisomycin SIS-10	NT	NT	NT	15
Streptomycin S-10	NT	63	NT	89
Tetracycline TE-30	32	79	26	87

NT= not tested

Table 2. Resistance pattern of *Shigella* and *Salmonella* species isolated from children

Antibiotics / Antimicrobial agents	Resistance (%)	
	Shigella spp. n= 47	*S. typhi* n=47
Ampicillin AM-10	87	88
Amikacin AN-30	0	5
Cephalothin CF-30	45	21
Carbenicillin CB-100	NT	74
Cefclor Cef-30	NT	43
Ceftriazone CTX-30	NT	19
Chloramphenicol C-30	75	79
Furazolidone Fx-100	36	12
Gentamicin GM-10	7	18
Kanamycin K-30	4	0.5
Minocycline MNO-30	15	NT
Nalidixic acid NA-30	11	11
Netilmicin NET-30	2	3
Neomycin N-30	0	NT
Norfloxacin NOR-10	6	11
Pefloxacin	NT	0
Septrin SXT	75	79
Sisomycin SIS-10	6	NT
Sparfloxacin	NT	0
Streptomycin S-10	75	90
Tetracycline TE-30	66	70

NT= Not Tested

DISCUSSION

This study recorded that bacterial pathogens isolated from different cases were highly resistant to most conventional antibiotics. This being the case, public should take the preventive measures which could avoid the transmission of these causative pathogens. Once personal hygiene and clean environmental conditions are maintained, the occurrence of this infectious disease could be reduced.

According to this study, ETEC and EPEC were found to be the commonest cause of diarrhoea. These were followed by *Shigella* spp. and *Campylobacter jejuni*, the latter being frequently underdiagnosed in the routine microbiology laboratory. *Vibrio cholerae* and *Plesiomonas shigelloides* were sporadic causal organisms for childhood diarrhea.

Some of the diarrhoeal bacterial pathogens were also isolated from asymptomatic controls who did not have diarrhea. Therefore, disease manifestation is due not only to the presence of pathogen, but may also be due to virulent factors of the bacteria, the dosage and the immune status of the host.

Serogroups of *Esch. coli* isolated from children in 1996 and 2003 showed that certain serogroups were increasingly isolated after a period of 7 years. Serogroups O6, O26, O119, O125, O126, O127, O128, O146 and O159 were more prevalent in 2003. There were 14 patients with serogroup O1 in 1996 but, there was none in 2003. One noteworthy result is the isolation of O157 enterohaemorrhagic *E. coli*(EHEC) in both 1996 (2/204) and 2003 (5/532). It is the strain that causes haemorrhagic colitis and even haemolytic uraemic syndrome, a potentially lethal condition.

The antibiotic profile of ETEC and EPEC shows a changing resistance pattern from the year 1980 to 1995. Resistance of ETEC strains to ampicillin increased from 18% to 100%. Similarly, resistance of EPEC strains increased from 18% to 89%, indicating the virtual ineffectiveness of this antibiotic to *Esch. coli* infections. They were also resistant to chloramphenicol and tetracycline with the same trend. Nowadays both *Shigella* spp. and *S.typhi* were resistant to ampicillin, chloramphenicol and septrin.

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Counselling needs of hepatitis B surface antigen (HBsAg) positive persons

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An action-oriented study was carried out in December, 1998 to explore the knowledge, perception, emotional reactions, and behavioural intention regarding hepatitis B surface antigen (HBsAg) positive test result. Opinions on counselling needs of the hepatitis B surface antigen positive persons were also explored. A qualitative approach using In-depth Interviews (IDIs) was conducted among 10 recently diagnosed hepatitis B surface antigen positive persons attending the Department of Medical Research (DMR) Vaccine and Diagnostic Clinic. Five men and 5 women participated in the study. Their age ranged from 17 to 51 years. All the respondents came to DMR Vaccine Clinic for voluntary testing of hepatitis B surface antigen. Some visits were job related and others were not. Most of the people tested to be hepatitis B surface antigen positive usually perceived the test result as a great threat to life. Common responses to the positive test result were surprise, fear, denial, and worry. The majority of the respondents did not know what the test positive result means. Even though the respondents were educated, they could not identify the disease transmission and consequences of hepatitis B surface antigen positivity properly. Their future behavioural intentions were vague and unclear. They all suggested that counselling to hepatitis B surface antigen positive persons was essential. When explored the counselling needs of hepatitis B surface antigen positive persons, the majority preferred being counselled in a group by a same sex counsellor. They also stressed that counselling service should be supported by a clinical and laboratory service.

INTRODUCTION

The hepatitis B virus (HBV) has infected more than 2,000 million persons alive today and 350 million persons are chronically infected carriers of the virus, at high risk of death from active hepatitis, cirrhosis, and primary hepatocellular cancer. Each year approximately 1 million people die from the acute and chronic sequelae of HBV infection [1]. Therefore, most people tested to be hepatitis B surface antigen positive (HBsAg) perceived the positive result as a great threat to life, and suffered from undue burden of emotional stress, resulting in reduced quality of life. As many HBsAg positive people may lack information and support, it cannot be taken for granted that they would change certain life styles and behaviors with a view of preventing transmission of HBV infection to others.

HBV infection is hyperendemic in Myanmar with a 10 percent carrier rate, 60-70 percent infection rate [2], and Myanmar is trying to integrate hepatitis B vaccine into the Universal Child Immunization Programme. In the meantime, the Department of Medical Research (DMR) Vaccine and Diagnostic Clinic is providing vaccination with plasma derived HBV vaccine and diagnostic testing for HBsAg status, to clients at a very reasonable price.

From experiences of the clinic medical officers, these newly diagnosed HBsAg positive persons were greatly concerned about the positive result and were at a loss

to adopt an appropriate plan of actions to cope with the problem. This may be due to the fact that these people may lack appropriate information concerning HBV infection and support to enable them live positively with HBV infection.

Despite an unmet need, counselling services for HBsAg positive persons are not well developed in our country. Thus, it is necessary to explore as a baseline data about knowledge, perceptions and emotional reactions of newly diagnosed HBsAg positive people for the training and conducting of counselling sessions. This is, therefore, an action-oriented study to support the future hepatitis B prevention programmes aiming towards HBsAg positive persons.

General objective

To explore the knowledge, perception, emotional reactions and behavioural intentions concerning HBsAg positively and counselling needs of hepatitis B surface antigen positive persons.

Specific objectives

1. To assess the emotional reactions of the clients to a positive HBsAg test.
2. To explore the perceptions of HBsAg positive test result in respect to disease meaning, transmission, complication and social implications.
3. To find out behavioural intentions of the clients and opinion regarding counselling.

MATERIALS AND METHODS

An exploratory qualitative study design was used in this study. Using the dimensional sampling method, a total of 10 adults both male and female, married and unmarried were included in the study. The study was conducted at Department of Medical Research Vaccine and Diagnostic Clinic in December, 1998.

In-depth interview for each client was conducted by a trained moderator with the assistance of a note taker and a cassette recorder. The discussions were transcribed, translated and mathematically coded. Commonalties were extracted through words, phrases and themes and re-coded and summarized. Then, the analysis framework was developed. The final analysis was done according to the major themes and sub-themes.

RESULTS

It is acknowledged that the information presented in this study may not be entirely representative of the general population. However, due to the qualitative nature of the study, the emphasis is on the content of the information gathered.

Reasons for testing

Among the studied subjects, all came to the diagnostic clinic on a voluntary basis. Most of the men were job related in testing the HBsAg. Some came to the clinic with family members for testing because it was a popular and dangerous infection.

"A *lot of people are saying about the disease, and they said it is highly infectious. So I came and tested for it with a friend."*

(An ongoing deck cadet, single)

"My husband is going to Singapore as a carpenter, I came with him and he told me to take the test while we are here."

(A pregnant mother of 2 children)

Emotional reactions to a positive HBsAg test

Most of the people tested to be the HBsAg positive usually perceived the positive result as a great threat to life. The most common responses are surprise, fear, denial, and worry.

"I *came here to take a test and to vaccinate myself. I was so sure of myself that I won't have the disease. I feel something. I don't know how to express it. I can't believe the result. It's so surprising.*"

(A 32- year- old single deck cadet)

"I'm so afraid, I heard about the disease on the television by a liver specialist

Saya U Khin Mg Win. I'm trying to keep calm. But still I'm so afraid." (Very afraid and so enthusiastic to inquire questions)

(A 37-year-old school teacher, single)

Perceptions about the positive HBsAg test

Majority of the respondents did not know what the test positive result means. They were mixed up with the disease. Some knew that it was due to an infectious organism. Followings are their responses.

"It's having a disease. But I don't mind. I'm old. I think it's bacteria. I've read and it's some form of bacteria."

(A 51-year-old, government officer, married man)

"I don't exactly know what it is. I've heard that it was due to pork. You see I eat a lot of pork."

(A single adolescent from Institute of Marine Technology)

Regarding the questions on hepatitis virus infection and its type, majority think that if they have the positive result they are going to suffer from some forms of liver disease in near future. When asked about the types, some respondents could answer correctly but some of them mentioned others such as liver cancer and dry liver (a local term used for cirrhosis).

"I don't know. I think it affects the liver. I know five types. They are crunchy liver, hard liver, dry liver. I don't know that if they are related to hepatitis or not."

(A pregnant mother of 2 children)

"I understand that it may be inflammation of liver. It is infectious. I don't feel anything"

(A 27-year-old single female)

Social implications

HBsAg testing in relation to work was one of the strongest reasons for testing HBsAg and about half of the respondents were job related in testing. Two youngsters were to enter a school, 2 men were to go abroad for working. A man who is a government officer came for testing without any reason in relation to job. Most of the women came along with their spouses or friends to be immunized.

*"*I *am worried, because I want to be a sailor. For the time being it's O.K., but people say that when you enter some country you need the vaccination certificate. I don't know what will happen as a consequence, but still I got depressed."*

(A 32-year-old married male)

"Yes it does affect my work. I was going to attend the Institute of Marine Technology. My dreams were wiped away."

(A 17-year-old adolescent)

Among the interviewed respondents, half were married and others were single. Almost all of the respondents were not clear of the relationship between HBsAg positivity and marriage. Those who came with their spouses who were negative at the time of diagnosis took the immunization immediately.

*"*I *don't know if there is connection. My wife is negative and she has been immunized."*

(A 51-year-old government officer)

Possible mode of transmission

It was very interesting to hear from the interviewed respondents regarding their belief in possible mode of transmission.

"You see I eat a lot of pork. I have heard that it is due to pork. I myself like pork very much"

(A 17-year-old adolescent)

"I don't know how I got the disease. I have no idea about the transmission. You see I don't usually take injection. The only thing is I told my children to take out my heat"

(A pregnant mother with 2 children)

Knowledge about HBV in relation to transmission

Regarding to knowledge about HBV transmission, majority of the respondents could not identify exactly the mode of transmission. Irrespective of their educational status, they could only mention few types very vaguely.

"I *think it can be transmitted through injection. I usually ask the doctor to use the disposable needle. I think you should also avoid eating pork. Because people say that if you eat pork it's easier to die."*

(A 39-year-old married male with 3 children)

"I *don't know much, but I have heard of some things, injection needle, drinking water, food and chilli sauce can cause hepatitis. When people have low resistance the disease can occur."*

(A 27-year-old graduate women)

Knowledge about HBV in relation to its complication

The interviewed respondents could not differentiate between the disease and its complication. Most of them thought that if they have the HBsAg they were having the disease inside them.

"The complications are crunchy liver, dry liver, liver cancer. But you see I suffered from hepatitis when I was very young. If there were complications, during these years I would have died. But you see nothing has happened to me."

(A 39-year-old married male)

"I *know liver cancer, dry liver but you see all are mainly due to alcohol. A person may or may not develop the complications."*

(A 32-year-old single deck cadet)

Behaviour intentions

Most of the respondents wanted to get rid of the disease or organism completely. Almost all have positive behaviour intentions (apart from one old man). Even though the respondents were quite high in educational level, their behavior intentions were vague and unclear. They were also not clear of the preventive and healthy living in relation to the disease.

"I *want to get cured. I've heard a lot of people saying indigenous medicines are more superior than western medicines in treating hepatitis infection. An indigenous healer who lives in front of Bogalay Market is famous. I am going there to see. And I have to watch my diet. I will advice my family be tested and vaccinated."*

(A 39-year-old father of 3 children)

"*I will separate my cup for drinking water. The main cause is water. I will also separate and use my own belongings. I will buy those big sterile water bottles for myself and use them as my own. I have to avoid another thing and it's cold. To increase my immunity I will try to eat a lot of organ meat."*

(A 19-year-old single male)

Opinion regarding counselling need

All the interviewees said that health education is essential in prevention and control of hepatitis B viral infection. They thought that it should be strengthened to reach the general public. They also stressed that counselling of individuals with positive HBsAg is also in great need. Because when they were diagnosed they did not know what to do, to whom to go and how to prevent further spread. They all have different views in counselling and the responses are as follows:

	How	Sex	When	Where	What
1	Groups	Same sex	After alcohol hours	No preference	Disease process and treatment
2	Groups	Same sex	Suitable time	Private place but not too private	Counselling and service
3	Single	No sex differen-tiation	Appoint-ment	No preference	Counselling service and support
4	Single	Same sex	Appoint-ment	No preference	Disease process
5	Groups	No sex differen-tiation	No preference	No preference	Disease process media aids and its outcome
6	Groups	Same sex	Any time	No preference	Disease process
7	Single	Same sex	Any time	No preference	Counselling and service
8	Groups	No sex differen-tiation	Any time	No preference	Disease process and how to live with the positive result
9	Groups	No sex differen-tiation	No preference	No preference	Disease process and prevention
10	Single	Same sex	Appoint-ment	No preference	Disease process

Those who preferred to have counselling given in a matter of groups or single had their own reasons.

"It would be better to give counselling in groups so that I can feel that I'm not alone and we can share our experiences."

(A 32-year-old single deck cadet)

Majority expressed that counselling should be given by same sex persons so that they could discuss more openly and frankly.

"I *think it should be given singly by a same sex health personal. So I can also ask private questions and discuss more freely."*

(A 19-year-old single male)

"I *feel more safe talking to a same sex personal*"

(A pregnant mother with 2 children)

Regarding time and place for counseling, majority had no preference. Almost all of the respondents stressed that counselling should be supported by service like clinical examination and blood testing.

"If you just talk and explain I would only come once or twice, no more. You should include a blood testing service and do clinical examination and explanation about the disease progress."

(A 51-year-old government officer)

DISCUSSION

In this study, it was evident that majority of the subjects who came for HBsAg testing were on a voluntary basis. Among them, most of the men were job related in testing the HBsAg status. This highlighted that job related HBsAg testing has become an important measure among the working community nowadays. Therefore, proper health education concerning hepatitis infection and its consequences should clearly be given to the high risk working communities (in a very positive manner).

According to our study we have explored that most of the HBsAg positive persons misunderstood about the disease process and HBsAg positivity. They all thought that they will suffer from some form of sequelae relating to hepatitis. They perceived the HBsAg positive result as a great threat to their life. This suggested that threat caused by the disease/infection has already been alarmed into the community. But there are still misperceptions about the disease transmission and disease process. There are in two extremes, some perceive the HBsAg positive result as a life threatening condition and some think it's no use worrying about the disease. Thus counselling should be encouraged to the HBsAg positive persons, about the misperceptions and carrier status. During adulthood infection rarely leads to the carrier state in which only 10% of adults turn out to be chronic carriers. And among them only 25% of them are at risk of serious illness and eventual death from its consequences [2].

Counselling regarding hepatitis infection has not been properly performed in our country up to date. Not only in our country but also in many parts of the endemic world, preventive efforts mainly aiming at immunization have dimmed the importance of the carriers who are the major source of infection. Therefore, counselling of HBsAg positive persons should be done as an essential component in prevention of hepatitis infection.

In this study, all of the interviewees pointed out that counselling is essential and they have stated the various ways of counselling like individual and group counselling. Majority preferred to be counselled by the same gender (sex) so that they can discuss more openly about personal matters. They had no specific preference in regard to time and place for counselling. They all suggest that counselling should be done in accordance with a clinical service like blood testing and clinical examination. Thus, voluntary HBsAg testing accompanied by counselling is a vital role to play within a comprehensive range of measures for prevention and control of hepatitis.

Hepatitis B prevention and support should be encouraged widely to reach the general public. The potential benefit of testing and counselling for the individual include improved health status through good nutritional advice and earlier access to care and treatment/prevention for hepatitis B related illnesses. Counselling also plays a vital role as an emotional support for better ability to cope with the disease (or) test positive related anxiety. Other preventive measures like awareness for safer options for reproduction and child bearing, motivation to initiate and maintain safer sexual practices and safer blood donation practices should be included in the counselling discussions.

Recommendations

1. Counselling service and training of counsellors for hepatitis infection should be incorporated into the hepa titis prevention programme.
2. Clear and precise information regarding hepatitis infection should be given to the community.

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Effect of neem insecticide on *Aedes aegypti* larvae in the laboratory

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Prospective controlled laboratory trials were conducted to determine the effect of neem insecticide (a.i. *azadirachtin* 0.75% SC) on third and fourth instars of *Aedes aegypti* larvae using six different concentrations (*aza* 0.0002% to 0.0064%) in accordance with WHO instructions on larval susceptibility test. These trials were performed in Health and Disease Control Unit, Mingaladon in September, 2001 and included six replicates, each consisting of total number of 150 larvae. It was found that the larvae were highly susceptible to *aza* and LC_{50} and LC_{90} were 0.00038% and 0.00087% respectively. The residual effect of insecticide was calculated applying the insecticide stock solution (*aza* 0.15%) of shelf-life day 1, 2, 3, 7 and 10 and it normally persisted for three days. Optimal concentration 0.0016% had the efficacy for seven days. The local product neem insecticide was found to be effective against *Ae. aegypti*, non-toxic to human and environment, easily available and cheap. Therefore, neem insecticide was found to be very promising to be used effectively in the field in treatment of miscellaneous water containers such as discarded unused tires, batteries, tins, coconut shells etc. – well-breeding sources of the vectors *Ae. aegypti* responsible for occurrence of dengue haemorrhagic fever.

INTRODUCTION

Phytochemicals derived from various botanical sources have provided numerous beneficial uses ranging from pharmaceuticals to insecticides. Synthetic organic insecticides, although highly efficacious against target species such as mosquitoes, can be detrimental to a variety of animal life including man. In addition to adverse environmental effects from conventional insecticides, most major mosquito disease vectors and pest species have become physiologically resistant to many of these compounds. These factors have created the need for environmentally safe, degradable and target-specific insecticide against mosquitoes. The search for such compounds has been directed extensively to the plant kingdom [1]. The most prominent phytochemical pesticides in recent years are those derived from neem trees, which have been studied extensively in the field of entomology and phytochemistry, and have uses for medicinal and cosmetic purposes [2]. The neem tree was described as *Azadirachta indica,* belonging to family *Meliaceae* (mahogany family). Neem is called *Tamar* in Myanmar where the neem tree could have originated. Among active ingredients in neem tree *azadirachtin* (*aza*), a very complex tetranortriterpenoid ($C_{35}H_{44}O_{16}$) is the main component which is present in its seed kernel in highest concentration [3]. In Myanmar, neem insecticide (a.i *aza* 0.75% SC) is produced mainly for agricultural pests from Neem Insecticide Factory in Paleik, Mandalay Division.

The currently used insecticides to control *Aedes* are temephos (abate) as a larvicide and malathion and fenitrothion as

adulticides. They are effective but not locally available and purchased from foreign countries at the large expense of foreign exchange. They also have chemical toxicity to man if not properly used though they have immediate lethal action on the larvae. The routine vector control methods are easy to carry out but there are many difficulties on consumers' side in treatment of *Aedes* breeding water containers out of which miscellaneous containers play an important role in producing *Aedes*. In that situation neem insecticide was focused to study with the objectives of : (a) to determine the 24 hours exposure effect and residual effect of neem insecticide on the *Ae. aegypti* (L) larvae and (b) to evaluate its potential use for the control of dengue infection.

MATERIALS AND METHODS

Study design was a prospective controlled laboratory trial conducted in Health and Disease Control Unit, Mingaladon from September, 2001 to November, 2002 and field sample collection site was Ward 3 Yanpye, Thaketa Township, Yangon Division.

Ae. aegypti larvae were collected randomly from at least thirty houses in the collection site for representative purposes. Neem insecticide (a.i. *aza* 0.75% SC) was purchased from Neem Insecticide Factory. Methanol 5% as control agent was purchased from Myanma Pharmaceutical Factory and disposable plastic cups from local markets.

Larval susceptibility tests were conducted against third and fourth instars of the larvae by using the insecticide. Six concentrations of insecticide commencing from 0.0002% rising double strength up to 0.0064% were used for six replicates. The tests were carried out according to the WHO instructions on larval susceptibility test. Larvae were collected from domestic water containers (e. g. metal drums) in collection sites using a clear plastic bucket (diameter 24.7 cm and height 25.0 cm) and they were kept, in the laboratory for one day for adaptation purposes, in plastic trays (34.5 cm x 24.5 cm x 6.0 cm) containing rain water before carrying out the test.

Stock solution of insecticide was made for one replicate containing six concentrations as follow: Five mililiter of insecticide was put into a clean disposable plastic bottle (diameter 7.5 cm and height 26.5 cm) containing 20 ml of rain water. Therefore the resultant stock solution was *aza* 0.15% solution. 0.33 ml of stock solution was taken and added into first clean disposable plastic cup (diameter 7.5 cm and height 11.5 cm) containing 224.67 ml of rain water and it was thoroughly stirred for 30 seconds with a glass-rod. Then 0.66 ml of stock solution was added into second cup containing 224.34 ml of rain water. Likewise double the amount of stock solution was added till the sixth cup was completed. Being a methanol neem seed extract, for control 0.5 ml of methanol 5% was added into the cup containing 224.5 ml of rain water. At the same time each batch of active and vigorous 25 larvae (13 third and 12 fourth instars) together with natural food from their habitats was transferred from plastic trays to 7 small clean disposable plastic cups (diameter 6.0 cm and height 4.8 cm) each containing 25 ml of rain water. Next each batch of 25 ml of rain water together with 25 larvae was poured

Table 1. Preparation of six neem insecticide concentrations (*aza* 0.75% SC)

No.	Initial rain water (ml)	Added stock solution (ml)	Added rain water (ml)	Resultant *aza* concentration (%)	Resultant *aza* concentration (ppm)
1	224.67	0.33	25	0.0002	2
2	224.34	0.66	25	0.0004	4
3	223.68	1.32	25	0.0008	8
4	222.36	2.64	25	0.0016	16
5	219.72	5.28	25	0.0032	32
6	214.4	10.6	25	0.0064	64

Aza concentration in stock solution of neem insecticide is 0.15%

into the six cups each containing 225 ml of insecticide solution and one control cup. The final *aza* concentrations in the cups were 0.0002%, 0.0004%, 0.0008%, 0.0016%, 0.0032% and 0.0064% (Table 1). Moribund and dead larvae were counted as dead after 24 hours exposure. The tests were done in six replicates.

For residual effect of insecticide, stock solution (*aza* 0.15%) was kept for one, two, three, seven and ten days and then tested against third and fourth instars (10 in numbers) separately using three replicates at each concentration. Then mortality percenttages were calculated after 24 hours exposure and compared.

The temperature (21°C to 35°C) and relative humidity (64% to 92%) were recorded throughout the test period. Data analyses were done by using Epi Info Version 6.04 and S. Swaroop's statistical method for χ^2 test to determine correlation co-efficient and LC_{50} and LC_{90} values [4].

RESULTS

The test results showed that LC_{50} and LC_{90} values of *aza* for thrid and fourth instars of *Ae. aegypti* larvae were 0.00038% and 0.00087% respectively and 95% confidence upper and lower limits were described (Table 2). Correlation between dose and effect was in a strong degree but not statistically significant ($r = 0.66$, $p > 0.05$) due to a small sample size of concentration of insecticide (n= 6). The χ^2 test of goodness of fit of the regression line showed that the mosquito populations were significantly heterogeneous ($p< 0.01$). Regarding residual effect of insecticide, its effects were more or less the same up to 3 days in all concentrations except 0.0004% at which larval mortality gradually decreased. After 3 days mortality decreased in all concentrations up to seventh day except at the last two concentrations. After seven days, mortality increased again in first three

Table 2. Fitting a regression line and testing the goodness of fit [Data on susceptibility of *Ae. aegypti* larvae to neem insecticide (*aza* 0.75 % SC)]

No.	*Aza* concentration (%)	Larvae dead/ tested	Observed mortality rate, (%) (corrected)	Expected mortality rate (%)	Observed minus expected rate	Contribution to χ^2
1	0.0002	12/150	8	4	4	0.04167
2	0.0004	83/150	55	54	1	0.00040
3	0.0008	111/150	74	88	-14	0.18561
4	0.0016	146/150	97	97.1	-0.1	0.00004
5	0.0032	150/150	100 (99.86)	99.4	0.46	0.00355
6	0.0064	150/150	100 (99.86)	99.84	0.02	0.00003
7	control	6/150	4	-	-	-
			Total			0.2313

LC_{50} = 0.00038 %
95 % confidence limit, lower = 0.00035 %
95 % confidence limit, upper = 0.00041 %
LC_{90} = 0.00087 %
95 % confidence limit, lower = 0.00077 %
95 % confidence limit, upper = 0.00098 %

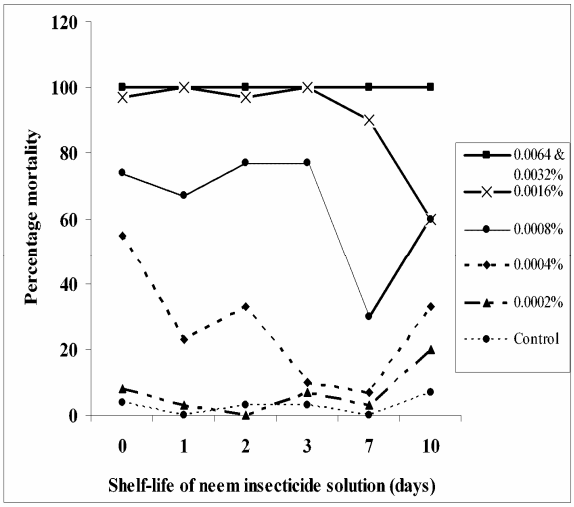

Fig. 1. Residual effect of six neem insecticide concentrations on third and fourth instars of *Ae. aegypti* larvae

concentrations, probably due to water pollution rather than the larvicidal action of insecticide, but decreased in 0.0016%. Cent per cent mortality was found at the last two concentrations in all days (Fig.1). Therefore neem insecticide is effective for three days

in all concentrations. After three days, percent reduction of residual effect in terms of larval mortality was between 10% and 61%. Optimum concentration for seven days was 0.0016% to obtain 90% larval mortality.

DISCUSSION

The effects of neem insecticide on insects are: phago- and oviposition-deterrent, repellent, antifeedant, growth retardant, molt-inhibitor and sterilant. Zebitz (1984) suggested that *aza* acts as an anti-ecdysteroid or affects the neuroendocrine control of ecdysteroids [1, 3]. Exposure of fourth instar larvae of *Ae. aegypti* to water extracts of neem resulted in conspicuous disruption of growth whereas exposure of first instar larvae caused a prolongation of the larval period and eventually about 90% mortality [5]. Aqueous extract of neem seed gave LC_{50} value of 78 mg/L when it was tested on first and fourth instars of *Ae. aegypti* larvae and LC_{50} value of methyl-tert-butyl-ether extract was 2 mg/L [3]. Effect of 'Margosan O' neem insecticide produced from USA (*aza* 0.3 %) on third and fourth instars of *Ae. aegypti* larvae showed that LC_{50} values were 12 ppm and 14 ppm respectively [6]. The results from the present study are much lower than those of above-mentioned study. The present study shows that effect of neem insecticide on *Ae. aegypti* larvae was very satisfactory. Residual effect also showed that the insecticide efficacy still persisted for three days. It is the same as the data in the study by IM Scott *et.al*, which showed that in the residue analysis, *aza* had a half-life of 36-48 hours in water exposed to normal sun light [7]. About 39% to 90% of third day efficacy remained till seventh day. Optimal concentration was 0.0016% for seven days.

Neem trees are widely and abundantly distributed in Myanmar where neem seed kernels were supposed to have highest concentration of *aza* (i.e. 6.1 ± 0.7 mg/g kernel) among many samples from 22 countries in the world [3]. The State has invested on a large scale in neem plantation in the country, for example in Shwe Pontaung region, Chauk Township, Magwe Division where nearly 0.5 million of neem trees are to be cultivated (300 neem trees per acre) from year 2000 to 2002 under the special project. In the country the existing accessible mature neem trees in central part alone can give about 766,000 bottles (500 ml) of neem insecticide annually [8]. At present, the factory produces 90,000 bottles (500 ml) annually. The local price is 500 kyats per bottle and four US $ per one liter bottle for export. Therefore, local price is cheap (Aye Ko, personal communication, 2001).

Dengue haemorrhagic fever (DHF) is one of the health problems in Myanmar especially in 5-15 years age group. It is endemic here with 3-4 years epidemic cycle. Reported cases are 1000-3000 every year in non-endemic year and 3000-15000 in epidemic year. Case fatality rate is 3.9 % [9]. At present, it is under 1 % probably due to the early referral of the cases to the hospitals. There are three categories of container type-major, minor and miscellaneous, which are all the breeding sites of *Ae. aegypti* (W. Tun Lin, unpublished data, 1995). Among many types in last category, tires may be key containers because they are used widely in various sizes from small to large since market-oriented economy is enacted and put into effect in our country. In a study by W. Tun Lin *et.al*., key container types, for example, tires and drums comprising 10-20 % of total containers were responsible for 82.6 - 99% of the *Aedes* being produced [10].

Regarding present vector control programme, the methods are simple but reportedly not carried out by local people regularly due to economic and administrative reasons, for example, going out for work, shortage of municipal workers to clean the solid waste and unwillingness of people to do. The insecticide has an unpleasant odour but the people normally accept it, if it is used in outdoor discarded

materials like unused tires, batteries, jars, cisterns, plant containers and coconut husks and temporary small water pools when removal and destruction is not feasible. Neem insecticide alone does not complete but it will be complement for the programme. Even if the larvae are alive after being exposed to *aza* their growth will be delayed and male adults will become sterile. Such slow action of botanical insecticide is not well understood by most of the people who generally like synthetic insecticide, which gives immediate toxic effect on the insects. So the effect of botanical insecticide should be thoroughly explained to the consumers if it is used in the vector control programme. Dosage of neem insecticide (*aza* 0.75% SC) for discarded container is 1 ml per liter of water held to have 90% mortality of larvae. One millilitre of neem insecticide costs only one kyat. Conventional insecticides like fenitrothion and malathion are expensive. By using local insecticide the country will be able to save much foreign exchange.

Neem insecticide is effective, cheap and in accordance with State policy. So, this study would support the present vector control programme in the country to a certain extent. The findings in the present study are compatible with those in other studies and give useful basic research data for appropriate technology for DHF control in Myanmar. Therefore it is recommended that neem insecticide with extraordinary characteristics of being safe, effective, locally available, economical and non-toxic to human and environment should be used to control *Ae. aegypti* mosquito, the vector of DHF which affects thousands of children under fifteen in our country annually.

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Comparing the efficacy of initial single dose rectal artesunate versus single dose intravenous artesunate at 24 hours and after full consolidation treatment in both groups with intravenous artesunate in severe falciparum malaria in adults

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The mortality rate of severe falciparum malaria, once vital organ dysfunction occurs, is as high as 30%. In remote areas, transport problems lead to high morbidity and mortality. Rectal artesunate, single dose is recommended for initial management of severe falciparum malaria especially in remote areas, to be followed by consolidation treatment at the nearest health care facility. A randomized controlled trial of rectal artesunate and parenteral artesunate was studied on 60 severe falciparum malaria cases admitted to DSGH. Test drug was Plasmotrim 50 mg rectocap 200 mg stat dose. Control drug was injection Artesunate 120 mg intravenous infusion, both arms followed at 24 hours by injection Arte-sunate 60 mg intravenous infusion, repeated 12 hourly (total=480 mg), plus tetracycline or clindamycin 250 mg 6 hourly for 7 days. Parasite clearance time in rectal artesunate was 46.3 hours and IV artesunate group was 49.1 hours and the 24 hours parasite clearances were 81.7% and 76.3% respectively ($p>0.5$). There was no difference in the 48 hour parasite clearance which were 96.2% and 96.9% respectively. Fever clearance time (FCT) was 51.5 hours with rectal artesunate compared to 30.3 hours with IV artesunate ($p>0.1$). The clinical success rate for rectal artesunate was similar to that of parenteral artesunate at 24 hours in this study, highlighting that it can be used effectively prior to definitive treatment to reduce malaria morbidity and mortality.

INTRODUCTION

Falciparum malaria remains a major cause of death in the tropics. Cerebral malaria,the most prominent manifestation of severe malaria carries at 16-20% mortality [1]. Once multiple vital organ dysfunction occurs, the mortality rate, even after treatment, is as high as 30%(WHO, 2000) [2]. Despite many treatment trials, no interventions have been shown conclusively to reduce this high figure.

The artemisimine derivatives are now the most rapidly acting, safe and potent of all the antimalarial drugs for treatment of *P. falciparum* malaria. In rural areas, where the transport facilities are poor, the mortality and morbidity rate is higher because of late referrals. The early initiation of artemisinin compound can save lives. WHO (UNDP, World Bank, and WHO Special Program for Tropical Diseases Research) selected rectal artesunate for investigation as a candidate to provide therapeutic cover for the initial 24 hours after presentation. Studies in Africa reported artesunate in rectal preparation in adults and children had a median parasitaemia reduction of 99% at 24 hours [3]. Rectal artesunate single dose

has now been approved by the United States Food and Drug Administration (US-FDA), for the initial management of severe falciparum malaria patients, who are unable to take oral medication and where parenteral anti-malarial treatment is not available. It was to be followed by further consolidation treat-ment at nearest health care facility. Hence, rectal artesunate makes a good stopgap until patient reaches health facilities [4].

In Myanmar, the usefulness of artesunate suppositories (Plasmotrim^{TM} rectocaps) in severe falciparum malaria (including patients in un-rousable coma) has been reported by our group in a prospective double blind randomized controlled study on 100 adult patients, on two dosage regimens. Artesunate suppository total dose 800 mg and 1200 mg given over 3 days each, was well tolerated, effective and cleared parasitaemia within 60 hours in both dosage regimens. The addition of mefloquine ensured a satisfactory 28 day cure-rate of 100 % [5].

However, there are no reported data on the 24 hours efficacy of single dose rectal artesunate in Myanmar yet. This study was aimed to determine whether administration of single dose rectal artesunate would provide beneficial initial antimalarial cover, indicated by a rapid fall in the density of parasitaemia and clinical improvement without serious adverse reactions, compared to a standard i.v. artesunate regimen. It will also look into the outcome at 14 days, after giving consolidation treatment of i.v. arte-sunate (in accordance with the National malaria treatment guidelines for severe malaria).

Objectives

1. To compare the therapeutic efficacy at 24 hours, of a single dose rectal artesunate with a single dose iv. artesunate.
2. To compare the final outcome between the two groups at 14 days, after giving identical consolidation treatments of i.v. artesunate.

PATIENTS AND METHODS

Laboratory confirmed, severe falciparum malaria patients admitted to Clinical Research Unit (Malaria) Defence Services General Hospital were recruited for the study.

Inclusion criteria

- Both sexes
- Age between 10 - 60 years
- Positive peripheral blood film for trophozoite forms of pure *Plasmodium falciparum*
- Informed consent

Exclusion criteria

- Patients who received parenteral anti-malarials (quinine >1200 mg) within the past 24 hours
- Patients with other concomitant diseases
- Patients with severe diarrhoea, bleeding per rectum, dysentery

Withdrawal criteria

- Patient's request
- Any serious adverse effects to drugs
- Serious or repeated non-compliance with protocol specifications

Sample size

60 patients

Study design

A hospital–based randomized controlled study.

Study period

July 2004 to September 2005

Drug regimens

A. Initial treatment

Test drug (30 patients)

- Plasmotrim 50 rectocap (Mepha-Switzerland. Batch No. 0250638
- 200 mg (4 rectocaps) single stat dose

Control drug (30 patients)

- Artesunate injection 60 mg (Guilin – pharma, China, Batch No. 020702)
- 120 mg single dose i.v. infusion

B. Consolidation treatment at 24 hours followed by full course of treatment

Artesunate injection 60 mg (Guilin pharma-China) 60 mg i.v. infusion at 24 hours and then 12 hourly (total dose for full course: - 480 mg) was given for both arms of treatment.

C. Combination drug

For adults:- Tetracycline 250 mg (MPF-Myanmar) 6 hourly for 7 days

For pregnant females & children: -

Clindamycin (Kalbe - Farma, India)

250 mg 6 hourly for 7 days was given when the patient could take by mouth

Procedure

Eligible patients were subjected to the following procedure. Routine history taking , clinical examination and relevant investigations were done and recorded in the standard proforma. Patients were randomized to the drug regimens, using sealed envelopes.

The following assessments were made.

1. *Clinical* (Day 0,1,2,3,4,7 and 14)
   - Symptoms review
   - Adverse effect review (according to the check-list)
   - Physical examination
   - Body temperature recorded 4 hourly until normal for 24 hours and then daily up to Day 14.

2. *Parasitology* (Day 0,1,2,3,7 and 14)

   Giemsa's stained thick and thin blood smears were examined and parasite counts performed 6 hourly until negative for 24 hours and daily up to 3 consecutive negatives, then weekly up to Day14.

3. *Haematology* (Day 0, 3,7 and 14)
   - Hb%, T & DC

4. *Biochemistry* (Day 0, 3,7 and 14)
   - Serum bilirubin
   - Blood urea, sugar

5. *ECG* (Day 0,3, 7 and 14)

Therapeutic response was assessed as follows:

a) For clinical response at 24 hours
   Parasite clearance
   Fever clearance
   Development of severe symptoms and signs
   Mortality
   Adverse effects
   Tolerability of drug

b) For final outcome after consolidation treatment (at 14 days)
   Early treatment failure (ETF)
   Late treatment failure (LTF)
   Adequate clinical and parasitoligical response (ACPR)
   Mortality
   Sequelae
   Adverse effects

Adverse effects were noted by means of a check - list clinically, haematologically, biochemically and ECG in the proforma.

Ethical considerations

The protocol was approved by the Ethical Committee, Department of Medical Research (Lower Myanmar). Informed written consent was obtained from all the patients/ relatives.

Statistical evaluation

Statistical evaluation comparing the regimens was done by computer using EPI-INFO software. Categorical data were compared by calculating the chi-square value with Yates' correction or by Fischer's

exact test. Normally distributed continuous data were compared by the Student's t test and analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U test.

Retreatment of failure

ETF cases were retreated with standard i.v. quinine dihydrochloride (10 mg of salt / kg 8 hourly).

LTF cases were retreated with standard oral quinine sulphate (10 mg of salt/kg three times a day).

RESULTS

Age, weight, initial temperature and initial parasite counts were comparable in the two groups. Initial parasitaemia was Mean (Range) 70,819 (1200 - 250,000) in artesunate rectocap and 50,933 (800 - 250,000) per microlitre in i.v. artesunate group. (p> 0.5) (Table 1).

Table 1. Baseline characteristics of the two groups

Parameters	Regimens		*'P'*
	PR Artesunate	IV Artesunate	
Age (years), mean	25.37	28.13	>0.5
(S.D.)	(8.71)	(11.37)	
Height (inches), mean	166.5	164.86	>0.1
(S.D.)	(10.09)	(8.28)	
Body weight (Kg) mean	49.22	50.79	>0.5
(S.D.)	(4.99)	(9.32)	
Initial temperature (°C) mean	38.7	38.67	>0.5
(S.D.)	(0.88)	(1.07)	
Initial parasitaemia / µl Geom mean(range)	70,819 (1200-250,000)	50933.33 (800-250,000)	>0.5

The rectal forms of artesunate were well tolerated and not expelled in adults, hence none were needed to be reinserted. At 24 hour after start of treatment, 43.3% of 30 patients who received rectal artesunate had a parasite density below 10% of baseline, compared with 36.7% of 30 on i.v. artesunate group. Percentage mean parasite reduction at 24 hours was 79.83 ± 22.38 % in per rectal group and 76.27 ± 21.78 % in the intravenous group. The median fractional reduction of parasitaemia at 24 hours was 88% and 79% in patients treated with rectal and intravenous artesunate, respectively (Table 2).

Table 2. Response at 24 hours and 48 hours for two groups

Response	Regimens	
	PR Artesunate	IV Artesunate
Response at 24 hours		
Percentage of patients with parasitaemia below 10% of baseline	43 .3	36.7
Percentage parasite reduction, mean (S.D)	79.83 ± 22.38	76.27 ± 21.78
Median fractional reduction of parasitaemia %	**88**	**79**
Tolerability of treatment	Good	Good
Adverse effect of drug	Nil	Nil
Mortality	Nil	1
Response at 48 hours		
Percentage of patients with parasitaemia below 10% of baseline	90	93.3
Percentage parasite reduction-mean (S.D)	92.47 (22.36)	96.89 (5.98)
Median fractional reduction of parasitaemia (%)	**100**	**100**
Tolerability of treatment	Good	Good
Adverse effect of drug	Nil	Nil
Mortality	Nil **	Nil

**(1 death at 72 hours)

At 48 hours, i.v. artesunate consolidation treatment started in both groups, 90% of 30 patients who received initial rectal artesunate, had a parasite density below 10% of baseline, compared with 93.3% of 30 patients who received initial i.v. artesunate. Percentage parasite reduction at 48 hours was 92.47 ± 22.36% in per rectal group and 96.89 ± 5.98 % in the intravenous group (p>0.5). The median fractional reduction of parasitaemia at 48 hour was 100% in both groups (Table 2). Parasite clearance time was 46.01 ± 21.36 hours and 43.39 ± 26.90 hours, and Fever Clearance time was 56.67 ± 46.85 hours and 27.87 ± 32.43 hours, respectively in the rectal and intravenous regimens (Table 3).

Adequate Clinical and Parasitological Response (ACPR) was 96.7% in per rectal group and 86.6% in intravenous group at14 days. Early treatment failure (ETF) was

3.33% in per rectal group and. 6.7% in intravenous group. Late treatment failure (LTF) was 0% in per rectal group and 6.7% in intravenous group and the results were not statistically different (Table 4).

There were no serious clinical, haemato-logical, biochemical, and electrographic adverse effects of therapy in the both groups within the 14 days observation periods.

Table 3. Clearance of fever and parasitaemia in two groups

Response	Regimens		*'P'*
	PR Artesunate	IV Artesunate	
Fever clearance time (hours) Mean (S.D)	56.67 (46.85)	27.87 (32.43)	>0.5
Parasite clearance time (hours) Mean (S.D)	46.01 (21.36)	43.39 (26.90)	>0.1

Table 4. Cure rates and outcome at 14 days

	PR Artesunate (n=30)		IV Artesunate (n= 30)	
	No.	%	No.	%
Early treatment failure (ETF)	1	3.33	2	6.7
Late treatment failure (LTF)	0	0	2	6.7
Adequate clinical & parasitological response (ACPR)	29	96.7	26	86.6
Sequelae	Nil		Nil	
Total	30	100	30	100

There were two cases that deteriorated in our study:

A 43-year-old female admitted with fever and jaundice and diagnosed initially as cholangiohepatitis in the surgical ward, was referred to the physician for development of acute renal failure with serum creatinine of 3 mg%. Peripheral blood film showed *P. falciparum* parasitaemia of 120,000/µl. In spite of giving intravenous artesunate, the patient rapidly became comatose GCS 3/15, with severe anemia and hypotension 70/50 and died of septicaemic shock within 72 hours after receiving treatment.

The second case was presented with jaundice, acute renal failure and serume
creatinine of 5 mg%, GCS 11/15 with peripheral parasitaemia of *P. falciparum* 9,000/ microlitre. He received i.v. artesunate and died at 24 hours after treatment.

DISCUSSION

Initial clinical outcome (Parasitaemia at 24 hours)

In Africa, Barnes *et. al* reported on 35 adults with moderately severe malaria, randomly assigned to rectal artesunate (single dose of about 10 mg/kg) or parenteral quinine treatment (10 mg/kg at 0, 4, and 12 hrs). Parasitaemia at 24 hours was less than 10% of baseline in 81% of patients allocated to artesunate, compared with 38% in the quinine-treated group. The median fractional reduction of parasitaemia at 24 hours was 99% and 72% in patients treated with artesunate and quinine, respectively [3].

In our study, on 30 adults receiving rectal artesunate 43.3% of patients had a parasite density below 10% of baseline, compared with 36.7% on i.v. artesunate group. The percentage of parasitaemia reduction at
24 hours from the initial baseline parasitaemia was 79.93% in per rectal group and 76.27% in intravenous group. The median fractional reduction of parasitaemia at 24 hours was 88% and 79% in patients treated with rectal and i.v. respectively. Hence, although the percentage of patients reaching below 10% baseline and median fractional reduction of parasitaemia are less than that of Barnes e*t. al*, the percentage reduction of parasitaemia at 24 hours in our study was quite satisfactory.

A single dose of artesunate suppositories at a dose of 200 mg was well tolerated in Myanmar adults, and as efficacious as a single dose of i.v. artesunate. There were no clinical, haematological, biochemical or

ECG adverse effects. After full consolidation treatment with standard therapy of i.v. artesunate, both treatment groups resulted in adequate parasite and fever clearance and satisfactory cure rates. Artesunate suppositories can be given safely and easily by anyone without need for much training, even in the home. However, as single administration, not a curative treatment for falciparum malaria, the need to watch for the expulsion of suppositories and to reinsert them, and of the need to ensure follow-up with an effective curative treatment should be properly informed to the care-givers.

It was concluded that per rectal artesunate is as effective as intravenous artesunate for rapid reduction of parasitaemia load at
24 hours, and is also as safe and effective as intravenous artesunate in the treatment of severe malaria in adults. Thus, single dose rectal artesunate in our study has equivalent clinical outcomes to that of single initial dose i.v. artesunate and also to those of Barnes *et al*. This treatment is of greatest relevance to communities in rural areas, where parenteral treatment is often not immediately available.

It was also recommended that in rural areas where facilities for parenteral treatment are unavailable, the single rectal artesunate which can be easily administered, may be used to serve as a stop gap treatment, before reaching health facilities for further consolidation treatment.

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Short Report

Two sea snake bite cases admitted to Yangon General Hospital

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Epidemiological studies of sea snake bite cases [1] revealed that the bites occurred in Letkokekone, Kyaikkami and Daedaye/ Pyapon / Bogalay. There were very few descriptive reports of sea snakebite. In this communication, we described clinical details and outcome of two sea snakebite cases admitted to Yangon General Hospital.

Case 1

A 21-year-old fisherman was bitten by a sea snake on dorsum of right hand while sorting out fishes at sea at 5 o'clock in the evening of 31 January 1999 (Khatiya, Twantay Township). A slight pain was felt following the prick and the wound was incised and a tourniquet was applied at the forearm. Fang marks were present. Ache and pain, paresis and stiffness of the limb, arm and trunk muscles, pain on moving limbs, ascending weakness, inability to open mouth and feeling of increased sweating developed 1½ hours after the bite. He was referred to Yangon General Hospital 12 hours after the bite. His blood pressure (BP) was 160/90 mmHg, and heart rate (HR) 100 per minute. He developed rashes after receiving 4 ampoules of cobra anti-venom for which he was given i.v. 200 mg hydrocortisone, i.v. 40 mg frusimide, 0.5 ml subcutaneous adrenaline. Complete bilateral ptosis developed at 15 hours after the bite was not responsive to three doses of i.v. 0.5 mg of neostigmine repeated at 5 minutes and 1 ½ hours after the first dose. Reflexes (ankle, knee, biceps and triceps) were reduced, all limbs were flaccid and he passed 700 mls of dark urine. He was incapable of lifting heel and head, unable to sit up unaided and trismus with failing vision were observed. His BP was 160/100 mmHg and HR 120/min. He had cardiopulmonary arrest 36 hours after the bite.

Results of investigations carried out on admission were: sodium 152 meq/L, potassium 3.8 meq/L, chloride 110 meq/L, urea 48 mg%, creatinine 107 µmol/L, glucose 7.6 mmol/L, haemoglobin 11.3 g%, total white cell counts 14500/dl, differential white cells, polymorph 82%, lymphocyte 9%, eosinophil 5% and monocyte 4%. ECG showed tall and persisted T waves 22 hours after the bite.

Case 2

A 56-year-old fisherman from Konewine, Twantay District was bitten by a sea snake while sorting out fishes under the light of an oil lamp at sea at right web space at 9 pm on 18 February 1999. It was a painless prick. An incision was made with a cut bottle and 2 tourniquets were applied at the arm. He complained of aching pain, inability to open mouth, heaviness and stiffness of the muscles, ascending weakness and pain on moving limbs 10 hours after the bite. On admission (24 hours after the bite), he complained of blurring of vision, drooping of upper eyelids, heaviness of all limbs, difficulty in swallowing and speech, sluggish voice and drowsiness. His BP was

150/100 mmHg, local swelling extended to the dorsum of right hand and weakness of muscle was detected in all limbs. Four ampoules of cobra antivenom were given. At 72 hours after the bite, his BP was 150/80 mm Hg and he was conscious, slightly drowsy with slurred speech with crepitation all over lung fields and passed 100 mls of dark urine. At 98 hours after the bite, he was conscious but drowsy with puffy face, no urine output, crepitation heard all over lung fields and had BP of 140/90 mmHg. He had cardiopulmonary arrest 100 hours after the bite.

Results of investigation carried out at 84 hours after the bite were: Sodium 147 meq/L, potassium 5 meq/L, chloride 105 meq/L, urea 10 mmol/L, glucose 4.8 mmol/L, serum creatinine 64 µmol/L, serum bilirubin 20 umol/L, alkaline phosphatase 35 iu/L. Total WBC 12000/dl, differential white cells, neutrophil 80%, lymphocyte 14%, monocyte 4% and eosinophil 2%. Ninety-eight hours after the bite, ECG shows persistent inverted tall T waves.

According to Reid [2], 80% of all sea snakebites have trifle or no envenoming. Of the remaining 20% of cases, about 40% were fatal and with antivenom therapy, this number should fall to almost zero. In the present study, both victims died of renal failure secondary to myoglobulinuria. Specific antivenom is not available in Myanmar. Both were fishermen and bitten while sorting out fishes under insufficient light. Local swelling of the victims was secondary to wound treatment and tourniquets. Usually it gives no local swelling [2]. Features of envenoming developed within one hour in one and 10 hours in another. In severe envenoming cases, these symptoms will develop in less than 2 hours [2]. Myoglobinuria is detected in 3-6 hours following envenoming [3] whereas in the present study it was detected at 12-13 hours after the bites. Hypertension developed in both cases. Hyperkalemia and moderate leucocytosis were observed. Cobra antivenom and anti-cholinesterase failed to reverse neurological symptoms. Secondary renal damage (blockage of renal tubules with myoglobulin) leading to renal shut down was observed in both cases. In the absence of specific antivenom, the patients' lives could be saved by performing renal dialysis and assisted ventilatory support.

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SHORT REPORT

Pharmacological effects on capillary permeability increasing activity of Russell's viper *(Daboia russelii siamensis)* venom

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Russell's viper bite is common in tropical countries. There is a geographical variation in clinical features of Russell's viper bites among countries and distinctive features of Russell's viper bite of Myanmar are pituitary haemorrhage and generalized increased capillary permeability evidenced by bilateral conjunctival oedema and facial oedema [1]. *In vitro* increased capillary permeability activity of Russell's viper (*Daboia russelii siamensis*) in Myanmar has been documented [2]. In order to find out the possible mechanism of this increased capillary permeability action of the venom, the following experiment was carried out in 1995.

Russell's viper(*D.r. siamensis*) venom used in the experiment was bought from Myanmar Pharmaceutical Factory. The minimum capillary permeability increasing dose was determined according to WHO recommended method on rodent [3]. A total of 150 male Wistar strain rats (200-230 gm body weight) from Laboratory Animal Services Division, Department of Medical Research (LM) were used. Five pharmacological agents (a) anti 5 hydroxy tryptinine (cyproheptadine, MSD, 4 mg/kg and promethazine hydrochloride, M&B, 10 mg/kg, (b) anti prostaglandin (indomethacin, MSD, 30 mg/kg), (c) antiphospholipase A_2 (hydrocortisone, UpJohn, 100 mg/kg, (d) anti H_1 receptor (diphenhydramine, Sigma, 10 mg/kg) and (e) anti-serotonin (methylsergide, Sandoz, 4 mg/kg) were used [4].

The pharmacological agents (0.1ml) dissolved in normal saline in varying concentrations (saline alone for the control) were injected intraperitoneally into the rats one hour before venom challenge. One minimum capillary permeability increasing dose (MCPID) (0.007 µg/ml) of the venom was dissolved in normal saline and then 0.1 ml/dose of the venom was injected intradermally into two marked sections on the depilated dorsal skin of the rat 20 minutes before 2 ml intravenous injection of 1% Evan's Blue dye (Sigma) in saline. Twenty minutes after the dye injection, the animal was sacrificed under light ether anaesthesia, then dorsal skin was removed and transverse diameter of the blue spots of the inner skin were measured in millimetre. Mean transverse diameters of four spots were recorded. The dose of the agent that gave 50% reduction of the diameter of the blue spot compared to the control (10x10 mm) was taken as end point. Three rats per dose were used and five doses with duplicate experiments were carried out for each pharmacological agent.

It was found that pretreatment of the animals with 2.5 µg/kg diphenhydramine, 125 µg/kg methyl sergide, 250 µg/kg indomethacin and promethazine hydrochloride, 500 µg/kg cyproheptadine and

1250 µg/kg of hydrocortisone resulted in 50% reduction of the size of the blue spot. It was evident that pretreatment of the rats with all pharmacological agents could inhibit the capillary permeability increasing activity of the venom. In other words, histamine, serotonin, 5HT, prostaglandin and PLA_2 play a role in causing increased capillary permeability of the venom.

Above all, diphenhydramine, anti H_1 receptor antagonist was the most potent agent. A similar finding was seen in *Trimeresurus mucroaquamatus* venom that histamine released from mast cells plays an important role in production of oedema and increased capillary permeability activity of the venom [5]. The proteases, phospho-lipases, membrane damaging polypeptides toxins and endogeneous autacoids released by the venom such as histamine, 5HT and kinins of the venom may induce increased vascular permeability [1]. It is concluded that histamine together with other agents namely serotonin, 5HT, prostaglandin and PLA_2 plays an important role in causing increased capillary permeability activity of Russell's viper venom.

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