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BLASTOCYSTIS HOMINIS: Commensal or Pathogenic?

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*Blastocystis hominis has been held to be a harmless or a common component of the normal human intestinal flora. Initially described as a trichomonad form by Prowazek it was given its current name in 1912 by Brumpt, who considered it to be a yeast. The classification of B. hominis remained controversial for several decades; it was assigned to the yeasts, the algae, and the protozoa by various investigators. It was finally resolved when Zierdt provided compelling evidence that B. hominis is a protozoan. One of the difficulties studying B. hominis is due to its morphologic heterogeneity. B. hominis found in faecal samples appears in a variety of cell forms with unexpected size ranging from yeast size (7 um) to giant cells of 20 to 40 microns. Structures like lack of a cell wall (but surrounded by fibrillar surface coat), possesses a membrane-bound central body and mitochondria gave Blastocystis sp. the protozoan morphology.

The presence of a cyst as part of the life cycle of Blastocystis has been extensively debated. It was earlier considered that the parasite was the cyst of other protists, notably Trichomonas but was refuted later. It was suggested that the granular form was the cyst but in a later report describing the life cycle, this was refuted by the same author, claiming that a cyst did not exist. The cystic stage of the parasite, obtained from the fresh faeces of a patient with acquired immune deficiency syndrome (AIDS) was described. It measures 5 - 10 um with as many as 4 nuclei, and mitochondria was not detected. It was shown that cystic stages obtained from the fresh faeces of a patient in their study, measured 3.5 - 7 um in size with only one nucleus, mitochondria, lipid inclusion and glycogen deposits. They also showed that these cystic stages possessed a thick wall with an outer multilayered fibrillar structure. The discrepancies in the description of the cystic stages by all these workers cannot be explained. It tends to imply that different species of Blastocystis perhaps exist.

Recent finding found that cysts form is more common in stored stools than fresh material. A cyst wall is secreted under the surface coat of the cell, while the surface coat and cell debris subsequently separated from the cyst. The infectivity of this stage to a new host was confirmed when they induced B. hominis strains to encyst using encystation media and infected it to the rats. They found that large numbers of Blastocystis were seen in the caecum and smaller numbers in the large intestine. In contrast, rats fed with vacuolar stage did not become infected.
A cyst form of *B. hominis* provides a possible mechanism of survival outside the host. The vacuolar and granular forms are both known to be sensitive to environmental conditions\(^5\) and unable to survive temperature changes, hypertonic or hypotonic environments or exposure to air\(^9\) and hence unlikely to provide a mode of transmission. It is generally assumed that *B. hominis* is transmitted by the faecal-oral route in a similar manner to *Giardia* and *Entamoeba*. Waterborne and foodborne transmission are other possible routes, but these have not been seriously examined. There was only a single report of a statistical association between *B. hominis* infection and the consumption of raw water\(^1\)\(^,\)\(^10\). The life cycles proposed by different workers are rather varied and based on very little experimental evidence and the exact position of the cystic stages in the proposed life cycles remains to be ascertained.

The life cycle of *B. hominis* is relatively simple\(^5\). Reproduction is asexual, usually by binary fission. Schizogony occurs in cultured cells. The amoeba form reproduces by plasmotomy. Other than in human, several studies have reported the existence of Blastocystis sp. in fresh faecal sample of mammals\(^10\),\(^11\),\(^12\), reptiles\(^13\), invertebrates\(^14\), birds\(^1\),\(^15\) fowls\(^12\), insects\(^16\) and in sewage\(^17\).

The association of *B. hominis* with human disease has been increasingly studied. Several studies from 1917 to 1937 reported the association of this organism with diarrhoea\(^18\),\(^19\). This organism then disappeared from the clinical literature for almost 40 years, until a severe case of *B. hominis* enteritis was reported\(^20\) in a patient who subsequently died of aspiration pneumonia. Over the last 15 years, several articles\(^21\),\(^22\), case reports\(^23\),\(^24\) and letters\(^25\),\(^26\) have dealt with this issue. The parasitological profile of chronic diarrhoea in Zairian adults suspected of Aids demonstrated that the frequency of protozoa was five times higher than that of helminths; where 86% of the protozoa were sporozoans of which *Isospora belli* was the most frequent followed by *Cryptosporidium* sp. and *B. hominis*\(^27\).

Several reports have suggested that *B. hominis* may be an opportunistic infection in immunosuppressed patients with AIDS. It was confirmed\(^28\) that *B. hominis* may be responsible for HIV-related diarrhoea when they observed 5 cases of blastocystosis in male homosexual subjects with symptomatic HIV infection. Prevalence study in French HIV-infected patients\(^29\) found that the prevalence of *B. hominis* was 13.7%. In Tanzania, it was noted\(^30\) that *B. hominis* was detected only in HIV-infected patients among three study groups (HIV-infected and non-HIV-infected children with chronic diarrhoea and controls without diarrhoea). Other studies showed that the prevalence of this parasite was as high as 44% in HIV patients and most of the cases were frequently symptomatic\(^31\).

Although *B. hominis* is now increasingly recognised as an agent of intestinal disease, it did not however show evidence of significant intestinal inflammation/injury\(^32\). Eosinophilia was reported among symptomatic patients. Clinical reports of human infection with *B. hominis* are now accumulating and one fatal infection caused by the protozoan has been
recorded, and in that patient colonic mucosal invasion was demonstrated. Several clinical studies of *B. hominis* have recently been initiated and the clinical presentation ranged from the symptomless to those symptoms associated with long-standing (more than one year) gastrointestinal disturbance such as crampy abdominal pain, nausea, passing loose stools or diarrhoea, flatus, anorexia, pruritus, vomiting, weight loss, dehydration, sleeplessness, inability to work, lassitude, dizziness, tenesmus, itching and slight acidocytosis, low-grade fever, malaise, chills and recurrent diarrhoea. Several case reports have suggested that *B. hominis* may be associated with a variety of diseases, including oligoarthritis, tropical pulmonary eosinophilia, reactive arthritis, colitis and terminal ileitis. Incidence of *B. hominis* in children with diarrhoea was not significantly different from the older patients. Attempts to assign pathogenicity to *B. hominis* by epidemiological studies have been criticised because of the impossibility of eliminating all other causes of symptoms, either infectious or non-infectious, especially when it is considered that approximately 25% of reported diarrhoea cases have no known aetiology. Experimental animal models is also lacking. Elimination of the parasite by drugs and subsequent subsidence of symptom is often taken to indicate that *B. hominis* is pathogenic. This is an unacceptable argument since the drugs used, chiefly metronidazole, are not specific and affect many other organisms, including bacteria. Although there have been many suggestions that *B. hominis* causes disease, there are a similar number of contrary reports. It is possible that the parasite is a commensal, but could be pathogenic under specific conditions such as immunosuppression, poor nutrition or concurrent infections.

On the other hand no statistically significant difference in prevalence of *B. hominis* was found between the asymptomatic study group and the symptomatic control group; and no correlation was found between the presence of *B. hominis* and that of faecal leukocytes. The presence of pathogenic and nonpathogenic strains of the parasite which are morphologically indistinguishable complicates diagnosis. The diagnosis of the infection depends either on the demonstration of the parasite or immunological assays to detect specific antibodies. The conventional technique based on morphological identification of this parasite is tedious and morphologically, pathogenic and nonpathogenic strains are similar. Therefore there is a need to utilize molecular biology techniques to differentiate these various strains for a better understanding of the disease.

**References**


Vibriocholerae is a gram-negative comma-shaped rod with a single flagella. It consists of toxigenic and non-toxigenic strains. The toxigenic strains carrying the ctx gene namely serotyped as *Vibrio cholerae* 01 and recently the *Vibrio cholerae* 0139 “Bengal strain” are the strains responsible for several cholera outbreaks and epidemics. Cholera is transmitted mainly by faecal-oral route or via contaminated food and water. Consumption of contaminated food supplies including raw, undercooked seafood especially oysters has also been responsible for cholera outbreaks and fatalities.2,3

*Vibrio cholerae* can be isolated from stools of patients during active disease and in suspected carriers. Human to human transmission had been reported involving psychiatric patients where personal hygiene and sanitation were not maintained.4

*Vibrio cholerae*, both the toxigenic and non-toxigenic strains have been found in polluted surface water, ponds, lakes and rivers. Literatures are available on the isolation of this organism in fresh water environment from Japan, Australia, USA, Spain and Russia but most of the work were carried out in Bangladesh and India where cholera is endemic.

*Vibrio cholerae* can thrive well in marine water and it has been isolated from almost all coastal areas throughout the world. Marine microbiota such as algae and plankton were shown to provide a reservoir for this organism. *Vibrio cholerae* attach well to various species of algae and long survival was seen with green algae, *Rhizoclonium fontanum* 4. The persistence of *Vibrio cholerae* inside the mucilaginous sheath of blue-green algae, *Anabaena variabilis* was observed for more than 15 months after experimental inoculation.5

Several instances of outbreaks of gastrointestinal illnesses due to consumption of seafoods contaminated with *Vibrio cholerae* have been reported. As this organism can be isolated in marine and estuarine environment, they may be present in the seafood at the time...
of harvest. The occurrence of *Vibrio cholerae* in brackish water ponds was monitored over a 2 year period in one of the studies carried out in a southeast asian prawn exporting country. It was shown that brackish water ponds and cultured prawns were inherently contaminated with the bacterial pathogens.

In communities where modern latrine systems were lacking, defaecation was carried out in the open soil. In a study on survival of *Vibrio cholerae* in soil by Khan in Bangladesh, the organism was isolated eleven days later from a spot of soil where a cholera patient defaecated, suggesting that *Vibrio cholerae* survive well in the soil.

Several aquatic bird species were also shown to harbour *Vibrio cholerae* in Colorado and Utah. The organism was also isolated from the water inhabited by these birds suggesting the birds can also play as an environmental reservoir.

**Detection of Vibrio cholerae from environmental samples**

Various methods are used to detect *Vibrio cholerae* in these environmental samples. These include the use of conventional culture techniques, monoclonal antibodies and molecular techniques.

**Conventional culture**

It is necessary to do prior enrichment with alkaline peptone water for all environmental samples before inoculating onto selective media such as thiosulphate citrate bile salt agar (TCBS) or Monsur's agar. *Vibrio cholerae* present in the samples may be too few thus enrichment in alkaline peptone water enhances growth and proliferation of this organism and inoculation into selective media inhibit growth of other organisms that may be present in the samples. However, reinoculation into a fresh alkaline peptone water followed by incubation for 4 to 6 hours before inoculating on the selective media is recommended as it was shown to give better yield. Conventional culture techniques normally require three days before an isolate can be confirmed to be *Vibrio cholerae*.

**Polymerase chain reaction**

Since the numbers of *Vibrio cholerae* in clinical samples may be too few to be detectable by conventional culture methods, many workers had turned to using molecular techniques viz polymerase chain reaction (PCR) to detect *Vibrio cholerae* DNA. As the organism has capability to survive in non cultivable state, PCR technique can be used to detect it. This technique is used to amplify cholera toxin gene known as ctx A and ctx B. PCR technique has been shown to be successful in detecting contamination of seafood with *Vibrio cholerae*. Direct PCR on lysate prepared from fish homogenates containing $10^3$ *V. cholerae/ml* gave a positive reaction. When combined with alkaline peptone water enrichment, homogenates containing 1.4 cells/ml gave amplification signal. The technique could also detect *V. cholerae* 0139, the recent epidemic serotype in the Indian subcontinent. An environmental isolate of non-01 *Vibrio cholerae* that produced cholera toxin was also positive in this assay. PCR-based techniques have great potential in rapid detection of toxigenic *Vibrio cholerae* in seafoods.
Fluorescent labelled monoclonal antibody Castillo A. et al \(^1\) reported the development of two monoclonal antibodies, termed 568 and 5012, belonging to the IgM and IgG class respectively suitable for the identification of \textit{Vibrio cholerae} 01 in environmental samples. In other studies, monoclonal antibodies were derived from acetone treated whole cells of the newly recognised \textit{Vibrio cholerae} 0139 serogroup which specifically recognised the lipopolysaccharide antigens of this organism suggesting possibility of screening in epidemic and endemic diarrhoea cases and environmental samples.

**Enzyme immunoassay**

A dot blot enzyme linked immunosorbent method was shown to be useful for screening for \textit{Vibrio cholerae} 01 even in specimens that were heavily contaminated with non-01 vibrios. The enzyme linked immunosorbent assay was performed using biotin-labelled antibodies and avidin-biotin-peroxide complex giving rise to brown dots developing in the wells containing \textit{Vibrio cholerae} 01.

The environment provides a reservoir for \textit{Vibrio cholerae} during inter-epidemic period and thus is an important host factor of the cholera disease. Rapid and sensitive method for the detection of \textit{Vibrio cholerae} should be available and used for prompt detection and control of cholera outbreak. Epidemiological measures directed against control of infectious disease such as prompt investigation, treatment of cases and carriers, personal hygiene, health education especially to food handlers if properly taken care of would limit future outbreaks and thus help prevent the eighth pandemic of cholera from occurring.

**References**:


The cutaneous mycoses are a group of fungus infections that are confined largely to the cutaneous tissues of skin, scalp and nails although these can occur on other areas of the body. Varying degrees of tissue reaction can occur from erythema and moderate scaling to heavily crusted suppurating lesions, or, rarely, granulomatous lesions. Skin and scalp lesions may be asymptomatic or extremely itchy and painful. The infected nails may be thickened and deformed.

The vast majority of cases occur in perfectly healthy people and it has been considered as the most common fungal communicable disease of man (2 out of 10 of the adult population in developed countries are affected at any time) (1).

Patients who have cutaneous fungal infections especially on the face or nail may develop psychological disorders about the abnormal appearance of their face or nails. Fungal infections at these sites may lead to the spread of fungus to adjacent and distant areas of the skin. This leads to further impairment of the quality of life, with mental and social implications leading to reduced self-esteem and limitation of interaction with others.

Treatment for nail infection may take several months and recurrent infections are common in cutaneous fungal infections. Cost of therapy would also include cost of treating recurrent infections. Thus, it is evident that cutaneous fungus infection is not merely a cosmetic problem and leads to impairment of quality of life, but one, which also has a marked economic impact.

The difficulty to treat some forms of cutaneous mycoses is mainly due to the fact that more than 60 species of fungi have been implicated from diseased cutaneous samples (2,3,4). One fungal species can infect many anatomical sites. Infection by mixed species of fungi may also occur (5). Since these fungal species vary in their susceptibility to antifungal agents (6), there is no single drug of choice. Choice of the appropriate antifungal agent(s) will depend on the combination of factors that include the causative agent(s), potential adverse effects and cost. Thus, confirmation of the exact etiologic agent(s) is required in order to initiate effective treatment.

Of more than 60 species of fungus implicated in cutaneous mycoses, the infections by a group of fungi called dermatophytes are most important and frequent. They are considered as primary etiologic agents of cutaneous mycoses in healthy as well as in the immunocompromised host. In one study of all the etiologic agents of skin and nail infection, only 10% of the cases where dermatophytes (5). Dermatophytes were isolated together with other fungi such as yeast in about half of the cases. In some cases, two dermatophytes co-exist. However, it is believed that majority of cutaneous mycoses were initiated by dermatophytes. Yeast and other mold implicated in cutaneous infections invade the cutaneous tissues after its destruction by dermatophytes.

Currently, more than 40 species of
Dermatophytes are recognized and placed into three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. Worldwide human infections are caused by eleven species of dermatophytes.

Determination of species of fungus causing cutaneous mycoses in patients attending Government Hospitals and private clinics in Malaysia has been carried out at the Mycology Section of the Institute for Medical Research (IMR) since 1985. IMR's Mycology Laboratory is located at the Division of Bacteriology. It receives about 200-300 cutaneous clinical samples from more than 12 referral hospitals in Malaysia for fungal diagnosis.

In order to determine the presence of fungus in cutaneous samples, it is important to culture the sample onto at least two different culture media. Sabouraud's agar containing cyclohexamide and chloramphenicol is used to isolate dermatophytes. Cyclohexamide inhibits saprophytic mold and most yeast except *Candida albicans, Candida pseudotropicalis* and *Candida guilliermondii*, while chloramphenicol inhibits bacterial contaminants. Growth of any non-dermatophytes needs to be inhibited as dermatophytes grow very much slower. To determine whether the sample harbors non-dermatophytes, the sample is also cultured on Sabouraud agar containing only chloramphenicol. In addition, a portion of the sample is treated with 30% KOH on a microscope slide. The slide is examined microscopically for fungal elements.

Table 1 shows species of *Trichophyton, Microsporum* and *Epidermophyton* isolated from the clinical specimens in the IMR's Mycology laboratory. All of the most common encountered dermatophytes worldwide had been recovered. *Trichophyton rubrum* (Figure 1) is the most common *Trichophyton* spp. isolated. Although *Microsporum canis* is the second commonly isolated dermatophytes, eighty percent of *M. canis* were isolated from scalp infection. Thus, *T. rubrum* and *T. mentagrophytes* are the most common etiologic agents of cutaneous mycoses in Malaysia while *M. canis* (Figure 2) is the major cause of scalp infection.

Although other species of dermatophytes have been reported to be endemic in certain countries, they are far less common in Malaysia. The reason for prevalence of *T. rubrum*, *T. mentagrophytes* and *M. canis* is because they are able to rapidly establish themselves in new geographical areas (4). Majority of dermatophytes species are geographically restricted (3).

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>126</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>27</td>
</tr>
<tr>
<td><em>Trichophyton tonsurans</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Trichophyton concentricum</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Trichophyton violaceum</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Trichophyton verrucosum</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Trichophyton schoenleini</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Trichophyton proliferans</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>101</td>
</tr>
<tr>
<td><em>Microsporum ferrugineum</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Microsporum gypseum</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Epidermophyton floccosum</em></td>
<td>10</td>
</tr>
<tr>
<td>Total clinical species</td>
<td>300</td>
</tr>
</tbody>
</table>
Figure 1: *Trichophyton rubrum* – the major etiologic agent of cutaneous mycosis

Figure 2: *Microsporum canis* – the major cause of scalp infection
Table 2 shows species of fungus other than *Trichophyton, Microsporum* and *Epidermophyton* isolated from the specimens. They were isolated more commonly than *Trichophyton, Microsporum* and *Epidermophyton*. However, they were separated from those in Table 1 because their role as causative agent in cutaneous mycoses in healthy people is still very much a matter of debate (7). Yeasts such as *Candida* are commensals of healthy skin and often found as saprophytes in nail tissue, but when host-parasite relations are disturbed, as occurs in states of immune depression, *Candida* can directly invade cutaneous tissue. Although *Aspergillus* spp were isolated frequently, they were mainly isolated from diseased nail. However, mold such as *Aspergillus* and *Fusarium* account for less than 5% of all nail infections (8). They are usually opportunists, which invade keratin that has already been altered.

Table 2: Other fungus than Dermatophytes isolated from clinical samples in 1989-1996

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>135</td>
</tr>
<tr>
<td>Other <em>Candida</em> spp</td>
<td>203</td>
</tr>
<tr>
<td><em>Aspergillus</em> spp</td>
<td>289</td>
</tr>
<tr>
<td><em>Fusarium</em> spp</td>
<td>39</td>
</tr>
<tr>
<td>Other mold</td>
<td>27</td>
</tr>
</tbody>
</table>

In the laboratory, isolates of *Candida, Aspergillus* and other mold could be convincingly considered as causative agents if (a) mycelium or spores of the fungus is observed on direct microscopic examination in the affected tissue, (b) no

*Trichophyton, Microsporum* or *Epidermophyton* is isolated, and (c) repeated isolation of the fungus from the same patient (9). Although we saw fungal elements in more than half of the specimens yielding these fungi, but we were unable to get a second specimen for culture.

Apart from nail infection, *Candida* and nondermatophytes mold may become important as cutaneous mycosis agents in the immunocompromised host as there is increased incidence of these fungal infections in this group of patients (10,11). More than 40 species of non-dermatophyte mold have been recognized to be etiologic agents of cutaneous mycosis in immunocompromised patients (12,13).

References


Tuberculosis (TB) remains a health problem in various parts of the world, including Malaysia. Active TB is diagnosed annually in more than 10,000 people in Malaysia.

Early laboratory confirmation of TB is essential for making therapeutic decision and avoiding usage of inappropriate, complex and potentially toxic drug regimens. Laboratory diagnosis of TB may be carried out by the following methods:

1) demonstration of Mycobacterium tuberculosis in clinical specimens

2) detection of bacterial genome in body secretions / fluids (sputum and CSF)

3) serological diagnosis.

**Demonstration of M. tuberculosis**

This usually means recognition of Acid Fast Bacilli (AFB) by microscopy utilising a standard acid-fast stain such as Ziehl-Neelsen. However, acid fastness is not specific to *M. tuberculosis* and it is common to all mycobacteria. It is not only an easy and quick procedure. It also gives a quantitative estimation of the number of bacilli being excreted. Unfortunately smear examination is insensitive and can only detect *M. tuberculosis* when they are present in number detect greater than 10,000 organisms per ml sputum (1).

For screening large numbers of clinical samples for mycobacteria in a short time, a sensitive but less specific staining method is available using rhodamine-auramine dyes and scanned for fluorescence using dark field microscopy.

Demonstration of AFB in smears made from a clinical specimen can be supplemented by isolation of AFB to provide essential information on the properties of mycobacteria seen on the smear.

The conventional method for detection of mycobacteria is by culturing decontaminated sputum samples onto solid media. However, this method is time consuming. It requires an average of three weeks to recover mycobacteria through culture from clinical specimens.

The newer method for detecting the growth of mycobacteria is by using Bactec system. This method was developed by Deland and Wagner in 1969 (2). It detects the growth of bacteria by measuring $^{14}$CO$_2$ liberated during the decarboxylation of $^{14}$C labeled substrates present in the medium. This principle of bacterial detection has been applied to detect the growth of *M. tuberculosis* by Cumming *et al* in 1975 (3). The main advantage of this method is the rapidity and increased isolation compare to conventional cultures (4,5). This method is in use in the Bacteriology Division of the IMR.
Detection of Bacterial Genome

Conventionally, the detection and identification of mycobacteria in clinical specimens often takes several days to several weeks before results are available to clinicians. The trend during the last several years has been to develop methods that would make clinically useful information available sooner within a clinically relevant period of time. The most current and most promising innovation for identification of mycobacteria is the use of the Polymerase Chain Reaction (PCR) and the nucleic acid probe techniques.

Manjunath et al (6) developed a system which obviates the need for post-PCR hybridisation confirmation, instead they used agarose gel electrophoresis. This makes the test simple. We are using this technique in our laboratory and have found it to be useful as a complement to our present Bactec system (for isolation and identification of M. tuberculosis).

The advent of PCR has opened new possibilities for diagnosis of microbial infections (7,8). In the field of mycobacteria the PCR has been used to identify M. leprae (9) and to detect DNAs extracted from various mycobacteria (10,11,12). The DNA sequence is amplified by PCR and identification of the target DNA is accomplished by using DNA probes.

Serological Diagnosis

Serological identification of infections are widely used in medicine. For diagnosis of TB, it is of great value in children and extrapulmonary disease where sputum examination is not available.

The first report of using serodiagnosis in TB appeared in 1898 (13). A serodiagnostic test for TB is inexpensive and rapid (test results are available within 1 day) and has the advantage of obviating the need for a clinical specimen from the site of disease (14). One of major problems associated with any serodiagnostic test is number of persons who have circulating specific antibody without further evidence of disease. The other drawback is the problem of non-specificity of the test. The tubercle bacilli contain several antigens which are widely shared amongst other species and genera. Because of this, false positive antibody results can occur frequently.

The serological tests that are available include Double diffusion test, Agglutination test and ELISA. These tests employ the use of either whole cell antigens or specific tubercle antigen. Analysis of IgG and IgM against SL-IV antigen of M. tuberculosis has been tried (15). The study showed that IgG analysis gave specificity of 96%. We are now evaluating the significant of the MycoDot test in serodiagnosis of TB. This is an agglutination test using specific tubercle antigen lipoarabinomannan (LAM).

Evaluation using serological diagnosis should be causiously interpreted especially in immunocompromised patients. It has been shown that the humoral response is noticeably decreased in the latency stage of HIV infection and it is practically useless in clinical AIDS (16).
References


GENETIC DIVERSITY OF HUMAN IMMUNODEFICIENCY VIRUS

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The World Health Organization (WHO) estimated that up to December 1996, more than 22 million people have been infected with HIV. By the year 2000 WHO projects that more than 40 million HIV infections will have occurred worldwide of which more than 90% will be in developing countries. The development of an efficacious vaccine is vital for stemming the spread of HIV. However reaching this goal is complicated by the high degree of HIV-1 genetic variability. The HIV is known to undergo genetic variation during its life cycle. Because of this high mutation rate HIV isolates from different individuals are found to be genetically distinct.

HIV genome

HIV belongs to the Lentivirus subfamily of Retroviruses, which produces chronic infection in the host and progressively damages the host's immune system. Two major HIV types in humans have been characterized: HIV type 1 (HIV-1), the predominant HIV type throughout the world, and HIV type 2 (HIV-2), first reported from and still primarily found in persons from West Africa. HIV-1 and HIV-2 contain three main structural genes, characteristic of all retroviruses. These genes are called gag which encode the core proteins of the virus, pol which encode the virus enzymes and env which encode the protein of the envelope surrounding the virus particle. Among the structural genes of HIV the envelope gene exhibits the highest degree of genetic variation. Most of the sequence variability found in the envelope gene is found in 5 regions V1-V5. The hypervariable region V3 in the

env is a major target of vaccine research because it is the principal neutralizing determinant. Antibodies elicited the gp120 of a virus isolate are type specific and neutralise only a small percentage of all virus isolates. Therefore to develop a vaccine that is widely applicable one must take into account global HIV variation.

Los Alamos National Laboratory Human Retrovirus and AIDS Database

Several methods based on analyzed nucleotide or deduced amino acid sequences have been developed to infer the phylogenetic relationships between different sequences. In 1985 the National Institute of Health in the United States (NIH) sponsored the HIV sequencing database project at the Los Alamos National Library. Myers and his team of researchers began collecting sequencing of HIV genes from all pandemic regions in gene bank computers. The computer analysis traces genetic changes in the gag and env genes, showing which of the strains are most closely related and likely to have emerged from common ancestors. Through the viral sequencing efforts of many groups of scientists, ten clusters or subtypes (alternatively termed "clades" or "genotypes") of HIV-1 have now been identified. These subtypes designated A through J, constitute the major group of HIV-1, group M. In addition a divergent or heterogenous group outside group M have been reported and are provisionally categorized as group O (Outliers) (Figure 1). HIV-2 has also been classified into subtypes, although the number of
sequenced isolates remains limited. International collaborations involving many institutions have augmented efforts to expand global surveillance. For example the WHO Network for HIV Isolation and Characterization was established in 1990 to collect HIV isolates from a number of countries worldwide to monitor genetic and antigenic variation for HIV vaccine development.

Figure 1: Simplified phylogenetic tree of HIV-1. (Adapted from Dale JH et al, 1996)

Geographic associations with HIV subtypes

The HIV sequences have been studied from isolates from a broad range of geographical regions and subtypes' associations with distinct geographical regions have been classified. Subtype B is mostly found in the Americas, Japan, Australia, the Caribbean and Europe; subtypes A and D predominate in sub-Saharan Africa; subtype C in South Africa and India; and subtype E in Central African Republic, Thailand and other countries of southeast Asia. Subtypes F (Brazil and Romania), G and H (Russia and Central Africa), I (Cyprus), and group O (Cameroon) are of low prevalence. In Africa, most subtypes are found, although subtype B is less prevalent.

HIV Subtypes in South-east Asia

Relatively few data have been reported on the extent of HIV genetic variability from South-east Asia. Ou et al in 1993 reported 2 distinct genotypes of HIV-1 in Thailand, segregating by mode of transmission viz. genotype A predominantly in people infected sexually and genotype B predominantly in intravenous drug users (IVDU). In 1995, Tsuchie et al in a collaborative study involving Thailand, India, Malaysia and Philippines found HIV-1 subtypes A, B, C and E in the region, with two IVDUs being reported in Malaysia to be of subtypes B and E respectively. An earlier collaborative study between the Centers for Disease Control and Prevention (CDC), USA, and the Institute for Medical Research, Kuala Lumpur in early 1992-1993 had first detected HIV-1 subtypes B, C, and E among parenterally and perinatally infected HIV-1 individuals in Malaysia. In addition an ongoing study in IMR of HIV-1 subtyping of infected IVDUs...
showed that out of 40 HIV-1 seropositive IVDUs, the majority (38 out of 40) had subtype B and one of each of subtype C and subtype E infections (IMR unpublished data).

**Diagnostic Tests**

The high genetic variation of HIV has important applications for the sensitivity and specificity of diagnostic tests. The isolation of HIV-1 in 1983 and the development of an antibody test soon thereafter were major breakthroughs. However, the subsequent identification of HIV-2 required significant modification of diagnostic tests. Somewhat analogous to the discovery of HIV-2 has been the characterization in 1994 of the highly divergent HIV-1 group O strains. Routine AIDS tests which are currently being used for blood screening and diagnostic purposes detect virtually all subtypes of HIV. Most companies have modified their assays to detect the newly identified HIV-1 group O strains. Active surveillance for and characterization of the prevailing HIV strains are essential to validate the sensitivity of HIV tests in clinical practice and research use. Prevailing HIV strains are essential to validate the sensitivity of HIV tests in clinical practice and research use.

**Global Surveillance of HIV variants**

There are important questions on the intriguing possibility that genetic and phenotypic differences in HIV may affect transmissibility, infectivity, pathogenicity and responses to therapy and vaccines. In addition, genetic recombination of different strains or subtypes during coinfection may allow for the formation of viral hybrids with altered pathogenic or transmissibility properties. Recombination also poses challenges for diagnostics and vaccine development.

These concerns emphasize the importance of establishing worldwide surveillance networks to monitor the molecular epidemiology of HIV to help understand the degree of genetic diversity within subtypes and what subtypes predominate within different population groups. As our understanding of the significance of different HIV genotypes and phenotypes increases, knowledge of their frequency and distribution will play an important role in a timely and effective response to the HIV pandemic.

**References**


DNA sequencing, basically is a technique where the correct order of nucleotides in a specific DNA is determined. With the advent of molecular techniques and tools, DNA sequencing plays a pivotal role in progressive research. Perhaps the discovery of chain termination by Sanger and Coulson in 1977 is the most important breakthrough in sequencing and thus in molecular biology. Among the various fields of research where sequencing plays important roles are geographical and molecular epidemiology, informations of mutations in relation to virulence/pathogenesis, drug-resistance, hereditary diseases/disorders and cancer research.

The two important techniques in sequencing are Chain Termination (Sanger & Coulson, 1977) and Chemical Degradation (Maxam & Gilbert, 1977). Even though both these techniques are very different from each other, they both work in accordance with the same principle. In summary, single stranded DNA is fragmented by certain techniques whereby these DNA fragments differ from each other by just a single nucleotide. Following this, the fragments which are labelled are separated by electrophoresis into distinct bands which can be detected according to the chemistry of labelling.

Sanger & Coulson's Chain Termination

The general principle of this method is quite similar to Polymerase Chain Reaction (PCR) but only a single primer direction is used. The template could be restriction enzyme-digested DNA cloned into plasmids such as PUC18 or PCR product itself. The first step is primer annealing to the region of interest. This primer acts as the starting point for new polynucleotide chain, complementary to the existing template. The chain elongation is catalysed by a thermostable DNA polymerase enzyme with the addition of DNA building blocks known as deoxyribonucleotide triphosphates (Adenine, dATP; Cytosine, dCTP; Guanine, dGTP and Thymine, dTTP). In addition a single modified nucleotide, deoxyxynucleotide, eg dddATP, is added into the reaction tube which can be incorporated into the growing polynucleotide strand just as efficiently as the normal nucleotide, but which blocks further strand synthesis. This is so because the deoxyxynucleotide lacks hydroxyl group at the 3' position of its sugar component. This group is needed for subsequent attachment of another nucleotide, thus forming phosphodiester bonds. Therefore chain termination occurs immediately a dideoxynucleotide is incorporated into the growing molecule.
The strand synthesis reaction is carried out four times in parallel with all four different modified nucleotides separately, i.e., ddATP, ddCTP, ddGTP and ddTTP. The result will be four distinct families of newly synthesized polynucleotides of various lengths, each ending with a dideoxynucleotide. The next step is to separate the DNA fragments which is done by polyacrylamide gel electrophoresis. A good sequencing gel should have the ability to separate the DNA fragments whose lengths differ by just a single nucleotide. This can be achieved by using ultrahigh resolution polyacrylamide gel electrophoresis. Urea is a denaturant and is added into the gel while the sample is resuspended in a loading buffer containing formamide, also a denaturant. Both urea and formamide can prevent the formation of hairpin loops which can give rise to compressed bands.

Each family of polynucleotides are loaded into a different lane of a polyacrylamide gel. During electrophoresis, negatively charged DNA will be migrating to the positive poles. The shorter fragments will travel faster then the longer ones, ending at the near bottom of the gel. As the sequencing gel is only about 0.3-0.4 mm thickness, the electrophoresis process can take about 2-3 hours of running depending on the length of our target DNA with the voltage maintained at about 2400 volts.

After the completion of electrophoresis, the gel is ready for Southern Transfer. This is a process where DNA bands are transferred from the gel to a membrane which can be a nitrocellulose or a nylon membrane. The membrane is placed on the gel and buffer is allowed to soak through, carrying the DNA from the gel to the membrane through capillary transfer. The membrane now carries the replica of the DNA bands from gel and a detection system can be used to detect the bands. The band that has moved the furthest is located at first as this represents the smallest piece of DNA. Hence the reading of the sequence starts from the first band from the bottom to the next mobile band which corresponds to a DNA molecule one nucleotide longer than the first. This continues all the way to the readable top (Figure 1).

**Figure 1. Flowchart for chain termination sequencing**

A. Termination with ddATP during strand synthesis

\[
\begin{align*}
5' & \quad \text{-----------------------------} \quad 3' \\
\text{ATCTGTTGCCGAGTGGCTAGCT} & \quad \text{Taq DNA polymerase} \\
\text{ddATP} & \quad \text{dATP, dTTP, dGTP, dTTP} \\
\text{ddATP} & \quad \text{and ddATP} \\
\text{ddATP} & \quad \text{-----------------------------} \\
\text{ddATP} & \quad \text{-----------------------------} \\
\text{ddATP} & \quad \text{-----------------------------}
\end{align*}
\]

B. The above step is repeated with ddCTP, ddGTP and ddTTP.

C. The four types of polynucleotides fragments are loaded into polyacrylamide gel and electrophoresis is carried out.

\[
\begin{array}{cccc}
A & C & G & T \\
- & - & - & - \\
- & - & - & - \\
\end{array}
\]

Hence, the above sequence should be read as AGCTACC
Maxam & Gilbert's Chemical Degradation Method

Basically the idea behind this method is similar to the chain termination method which is to get families of A, C, G and T molecules separately. Here, this objective is met by using various chemicals in different reaction tubes. These chemicals have the ability to cleave one particular nucleotide. For example if our target is to get polynucleotides ending with G, then the chemical dimethyl sulphate is used. Briefly, the starting polynucleotides that we intend to sequence must be labelled at the 5' end by radioisotopes for detection at the final step. Then these molecules can be treated with dimethyl sulphate which reacts specifically with G nucleotides, resulting in a chemical modification to the purine ring.

Strand cleavage occurs when a second chemical, piperidine is added which removes the G nucleotide and cuts the DNA molecule at the phosphodiester bond. This gives us a DNA fragment with the length being determined by the position within the original molecule of the G nucleotide that was modified during the chemical treatment. This step is repeated in different reaction tubes using different modification chemicals resulting in A, C and T families of polynucleotides. These families of polynucleotides are then loaded into the polyacrylamide gel and electrophoresed. Comparison of the banding pattern produced by A, C, G and T families are deduced by the same way as for the chain termination method.

General description of the ABI 373 DNA Sequencing System

The Applied Biosystems Model 373 DNA Sequencing System is an automated gel-scanning instrument, which consists of an electrophoresis module, a Macintosh Computer that includes software for data collection and data analysis, and a Tektronix Phaser II colour printer. The preparation of the families of polynucleotides are done using Sanger and Coulson's Chain Termination method. The dideoxy sequencing reactions are performed with fluorescent dye-labelled reagents. Dye labels can be incorporated into DNA using either 5'-dye labelled primers or 3'-dye labelled dideoxynucleotide terminators. There are fours colours for each ddNTP with green represents A, blue represents C, black represents G and red represents T.

As each dideoxynucleotide is colour-coded, the chain termination reaction is carried out in a single tube and can be loaded into a single lane in the polyacrylamide gel and electrophoresed. Hence, up to 36 clones(template) can be analysed simultaneously on a single gel. The dye-labelled DNA fragments electrophores through the gel and separate according to size. When the fragments reach a fixed position above the lower buffer chamber, the fluorescence of the dyes is excited by light from a laser, which scans back and forth across this area of gel.
A photo multiplier tube (PMT) detects the fluorescent light and converts it into an electrical signal. These signals are then transmitted to the computer and stored for eventual processing. Unlike the conventional method where sequencing pattern is determined by a ladder of bands, in this system a series of colourful peaks representing the sequence of our target DNA is produced with the order of nucleotides readily written (Figure 2). This greatly assist in getting the correct sequence.

Molecular techniques have important roles in defining the broad patterns of infections especially in emerging and re-emerging diseases. Techniques such as PCR, hybridisation and DNA sequencing have facilitated studies of the molecular epidemiology of several infections which were previously difficult to investigate. Recent published investigations have used a combination of PCR and sequencing to determine the extent of viral genome variation. Sequencing have attracted the epidemiologist’s attention, particularly in the transmission of HIV from infected patients to medical staff. The case of the Florida dentist, who was shown to have infected several of his patients, illustrates how sequencing can be used to establish relationship among HIV isolates. At the home front, the Division of Virology, Institute for Medical Research is carrying out research in the pathogenesis of dengue. Dengue virus isolates from Dengue Fever and Dengue Haemorrhagic Fever patients are sequenced at selected genomic region to look for mutations which could be responsible for the virulence of the virus.

References
Dengue fever (DF) and dengue haemorrhagic fever (DHF) are serious mosquito-borne diseases commonly found in Southeast Asian countries including Malaysia ever since their first description in 1902 by Skae. The first major national outbreak of DF and DHF in Malaysia occurred in 1973 at which time 969 cases and a case fatality ratio of 5.6/100 was reported (Ministry of Health, 1973). The next epidemic (3,005 cases notified, 1,001 laboratory-confirmed, 35 deaths) occurred in 1982. From 1982-1989, the cases fluctuated between 1000-2500 cases annually, but in 1990 and 1991, 4880 and 6458 cases respectively were notified to Vector Borne Diseases Control Programme (unpublished data). In 1995, a total of 6520 cases were notified to the VBDCP and these included 6150 DF and 370 DHF cases with 28 deaths. Since 1996, more than 10,000 cases are reported annually and the trend is still increasing. By the first 7 months of 1997 (31 July) more than 10,000 cases of dengue are reported to VBDCP. There is at present a lack of specific treatment for DF and DHF and the control of these diseases depends primarily on the suppression of the 2 important vectors namely, Aedes aegypti and Ae albopictus. Despite intensive and extensive Aedes control efforts by health agencies, the diseases continue to occur. This paper attempts to update various important research in vector control conducted in Malaysia recently.

1. VECTOR CONTROL
   Simultaneous adulticiding and larviciding

   Presently, the interruption of dengue transmission is dependent on the use of malathion, an adulticide which is highly effective when delivered by thermal or ULV fogging; while larviciding is effected through the use of temephos in containers. Traditionally, adulticiding is conducted by the health authorities and the application of larvicide is left to the community. However, this compartmentalisation of adult and larval control is unfortunately ineffective. In a nation-wide survey it was found that only about 30% of the households had used temephos at least once. Most of the households did not apply the larvicide regularly and was often under- or over-dosed. An ideal and effective dengue vector control operation should therefore incorporate both activity in a single operation. This concept of simultaneous adulticiding and larviciding is not new and has been known and used for many years in the control of other vectors such as malaria. It was found that ULV fogging of B thuringiensis H-14 was highly effective in Aedes larval control and when used together with malathion was able to induce also complete adult mortality. However, the discharge dosage used was 1.6 L/min of B thuringiensis H-14 and this was considered excessively high. In another trial, B thuringiensis H-14 was mixed with malathion (9V:1V) and discharged at 250 mL/min in a residential area in Pandamaran, Klang. It was found that complete mortality of caged Ae aegypti and Ae albopictus adults left indoor and outdoor was achieved. Similarly, high larval mortality of both species was obtained. Trials conducted in construction sites using a mixture of malathion-Bt induced complete adult and larval mortality in Ae aegypti (Lee & Seleena, unpublished data).

   A field trial in Johor using malathion-Bt mixture at a ratio of 1:1 and 3:7 indicated that the 3:7 ratio was more effective in inducing adult and larval mortality (VBDCP, unpublished data). From these trials, it is obvious that (1) existing ULV machines
are able to disperse the bacteria, (2) residual larvicidal activity was seen and (3) cost-effectiveness of this method needs to be assessed.

**Compatibility of Bacillus thuringiensis H-14 and chemical insecticides**

If Bti is to be mixed with chemical insecticides and used in dengue vector control, the compatibility of the bacteria with the chemicals need to be ascertained. Laboratory studies on the compatibility of Bti with malathion 96% TG; primiphos-methyl 50EC and Aqua-ResigenR (S-bioallethrin+permethrin+piperonyl butoxide) has been carried out. Malathion was found to be the most compatible insecticide with Bti. There was no loss in the larvicidal activity of Bti on mixing with primiphos-methyl. However, after 7 days the Bti toxicity was reduced by 3 folds. Larvicidal activity of Bti in Aqua-ResigenR was not observed at all, probably due to the rapid knock-down of the larvae by the pyrethroids. These limited tests indicated that certain insecticides can be mixed with Bti and used for dengue vector control.

**ULV application of microbial control agents**

Microbial agents especially *B thuringiensis* is highly effective against larvae of *Aedes*. However, the use of Bti in dengue vector control is not widespread due to difficulty in applying the bacteria effectively. In this respect, ultra-low-volume cold fogging is ideal for the mass scale dispersal of the bacteria for larviciding purposes. It was found that a flow-rate of 0.3 L/min was not effective, but at 0.5 L/min, very high larval mortality was achieved with long residual activity of the bacteria in test cups and tyres. Subsequently, a real-life trial using an aqueous solution of *B thuringiensis* H-14 (VECTOBAC AS) was conducted in an residential area in Selangor. The field dosage used was 0.5 L/min. At this dosage, complete larval mortality was achieved in outdoor containers and those containers placed about 5 ft. indoor. Obviously, to achieve more significant results, a higher flow-rate of probably >0.5 to 1 L/min of Bti should be used for larviciding only. More recently, Lee *et al* (1997, unpub data) found that a local isolate of Bt H-14 dispersed by a portable mist-blower at a discharge rate of 50 mL/min was highly effective in *Aedes* control and the residual activity of the bacteria lasted for about 1 month.

**Adult susceptibility to malathion**

Recently, Lee *et al* (1995, unpublished data) conducted a limited survey of the insecticide susceptibility of *Ae albopictus* adults (F-1) to DDT, malathion and permethrin. The mosquito larvae were collected from various locations in Johore and bred to F-1 adults which were subsequently tested using a modified WHO bioassay procedure. The results indicated that *Ae albopictus* adults were highly susceptible to both malathion and permethrin, though a high level of resistance to DDT was also detected. A survey has recently been initiated to determine the susceptibility of Malaysian *Ae albopictus* to these insecticides. Weekly changes in the adult and larva susceptibility of a Malaysian field strain of *Aedes albopictus* against several commonly used insecticides was monitored for 10 weeks. Weekly variations in the susceptibility against all insecticides was found. The adult mosquito was highly susceptible to malathion but multiple resistance to permethrin and DDT was detected. The larvae appeared to be susceptible to both malathion and temephos. Non-specific esterases did not appear to play a role in the multiple resistance of the adult.

**Effect of sublethal dosage of malathion on susceptibility to dengue infection**

No work on the effect of sublethal dosages of malathion on the susceptibility of *Ae aegypti* to dengue virus infection has been reported to date. Such information is of utmost importance in vector control because in the absence of adult mortality due to exposure to sublethal dosage of the insecticide, the potential of the surviving
adults to be infected with dengue virus will determine the subsequent possibility of transmission to human. The present study has recently been initiated in the laboratory to examine this possibility. Surviving adults of lab-bred *Aedes aegypti* (L.) exposed to sublethal dosages of malathion were membrane-fed with human blood containing dengue-2 virus. After an incubation period of 10 days, the fed mosquitoes were pooled and homogenised. The homogenate was inoculated into C6/36 *Aedes albopictus* cell line and the presence of the virus was detected by the peroxidase antiperoxidase staining. *Ae. aegypti* adults exposed to sublethal dosages of malathion and those survived were able to support the development of dengue-2 virus. This finding implicates that malathion fogging, one of the important methods of dengue vector control, should be conducted in strict accordance to the dosage and application techniques to ensure complete mortality of all adult vectors.

**Residual effect of insecticides**

Recently, it was reported that synthetic pyrethroids (alphacypermethrin and permethrin) sprayed on the wall of wooden trap huts were able to exert residual adulticidal activity against lab-bred *Ae albopictus* for at least 8 weeks. They thus proposed the use of residual spraying for *Aedes* vector control in the urban area. In a much earlier study, it was concluded that peritropical residual spraying of DDT and dieldrin was effective and the most economical means of controlling *Ae aegypti* adults in Port Swettenham (Klang). However, in view of the present urban set up in Malaysia, the operational practicability of residual spraying in dengue vector control needs to be considered carefully. In addition, the indoor resting behaviour of the vectors need to be studied before the effectiveness of this method can be ascertained.

**Effectiveness of ULV application of synthetic pyrethroids**

The efficacy of ultra-low-volume application of a synthetic pyrethroid was evaluated in 2 residential areas comprising linked terrace double storey houses (site 1) and multi-storey flats (site 2), respectively in Kuala Lumpur City. Air-borne bioassay of caged laboratory-bred *Aedes aegypti* and *Culex quinquefasciatus* indicated that in site 1, overall mean indoor and outdoor mortality was about 51% and 70%, respectively for both species. In site 2, highest mean mortality (81.5%) of *Ae aegypti* was achieved in the first floor (indoor). Low adult mortality of *Cx quinquefasciatus* was observed. There was a complete absence of larvicidal activity. The droplet profile analysis indicated presence of ULV droplets which were uniformly distributed. Generally, in ULV application of pyrethroid under real life situation, complete adult mortality was not achieved. Low adulticidal activity of synergised pyrethrins against *Ae aegypti* and *Ae albopictus* was reported in Penang Island; while low *Ae. aegypti* adult mortality in testing of ULV-applied Resigen (S-bioallethrin+permethrin+piperonyl butoxide) diluted to 1:16 with diesel was also reported. On the other hand, Gill (unpub data) reported in tests with Resigen, that very high kill of *Ae albopictus* in a residential area in Ipoh, Perak was observed. These data indicated that the effectiveness of synthetic pyrethroids applied by the ULV technique may be dosage-dependent and not due to machine performance. Hence to ensure the effectiveness of synthetic pyrethroids in dengue vector control, it is important to determine the optimal dosage of pyrethroid to be used in ULV application prior to their actual use in control operation.

**Combined adulticidal, larvicidal and residual activity of ULV-applied pyrethroid**

Ideally, a complete system of dengue vector control using chemical insecticides should utilise a chemical that exhibits all 3 crucial
insect-killing activities: adulticiding, larviciding and residual activity (on wall) in a single spraying. As mentioned above, simultaneous killing of larvae and adult is highly effective, while survived adults that rest on any surface that contained the insecticide will be killed. One such pyrethroid has been reported\textsuperscript{16}. The combined adulticidal, larvicidal and wall residual activity of ULV-applied bifenthrin, a synthetic pyrethroid was evaluated in houses in Kuala Lumpur, Malaysia against larvae and adults of lab-bred \textit{Aedes aegypti} and \textit{Culex quinquefasciatus}. A portable ULV sprayer was used to disperse a ULV formulation of bifenthrin at a discharge rate of 45 mL/min. The results indicated that bifenthrin sprayed at this rate exhibited all the 3 activities against the test mosquitoes. Complete adult mortalities were achieved, while very high larvicidal activity was also observed which persisted for 7 days. Wall bioassay with adults of \textit{Ae aegypti} also resulted in very high mortality which also persisted for 1 week. The combined mosquitocidal activities of bifenthrin is considered more effective especially in the control of dengue vectors.

\textbf{Effect of household aerosol on \textit{Aedes aegypti}}

The use of household aerosol in Malaysia is widespread and increasingly important. However, very few field studies on the effectiveness of these aerosol are reported. A limited field evaluation of the effectiveness of a commercial household aerosol can in 3 unoccupied houses in Kuala Lumpur was reported\textsuperscript{17}. Cages of lab-bred adults of \textit{Aedes aegypti} and \textit{Culex quinquefasciatus} were placed into each test house. One house each was sprayed for 5, 10 and 15 seconds and the knock-down rate of the mosquitoes was determined 10 min post-spraying. The mortality of the mosquitoes was recorded 24 h after spraying. The results indicated that a spray time of 10 and 15 s in these houses was highly effective against these mosquitoes. Thus household aerosol containing d-allethrin, if used correctly, may also play an important role in the control of dengue vectors.

\section*{2. VECTOR SURVEILLANCE}

\textbf{Surveillance of dengue virus in mosquitoes}

The detection of dengue virus in the vector mosquitoes is important since this serves as an early warning sign before the appearance of the first human case and the subsequent outbreak. Unfortunately, the detection of dengue virus in wild mosquito populations is rarely conducted due to various constraints. Recently, the detection of dengue virus from wild populations of \textit{Ae aegypti} and \textit{Ae albopictus} using cell culture techniques and detection by PAP staining and RT-PCR has been reported\textsuperscript{18}. Dengue virus was detected (by PAP) in a pool of adult \textit{Ae albopictus} collected in Kuala Lumpur. Dengue virus was also detected from adults originated from pools of larvae collected from various localities in K. Lumpur and other states; thus confirmed the maintenance of the virus in the larval stage through transovarian transmission.

\textbf{Laboratory and field studies on transovarial transmission of dengue virus in \textit{Aedes}.}

Experimental results in studies on the possibility of transovarial transmission of dengue virus in \textit{Ae aegypti} and \textit{Ae albopictus} has been reported\textsuperscript{19}. The Malaysian (Selangor) strains of \textit{Ae aegypti} and \textit{Ae albopictus} were studied to observe whether they can transmit dengue virus transovarially to their offspring. Virus isolation was done by using C6/36 cell culture, and virus in cell culture was detected by using PAP staining and RT-PCR. Transovarial transmission of dengue virus was demonstrated in the strain...
of *Ae aegypti*. Dengue virus was isolated from the fourth instar larvae (L4) reared from eggs collected 10-12 days after an infectious blood meal by the parental females. This finding suggests that transovarial transmission of dengue virus can play an important role in dengue virus maintenance in the urban areas where *Ae aegypti* is one of the vector species. This is the first report of transovarial transmission of dengue virus in Malaysia.

The occurrence of transovarial dengue virus in wild populations has been confirmed. Mosquito larvae were collected from dengue high risk areas and transported to the laboratory for identification. Identified mosquitoes were pooled according to the species, date and locality and stored at -70°C. A total of 835 pools of *Aedes albopictus* and 202 pools of *Ae. aegypti* were collected from major towns in 12 states in Peninsular Malaysia. Virus isolation was carried out using cell culture (C6/36 clone) of *Ae. albopictus* and detection of dengue virus by the peroxidase anti-peroxidase staining. All positive isolations were further re-confirmed by the reverse transcriptase-polymerase chain reaction (RT-PCR). Samples from 2 localities in Kuala Lumpur City were positive with PAP staining. On the other hand, samples from Kuala Terengganu, Balik Pulau and Johor Bahru were positive using both methods.

**A stochastic model of dengue transmission**

Weekly ovitrap surveillance of *Aedes* vectors was conducted continuously in three urban areas in Kuching, Sarawak to monitor the populations. The possible application of sequential sampling technique to analyse the ovitrap data was examined. The *Aedes* eggs/larvae were found to exhibit a clumped distribution fitted to a negative binomial distribution model without a common K value. The sample size of ovitraps required for decision to control *Aedes* vectors was determined using techniques of sequential sampling and its use in dengue vector control in Malaysia was examined. The year-to-year variations in the threshold of transmission of a particular locality may reflect the actual efficiency of the vector control operations; for if the threshold is increased, it would imply that higher vector population density is required to initiate an outbreak, as the original population is now less efficient in transmission due to the effective vector control operations. Thus, this stochastic model not only is able to determine the transmission threshold, but also can be used as an epidemiological tool to evaluate the effectiveness of dengue vector control operations in the field.

**3. VECTOR BIONOMICS**

**Biting activity**

Both species are generally day-time biters and are active during the day. However, *Ae albopictus* have also been observed to possess some night-time activity. During the day, both mosquitoes have peaks of landing and biting activity. Two such peaks i.e. just after sunrise and just before sunset were observed. Recently, it was reported that the peak biting activity of *Ae albopictus* occurred at 0915-0930 and 1815-1830. More recently, it was found that the first peak of biting of *Ae albopictus* occurred at about 1 hour after sunrise and reached another peak before sunset. Hence, it is obvious that both species is a day-bitter and there has been no behavioural changes in terms of biting activities in Malaysia.

**CONCLUSION**

Future research on the dengue vectors should be focused on finding better means of control and surveillance so that such knowledge can be used to enhance our present control methods. Such research efforts should: (1) look
into more effective methods of application of insecticides to ensure total and full coverage of target sites e.g. use of aerial spraying, (2) examine the role of simultaneous application of larvicide and adulticide especially the mixture of chemical insecticide and microbial control agent, (3) search for control agent that exhibits larvicidal, adulticidal and residual activities in a single operation, (4) determine transmission threshold so that remedial action can be taken to prevent an outbreak, (5) develop capability to detect dengue virus infection in the vectors and (6) to ascertain the effect of various insecticides on the susceptibility of the vectors to dengue virus infection.

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GENOTYPES OF JAPANESE ENCEPHALITIS VIRUS ISOLATED FROM THREE STATES IN MALAYSIA

H Tsuchie, K Oda, I Vythilingam, R Thayan, B Vijayamalar, M Sinniah, Jasbir Singh, T Wada, H Tanaka, T Kurimura and A Igarashi


Two hundred forty nucleotides from the pre-membrane gene region of 12 Japanese encephalitis virus (JEV) strains isolated from three different regions of Malaysia from 1993 to 1994 were sequenced and compared with each other and with the JEV strains from different geographic areas in Asia. These 12 Malaysian isolates were classified into two genotypes. The four JEV strains isolated from Sarawak in 1994 and the four JEV strains isolated from Sepang, Selangor in 1993 were classified into one genotype that included earlier isolated strains from Malaysia (JE-827 from Sarawak in 1968 and WTP/70/22 from Kuala Lumpur in 1970). The four JEV strains from Ipoh, Perak in 1994 were classified into another genotype that included JEV strains isolated from northern Thailand and Cambodia. In an earlier report, 10 JEV strains from Sabak Bernam, Selangor in 1992 were classified into the largest genotype that included strains isolated in temperate regions such as Japan, China and Taiwan. The data indicate that at least three genotypes of JEV have been circulating in Malaysia.

ABUNDANCE, PARITY AND JAPANESE ENCEPHALITIS VIRUS INFECTION OF MOSQUITOES (DIPTERA: CULICIDAE) IN SEPANG DISTRICT, MALAYSIA

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A 2-yr study of Japanese encephalitis (JE) virus in Sepang District, Selangor, Malaysia, was carried out to identify the mosquito vectors and to determine their seasonal abundance, parity and infection rates. In total 81,889 mosquitoes belonging to 9 genera and >50 species were identified from CDC trap collections augmented with dry ice during 1992 and 1993. Culex tritaeniorhynchus Giles and Culex gelidus Giles were the most abundant species and both increased in numbers with increases in rainfall. Overall, 45 JE virus isolations were made from 7 species - Cx. tritaeniorhynchus (24), Cx. gelidus (12), Culex fuscocephala Theobald (2), Aedes butleri Theobald (4), Culex quinquefasciatus Say (1), Aedes lineatopennis Ludlow (1) and Aedes (Cancraedes ) sp (1). Based on elevated abundance and JE infection rates, Cx. tritaeniorhynchus appears to be the most important vector of JE virus in Sepang.
Comparison of sequences of E/NS1 gene junction of dengue type 3 virus following culture subpassage in C6/36 cells to study the possible occurrence of mutations.

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The aim of this study was to determine whether mutations could occur in the dengue virus genome following three subpassages of the virus in a mosquito cell line. This was done because sources of virus isolates used for sequencing studies are usually maintained in cell lines rather than in patient’s sera. Therefore it must be assured that no mutations occurred during the passaging. For this purpose, sequencing was carried out using polymerase chain reaction (PCR) products of the envelope/non-structural protein 1 junction (280 nucleotides) of dengue type 3 virus. Sequence data were compared between the virus from a patient’s serum against the virus subpassaged three times in the C6/36 cell line. We found that the sequence data of the virus from serum was identical to the virus that was subpassaged three times in C6/36 cell line.

A NOVEL INSECTICIDAL SEROTYPE OF CLOSTRIDIUM BIFERMENTANS


A novel Clostridium bifermantans strains toxic to mosquito larvae on ingestion was isolated from soil sample collected from secondary forest floor. This strain was designated as serovar paraiba (C.b.paraiba) according to its specific H antigen. Clostridium bifermantans paraiba is most toxic to Anopheles maculatus Theobald larvae (LC50 = 0.038 mg/liter ), whereas toxicity to Aedes aegypti (Linn.) (LC50 = 0.74 mg/liter ) and Culex quinquefasciatus Say ( LC50 = 0.11 mg/liter ) larvae were 20 and 3 times lower, respectively. The toxicity to An. maculatus larvae is as high as that of Bacillus thuringiensis serovar israelensis. C.b. paraiba was also found to exhibit significant per os insecticidal activity towards adult Musca domestica ( Linn. ).

A MALAYSIAN ISOLATE OF BURKHOLDERIA PSEUDOMALLEI EXHIBITING MOSQUITOCIDAL ACTIVITY

P. Seleena and HL Lee ASEAN Journal Science Technology Development 1997; 14: 69-78

A gram negative, motile mosquitocidal bacteria ( Isolate No.448 ) was isolated from a roadside soil sample collected in northern Perak. This isolate, identified as Burkholderia pseudomallei, is toxic to Culex quinquefasciatus, Anopheles maculatus and Aedes aegypti mosquito larvae, with Cx. quinquefasciatus being the most susceptible. The mosquitocidal activity was detected in cultures grown in nutrient agar with glycerol at 28-35 °C. Mammalian toxicity studies in hamsters indicated that this isolate is non-virulent. A 24 hour broth culture left in the room temperature for 2 months continued to
cause 100% larval mortality. The stability of the mosquitocidal principle(s) at room temperature and its absence of virulence may make it a promising candidate of control agent. On agar plate, this isolate produces morphologically 2 different colonies: a smooth mucoid colony (SM) and a dry wrinkled colony (DW). Both colonies exhibit similar biochemical characters. The SM colony is non mosquitocidal while the other DW colony is toxic to mosquitoes. On subculturing, the SM colony produces either SM or DW or both types of colonies. Electronmicrograph showed the presence of dark granules in the DW colony which are absent in the SM colony. These granules may be related to mosquitocidal activity of the bacteria.

**Cockroaches from Urban Human Dwellings: Isolation of Bacterial Pathogens and Control**

I Vythilingam, J Jeffery, P Oothuman, Abdul Razak AR and Sulaiman A


A study was carried out to determine the distribution of cockroaches in two different housing areas with central sewerage or individual septic tanks in an urban area in Kuala Lumpur, Malaysia. Six species of cockroaches were present and of these Periplaneta americana and Periplaneta brunnea were found in greater abundance. Seventeen species of bacteria were isolated and of these Escherichia coli and Klebsiella pneumoniae were isolated in greatest numbers. Control measures carried out using lambda cyhalothrin showed that there was no significant difference between treated and control sites.
From 1993-1995, the Institute for Medical Research (IMR) and the Japan International Cooperation Agency (JICA) successfully conducted a collaborative research project on selected tropical diseases. During this period, the IMR benefitted tremendously through the transfer of expertise in biotechnology by Japanese experts. As a continuation of this linkage, it was felt that IMR researchers who were trained through the project could share their knowledge and expertise with the help of Japanese experts by training other scientists in the region; this would be carried out through the IMR-JICA Third Country Training Programme for an initial period of three years from 1995-1997. The title of the one-month training course was “International Seminar on Biotechnological Techniques in Tropical Medicine”. For each course, a total of 8 foreign participants who were fully sponsored by JICA were selected from countries in the Asia Pacific region. For the Malaysian participants (4 or more per course), they were sponsored by their host institutions.

The main objective of this training course was to provide participants from Asia and the Pacific countries with an opportunity to improve their knowledge and techniques in the field of biotechnology in tropical medicine. At the end of the course, the participants are expected to have acquired the latest techniques in relation to nucleic acid isolation, purification and manipulation, construction of genomic and cDNA libraries, production and use of nucleic acid probes, various molecular methods for characterization and analysis of nucleic acid fragments, and the production and use of monoclonal antibodies. In addition, it is hoped that through these courses, participants would be able to strengthen an Asia-Pacific network for the practice of biotechnological techniques in tropical medicine.

Three such courses have been conducted over the three-year period (1995-1997) and a total of 41 participants from 11 countries (China, Indonesia, Lao PDR, Malaysia, Maldives, Nepal, Philippines, Solomon Islands, Sri Lanka, Thailand, Vietnam) have been trained. The course lecturers included researchers in the IMR, invited lecturers from local universities/institutions and Japanese experts (two per course); the emphasis of these courses was hands-on practical training (which covers about 70% of the duration); the rest being lectures and tutorials.
From the feedback obtained from course participants at the end of each course via questionnaire and open discussions, they felt that they had gained a lot from the training they had received and they also found that the contacts and friendships forged between scientists of participating countries, course lecturers and Japanese experts to be extremely beneficial for future collaborations.

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