FACULTY OF MEDICINE, UDG GIRONA

AN UPDATE ON DENGUE SITUATION AND CASE CLASSIFICATION

Final Degree Project

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World Health Organization, Genève

September – November 2015

To Dr. Raman Velayudhan, Dr. Jose A. Ruiz Postigo, Caroline Reghelini, Lise Grout, Anna Drexler and Dr. Josep Garre for providing me such a wonderful opportunity of working at the WHO and providing me with guidance throughout the project. I'm also grateful to Dr. Olaf Horstick and Dr. Volker from Heidelberg University for providing technical and statistical support.

CONTENTS

ABBREVIATIONS

SEARO	Regional Office for South-East Asia
SEAR	South East Asia Region
PAHO	Pan American Health organization
EMRO	Eastern Mediterranean Regional Office
EMR	Eastern Mediterranean Region
AFR	African Region
WHO	World Health Organization
DENV	Dengue virus
EU	European Union
EEA	European Economic Area

1. ABSTRACT

This descriptive cross-sectional retrospective study is designed to update the global burden of dengue and to analyse if the new case management has been effective in reducing mortality and morbidity.

The world is divided in two groups, each one using different case definitions for diagnosing dengue. SEAR uses the old 1997 WHO case definition while PAHO uses the new 2009 WHO case classification.

A literature review will be carried out in order to update the pros and cons of each case definition and national data will be used to analyse the impact of both methods on the mortality. Statistical tests will be performed to quantify the results.

Due to time limit, only the top five representative countries will be studied for each region.

2. Background

2.1 Introduction

Dengue is the most rapidly expanding mosquito-borne viral infection in the World. The flavivirus is disseminated by the female mosquito mainly of the species *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*. It is mainly found in tropical and sub-tropical climates and has significant public-health impact. The early stages of the infection are flu-like, but can occasionally develop into a potentially fatal complication named severe dengue. Once infected, humans become the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes. The virus circulates in the blood of an infected person for 2-7 days, at approximately the same time that the person develops a fever. Patients who are already infected with the dengue virus can transmit the infection via *Aedes* mosquitoes after the first symptoms appear (during 4-5 days; maximum 12). (1)

Distinct genotypes have been identified within each serotype, highlighting the extensive genetic variability of the dengue serotypes. Among them, "Asian" genotypes of DEN-2 and DEN-3 are frequently associated with severe disease accompanying secondary dengue infections. (1, 2, 3)

The global incidence has been multiplied rapidly in recent decades and almost half of the world's population is now at risk. The severe dengue stage is a well-known cause of serious illness and death among children in Asia and Latin American countries. The treatment of dengue infection is rather symptomatic, but early detection and good health care system could lower fatality rates below 1%. Vector control measures could be the most effective way to control and prevent dengue. Research is in progress. (1)

2.2 Epidemiology

2.2.1 The virus

The dengue virus (DEN) is a small single-stranded RNA virus. There are four different serotypes known (DEN-1 to -4) and all of them belong to the Flaviviridae family.

2.2.2 The vectors

All the dengue serotypes are transmitted to humans through the bites of an infected Aedes mosquito, principally *Ae. aegypti.* The fly mainly acquires the virus while taking a blood meal from an infected person. Within the mosquito, the virus infects the mosquito mid-gut and spreads to the salivary glands in 8-12 days. Subsequently it can infect other human beings. The mosquito is well distributed in tropical and subtropical regions all around the World, mostly between latitudes 35°N and 35°S.

They do not survive the winter, and thus, they are rarely found above 1000 metres due to low temperatures. (4) These mosquitoes have a complex life-cycle. They lay their eggs on the inner, wet walls of water containers. The larva is engendered when the water inundates the eggs after a rain or a flooding (addition of water by humans). Later, the metamorphosis is triggered and the larva turns into pupa in a week and into an adult mosquito in approximately two days. The newly formed adult rises from the water after cracking the pupal skin. Thus, the life-cycle, completed in approximately 8-10 days, can be divided into two stages: the aquatic stage (eggs, larvae, pupae) and the terrestrial stage (adults). (5)

Not only in outdoor water containers, the Ae. Aegypti can also be found indoors in relation with artificial containers associated with human dwellings or even in dark cool closets. (6)

It is very difficult to control and eliminate this mosquito specie due to its capacity of adaptation. (9) The eggs can remain viable for many months without the presence of water. Dengue outbreaks have also been linked to other species like Aedes albopictus, Aedes polynesiensis and several other species of the Aedes scutellaris family. In recent decades, Aedes albopictus has spread to many regions from Asia to Africa, Europe and the Americas due to the increased international trade of used tyres. These tyres contain dried Aedes eggs and they emerge when in contact with water. The *Ae. albopictus* is primarily a forest species that has adapted to rural, suburban and urban human environments.

2.2.3 The host

Once a person is infected, there is an incubation period of around 4 to 10 days. After this time span, the dengue virus can produce a broad spectrum of illness. Most of the infections are subclinical or asymptomatic or even flu-like. After a primary infection, the organism produces life-long immunity for that specific serotype (7) To the contrary; individuals get cross-protective immunity only for a limited period, not for life time.

The dengue virus enters via the bite of an infected mosquito during a blood meal. During the acute phase of illness the virus is present in the blood and its clearance from this compartment generally coincides with defervescence. Humoral and cellular immune responses are considered to contribute to virus clearance via the generation of neutralizing antibodies and the activation of CD4+ and CD8+ T lymphocytes. In addition, innate host defence may limit infection by the virus. After infection, serotype specific and cross-reactive antibodies and CD4+ and CD8+ T cells remain measurable for years (4)

The severity of the disease depends on the individual risk factors such as age, ethnicity, immune status, secondary infection, and possibly chronic diseases like diabetes mellitus, bronchial asthma and sickle cell anaemia (4, 8, and 9).

Young children are at greater risk of dengue shock due to the lack of capillary leakage compensation (4). Severe dengue is characterized by plasma leakage, haemoconcentration and homeostasis abnormalities (4). Further details will be given in the next sections.

2.3 Burden of disease

Dengue is a menacing and expanding global economic and burden disease. It is a burden on health care systems in endemic countries. It is estimated that nearly half of the world's population is at risk of the disease.

There are no exact figures yet, only estimates considering the number of cases underreported and the cases misclassified. Although the numbers are uncertain, its epidemiological patterns are alarming. A recent study estimates that the current global disease burden is approximately 390 million infections per year (95% credible interval 284-528 million) of which around 96 million (67-136 million) manifest clinically (with any severity of disease) (10)

Table 1 below shows the estimates for apparent and inapparent global burden. These estimates are sensitive to changes in data.

	Apparent		Inapparent	
	Millions	(credible interval)	Millions	(credible interval)
Africa	15.7	(10.5 - 22.4)	48.4	(34.3 - 65.2)
Asia	66.8	(47.0 – 94.4)	204.4	(151.8 – 273.0)
Americas	13.3	(9.5 - 18.5)	40.5	(30.5 – 53.3)
Oceania	0.2	(0.1 - 0.3)	0.6	(0.4 - 0.8)
Global	**96.0**	**(67.1 - 135.6)**	**293.9**	**(217.0 – 392.3)**
Tropics	71.8	(50.9 - 100.1)	219.8	(16.4 - 29.0)
Not-tropics	23.4	(15.7 - 34.4)	71.7	(51.1 - 99.0)

Table 1. **Global disease burden estimates for dengue: apparent and inapperent (61)**

Table 2 below compares the global burden for dengue and malaria.

	Malaria[1]	Dengue[2]
Population at risk	3.2 billion	3.9 billion
Endemic countries	97	128
Infections /year	198 million	390 million
Severe Cases	3 million	2.1 million
Deaths/ year	584,000	21,000

Table 2. **Global disease burden estimates for dengue and malaria. (61)**

Approximately 21.000 deaths per year are attributable to dengue. Mortality is highest during the initial span of the outbreak or epidemic. Children are considered to be the most vulnerable. The worsening in the dengue situation is due to unplanned urban development, poor water storage practices and deficient sanitary conditions. (12)

A recent study on the prevalence of dengue has shown estimates of 3900 million people living in 128 countries are at risk of being infected by dengue (11)

The dengue vectors have been silently expanding their distribution around the world and are now present in 150 countries. International trade of used tyres and plants with axils have facilitated the spread. Both these products are a reservoir of dried mosquito eggs. (12)

The main dengue vectors also transmit other closely related arboviruses such as Chikungunya and Zika viruses.

AFRICA

Although the total burden of disease is still unknown, 22 countries have reported outbreaks. A serological survey was carried out and showed that there is high prevalence of antibodies to dengue viruses, thus this suggests that dengue is endemic in many parts of Africa (12)

Dengue is still underreported in Africa. Many reasons contribute to that such as lack of awareness among health-care workers, the existence of similar febrile illness (malaria) and insufficient clinical diagnosis, laboratory testing and case reporting that hinders systematic surveillance. Angola, Mozambique, Mauritius (12, 13) and United Republic of Tanzania have reported dengue outbreaks since 2013.

Around 32 African Countries have been reporting cases since 1960 although the burden of disease and distribution are still uncertain. DENV-2 has been the most common but all four serotypes have been found in Africa. Co-circulation of three serotypes simultaneously has been documented in Gabon (31). There is research data collected showing presence of both dengue vectors present in Africa: *Ae. aegypti* and *albopictus.* The lack of comprehensive surveillance and correct diagnosis is still a major problem (16).

EASTERN MEDITERRANEAN REGION

Since the last two decades, laboratory-confirmed cases have been reported in the EMR. Dengue is considered to be an emerging disease. Most of the cases have been detected near the coasts of the Red Sea and the Arabian Sea. It is considered as a major public-health problem in Yemen, Pakistan and Saudi Arabia. Multiple virus serotypes co-circulate in Djibouti, Sudan and Somalia and these countries have therefore reported frequent outbreaks.

Oman has reported imported cases. (12). Since mid-1990s, Yemen and Saudi Arabia have been reporting epidemics. Dengue haemorrhagic fever and dengue shock syndrome have caused some deaths as well. *Aedes aegypti* is still found in some places even though there is low rainfall and the climate is unfavourable in these areas. It is supposed that construction sites are apt for breeding. The risk in these regions is higher due to mass gatherings (pilgrims to the Hajj) and trade. Urbanization is also increasing. In 2013, Saudi Arabia reported 4411 dengue cases which were four times higher than 2012 and the peak transmission happened in May (29). In these regions, DENV-1, DENV-2 and DENV-3, the three serotypes, have been recorded. Yemen has documented co-circulation of dengue and chikungunya just like Africa, Asia and the Pacific Islands (30)

REGION OF THE AMERICAS

During 1970, this region had been successful in interrupting dengue transmission as a result of the *Ae. aegypti* eradication campaign against yellow fever. Outbreaks reoccurred in the Caribbean and in Central and South America due to poor sustained vector surveillance (12, 14).

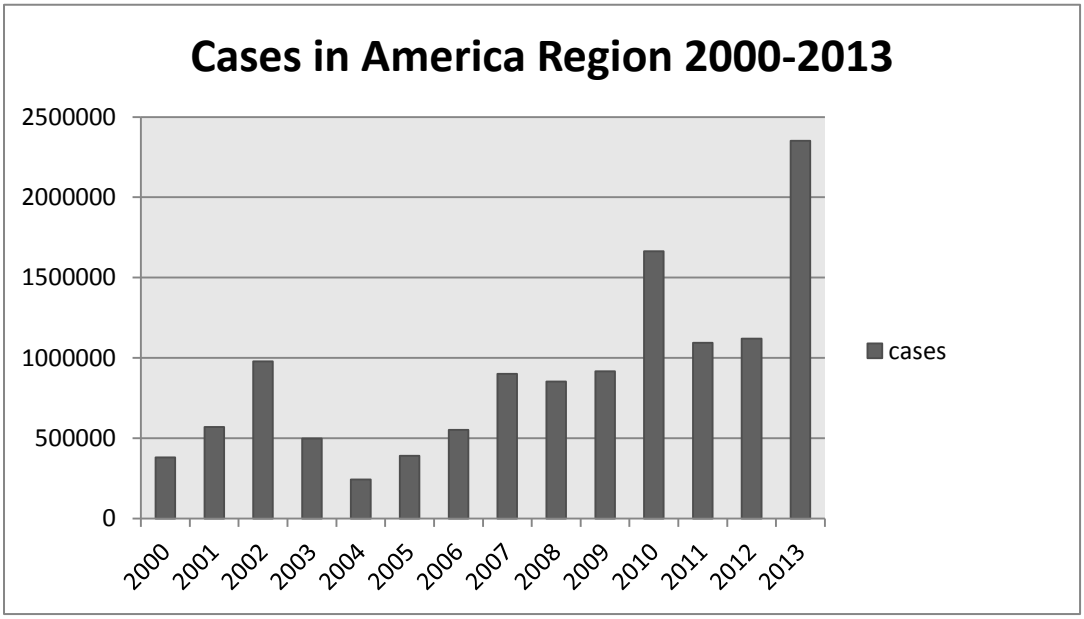
These regions are now in a hyperendemic state, with transmissions happening in almost all countries (12, 15)

USA has undergone massive epidemics in the past, but in recent decades only sporadic cases related to local transmission have been reported (17) (*see graphs A & B*). Since 2009, Key West (Florida) has been having dengue transmissions (16, 18), mainly DENV-1 serotype (16, 19). Dengue is increasing in the Americas. It has increased almost eight fold in the past 30 years (16, 20, and 21). Peru underwent an outbreak in 2010-2011. DENV-2 virus has been circulating in the region since 1990, but research showed that the responsible was a DENV-2 of lineage II of Southeast Asian/ American genotype that differed from the original one (16, 22)

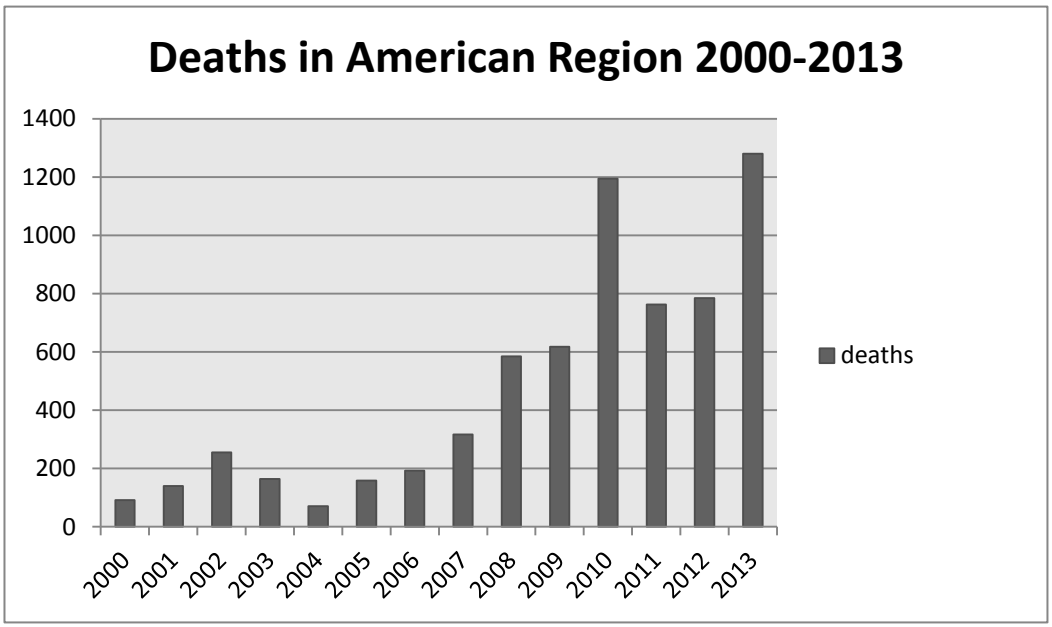
The secondary vector, Aedes Albopictus, can currently be found in New Jersey, southern NY and in Pennsylvania. It is predicted that around 30 million people could live in infested areas with *Ae. albopictus.* It is important to remember that this mosquito is a common vector for dengue transmission as well as Zika virus and Chikungunya (a newly introduced in the Western hemisphere). (16, 23) Araujo and colleagues studied the evolution of DENV-3. They reckon that probably this serotype emerged during mid-1970s and then spread and its introduction from the Caribbean to Brazil is quite frequent. DENV-3 was first detected during outbreaks in Nicaragua and Panama in 1994 (16, 24). The serotype DENV-4 reappeared in Brazil during 2010 specifically in Roraima state. It hen spread to Rio de Janeiro and other regions (16, 25, and 26). There has been an increase of new cases and a rise of severe cases in Brazil between 2000-2010 (16, 27, and 28).

The epidemiological pattern has been complex overall. Much attention was given on Brazil during the FIFA World Cup event in 2014. Prosperously, no outbreaks were reported during and immediately after the World Cup in Brazil (16)

Graph A. Cases in American region 2000 to 2013



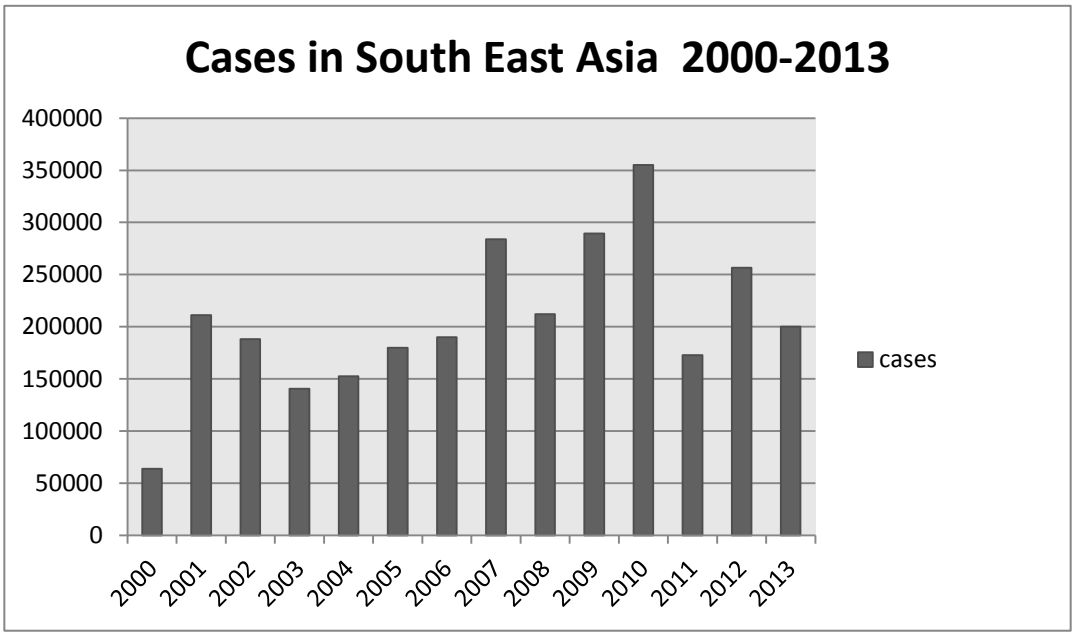
Graph B. Death in the American region 2000 to 2013



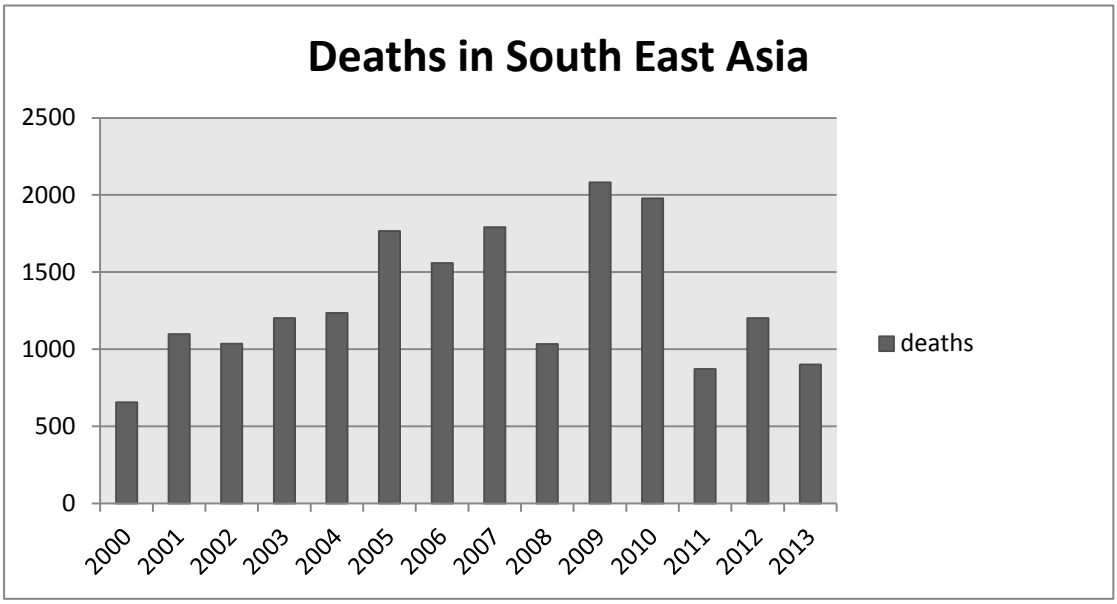
SOUTH EAST ASIA

Dengue is considered to be endemic in this region, although the number of cases varies greatly among countries and within each country. Around 1.8 billion people are estimated to be at risk in Asia pacific countries. This region bears the heaviest burden. (12) (*see graph C & D)*

Graph C. Cases in South East Asia 2000 to 2013



Graph D. Deaths in South East Asia 2000 to 2013



EUROPEAN REGION

Global trade of used tyres and lucky bamboo has aided the *Ae. albopictus* expansion into more than 25 countries. Therefore, dengue outbreak threats exist in Europe. Croatia and France reported local transmission cases for the first time in 2010; several other European countries reported imported cases. "*An outbreak in Madeira island of Portugal (2012) resulted in more than 2200 cases and importation of cases into 17 other European countries "(*12). Some sporadic cases have been documented in mainland Europe, France and Croatia (39, 40). Since 2007, *Aedes albopictus* has been established in southern France and also found in varied parts of southern Europe. In less than four months, France reported 370 confirmed cases of dengue and chikungunya during summer of 2014. Aedes aegypti is also found in parts of Europe (41).

Madeira Island was affected by a major outbreak back in 2012 (42). The cases started to appear in October 2012 and finished in March 2013. There were more than 2100 cases reported, all due to DENV-1.

WESTERN PACIFIC REGION

This region reported around 348 452 dengue cases and 1199 deaths in 2012. The maximum number of cases was reported from the Philippines, Malaysia, Australia and Cambodia. Island nations are predisposed to have epidemics. (*see graph E & F)*

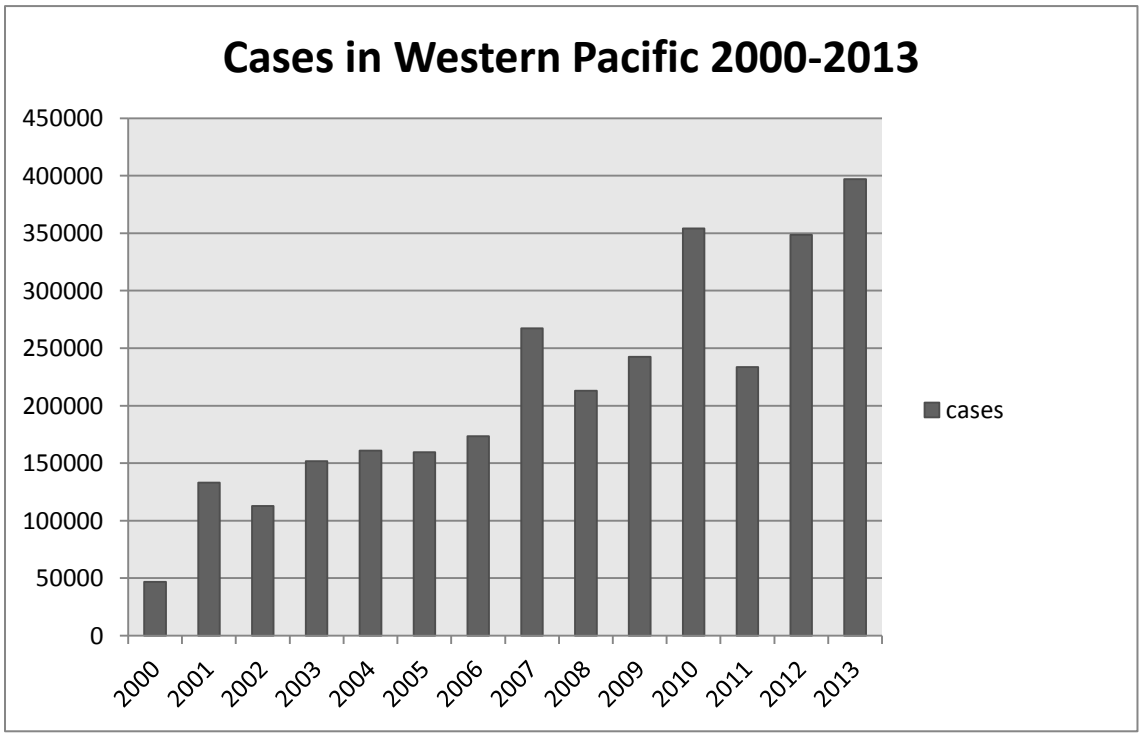
During 2013-2014, the DEN-3 serotype was found in many islands, such as Fiji. Singapore and Malaysia insinuated sustained epidemic activity during the same period. Since late 2013, some countries in the Pacific Region have reported concurrent outbreaks of dengue, Zika and chikungunya viruses. (12)

Dengue is widespread in Asia. Japan (32) recently documented local transmission, including Tokyo (33) which had a chunk of infections. DENV-1 was found responsible in the first suspected outbreak in Shenzhen, China in 2010. The same serotype had also been responsible for infections in Malaysia, Singapore and Vietnam. A province in China named Guangdong is known to have dengue transmission yet Pearl River Delta and the Han River Delta Regions have proven to be highly endemic (34). A multi-centre study interestingly showed that infections due to *Salmonella typhi* and *Chikungunya* have been more common than dengue infections (35)

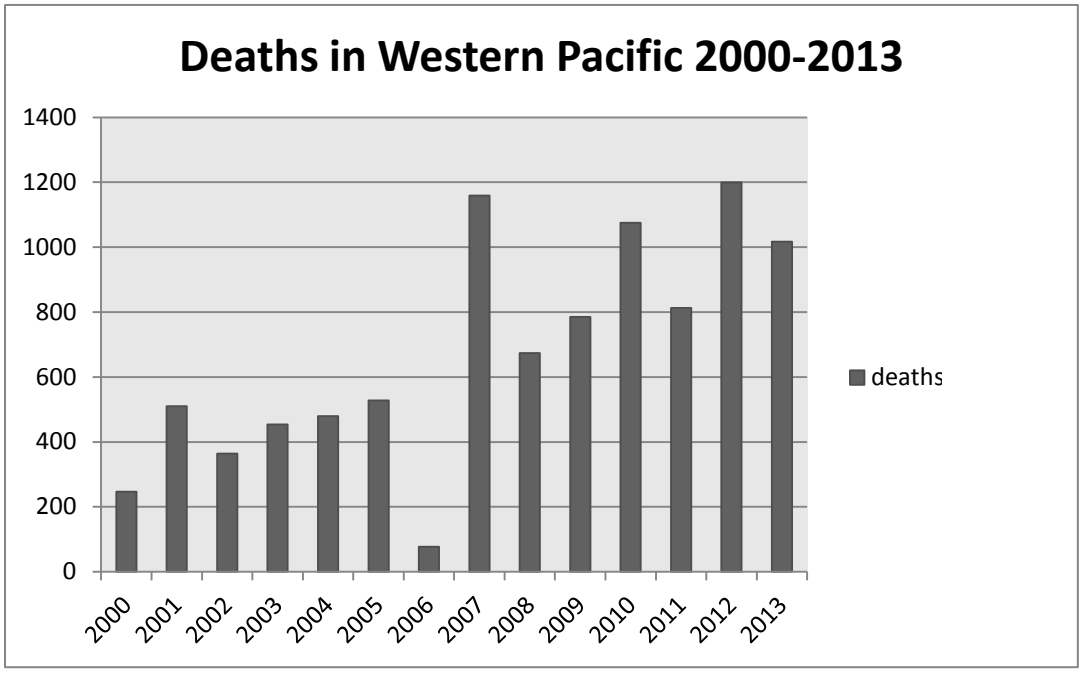
Recently, during 2012-2013, Kosrae in the Federated States of Micronesia (36) reported an intense outbreak where Aedes albopictus predominated. The last outbreak in this region was reported back in 1998.

In 2013-2014 dengue serotypes 1 and 3 were found circulating in the South Pacific Island of French Polynesia. At the same time, Zika virus, also transmitted through the same dengue vectors, was found implicated in a massive outbreak causing around 28.000 cases (37, 38)

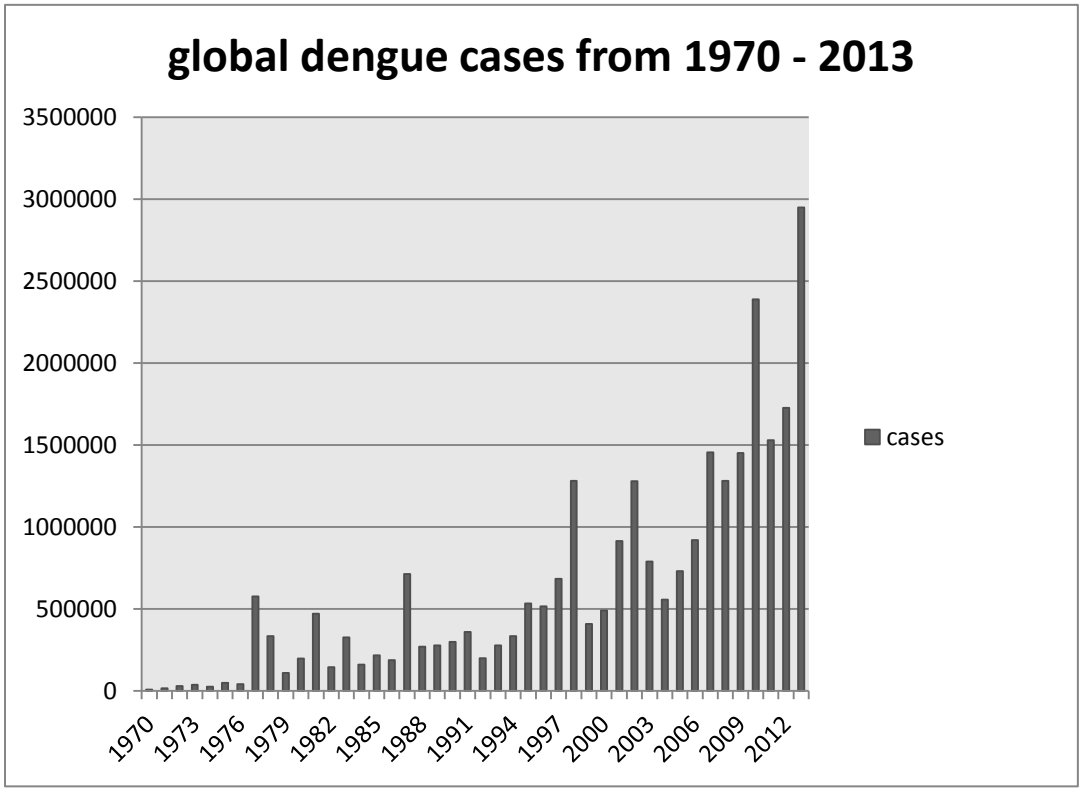
Graph E. Cases in Western Pacific 2000 to 2013



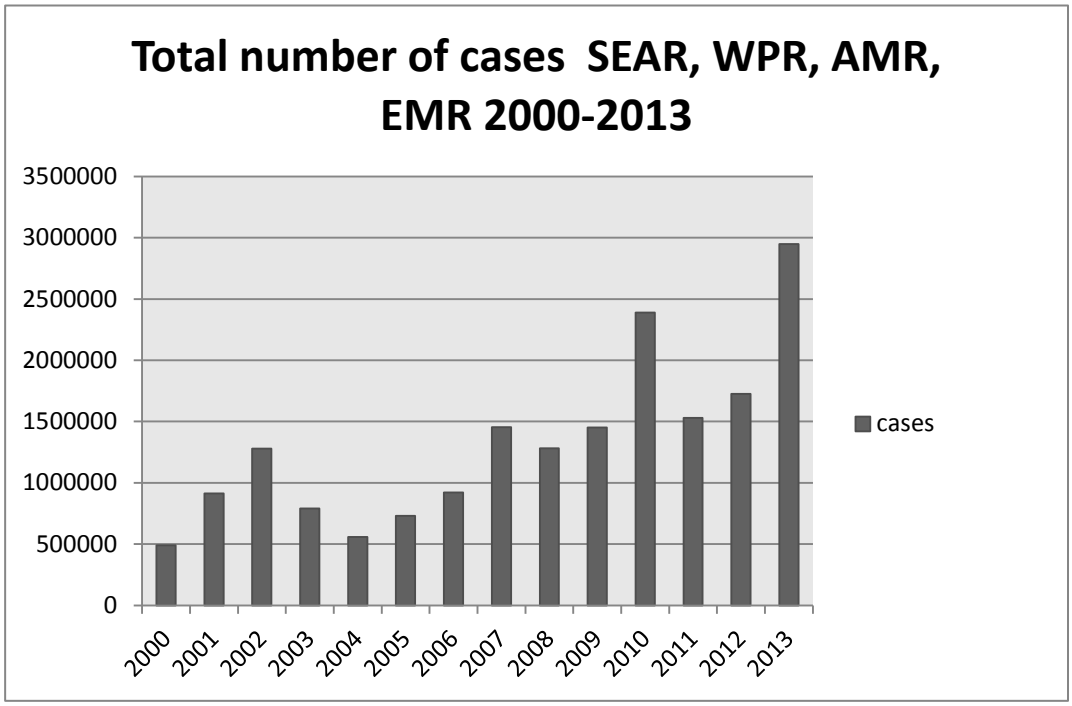
Graph F. Deaths in Western Pacific 2000 to 2013



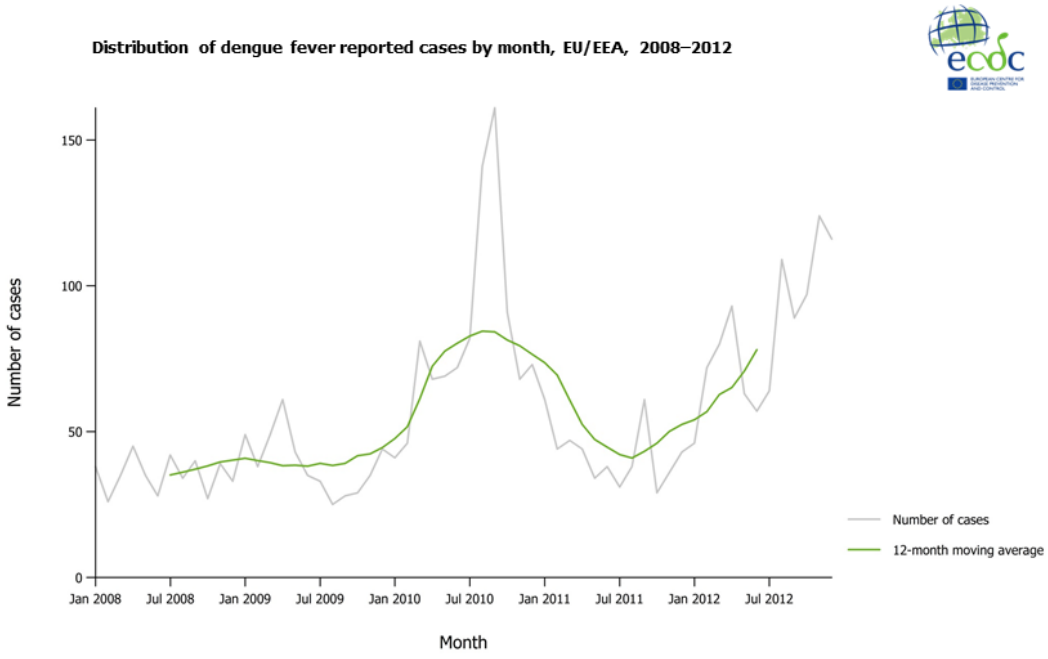
Graph G. Global Dengue cases from 1970 to 2013



Graph H. Total number of cases in the four main Dengue Endemic Regions



Graph I. Distribution of Dengue Fever reported cases by EU/EEA



Source: Country reports from Austria, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Malta, Poland, Slovakia, Slovenia, Spain and Sweden.

Source: European Centre for Disease Prevention and Control. Annual epidemiological report 2014 – emerging and vector-borne diseases. 2014

3. Global strategy for dengue prevention and control

The main aims of the global strategy are (43):

- Reduce mortality by at least 50% by 2020
- Reduce dengue morbidity by 25% by 2020
- Estimate the true burden of disease by 2015

REDUCE THE BURDEN OF DENGUE

Progress has been seen towards the goal of reducing dengue mortality, with data indicating the decline in recent years (Figure 1). Nonetheless, the number of cases per year continues to rise, based on data reported to the WHO from four regions (EMRO, AMRO, WPRO and SEARO). No data is available from AFRO and EMRO. (61)

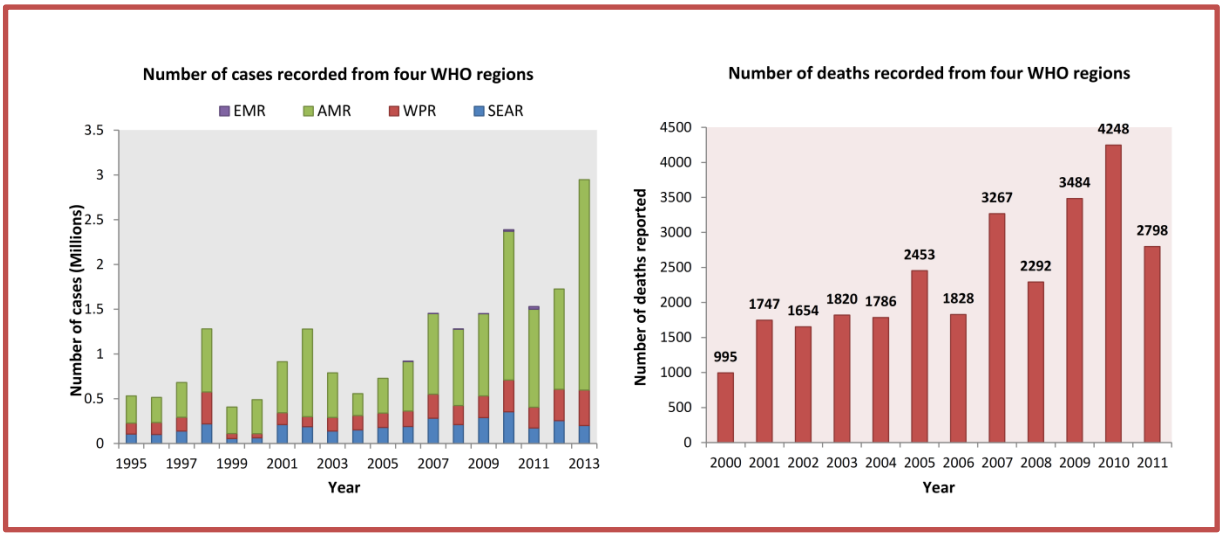


Figure 1. Dengue morbidity and mortality, World Health Organization 2015 (61)

For achieving the global strategy goals, these five key elements must be accurately evaluated and used.

3.1 The five technical elements of the global strategy

1. **Diagnosis and case management:**

This first technical element is crucial for achieving the reduction of mortality by at least 50% by 2020. It is acknowledged that by implementing proper clinical management, which includes early case confirmation, laboratory diagnosis, intravenous rehydration, hospital restructuring and staff training, can reduce the dengue mortality to almost zero.

For a clinical outcome to be successful, it requires (43):

- Early diagnosis
- Accurate differential diagnosis
- Rapid laboratory confirmation
- Early action on severe dengue cases

Dengue is a complex disease due to its wide spectrum of illness yet its management is quite simple, inexpensive and extremely effective in saving lives if interventions are timely and correctly initiated.

The diagnosis of dengue can be done by clinical signs and symptoms; however, it can easily be confused by another febrile vector-borne viral/parasitic disease such as Malaria, Chikungunya or Zika viruses (43).

Diagnosis involves (43):

- Virus culture/ virus detection
- Nucleic acid detection
- Antigen detection:
    - ELISA
    - Rapid tests
    - NS1 (non-structural protein 1) antigen: useful for early diagnosis in febrile patients
- Serology
    - ELISA
    - Rapid tests

After the onset of the disease, the virus can be detected for 4-5 days in serum, plasma, blood cells or any other tissue. During the acute phase, virus isolation, nucleic acid or antigen detection can be useful for the diagnosis. Once the acute phase is over, serology is used as the method of choice for dengue detection.

These serological tests detect specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to dengue virus and can be used as an alternative to virus isolation and/or PCR to support the diagnosis of dengue fever.

In primary dengue infections IgM is the first one to appear. They can be detected in 50% of the patients by day 3-5 after the onset of the disease, 80% by day 5 and 99% by day 10. *"IgM levels peak about two weeks after the commencement of the symptoms and then decline generally to undetectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life"* (44, 45).

To the contrary, in secondary infections the IgM response is weak but has a stronger IgG. This IgG persist for periods lasting from 10 months to life. IgM levels are lower in secondary infections than in primary ones and may be undetectable in some cases, depending on the test used (46). To distinguish between primary and secondary dengue infections, IgM/IgG antibody ratios are now used rather than the haemagglutination-inhibition test (HI) (47, 48)

Primary and secondary care levels, where triage and management decision take place, are crucial in determining the clinical outcome of dengue because these are where patients are first seen and evaluated. *"A well-managed front-line response not only reduces the number of unnecessary hospital admissions but also saves the lives of severe dengue patients. Early notification of dengue cases seen in primary and secondary care facilities as well as commonly accepted definition of outbreak indicators (triggers) is crucial for identifying outbreaks and initiating an early response".* (43)

2, Integrated Surveillance:

Surveillance is a key element for any prevention and control programme. It gives the information necessary for estimating risk. Regarding dengue, it has different objectives (43):

- Rapidly detect epidemics for a further initiation of the cure in process
- To estimate burden of disease and provide data for evaluating its impact on the population
- Detect dengue trends (distribution and spread)
- Supervise environmental risk factors
- Assess if the prevention and control programmes for dengue are effective
- Help planning

Data collected from surveillance programmes can help evaluate success and set new goals.

For a country to have improved dengue surveillance, it should:

1. Promote communication of dengue surveillance indicators
2. Improve data on mosquito breeding sites and environmental risk indicators.
3. Continuous studies on serotype changes

Many new systems of predicting dengue epidemics are in the pipeline. In Peru some investigators use clinical, meteorological, climatic and socio-political data to predict dengue. The future incidence is predicted as high or low (49)

In Colombia, they came up with an environmental-based method that allowed anticipating dengue infection from 2 weeks to 6 months before the occurrence. They used it from 2000 till 2011. They found that infections occurred during warm-dry periods with daily temperatures between 18 and 32°C (50).

In Thailand, Campbell and colleagues gave more importance to the weather (51). They discovered that the range of temperature informed on the dengue transmission viability and that humidity strengthens the capacity of transmission. The results showed that in Thailand, 80% of the infections occurred when humidity was above 75% and the mean temperature was between 27 and 29.5°C (51)

In Singapore, Xu and colleagues concluded that among all the climate variables, humidity seemed to be the key factor (52). Importance is also given to the daily fluctuations of temperature. The vectors seem to live longer and infect more if the daily fluctuations are not extreme (53)

Liu- Helmersson and colleagues mentioned the importance of diurnal temperature range in the potential of dengue epidemic (54)

A Vietnam study found that *"high early plasma virus levels were associated with longer duration infectiousness of the mosquitoes. Hospitalized patients have higher viraemia levels than ambulatory dengue cases but virus levels in ambulatory cases were still sufficient to infect mosquitoes"* (55)

Many speculations have been done on host genetics being a factor that influences in dengue infection severity.

A recent study suggests that having an African ancestry protects against severe outcomes of dengue infection (9, 56). To the contrary, having less African ancestry was associated with more severe clinical symptoms was reckoned by a Colombian study.

3, Sustained Vector Control:

Vector control is crucial for reducing dengue morbidity. We define morbidity as the relative frequency of occurrence of dengue. The main purpose is to decrease dengue transmission thus, decreasing the infection incidence and the number of outbreaks.

It is seen through research that the primary vector, mosquito Aedes Aegypti, is a daytime feeder. Biting peaks occur early in the morning and before dusk in the evening. The female aegypti takes multiple bites during each feeding time (43)

The secondary vector, Aedes albopictus, seen in Asia and now spread to North America and Europe can tolerate temperatures below freezing. It also has the capacity to hibernate and can huddle in small habitats.

Other known vectors are: Aedes polynesiensis and Aedes scutellaris (Rodhain F, Rosen L, 1997). *Aedes hensilli* was identified as an epidemic vector in the Federated States of Micronesia (Savage et al., 1998). *Aedes furcifer and Aedes luteocephalus* are among probable sylvatic vectors in western Africa (43)

Dengue has been mainly controlled by source reduction, as simple as eliminating containers which are favourable for vector development. However, fitting lids or covers over containers can be quite of help. Insecticides are used for killing the aquatic stages of the vector (43)

There are some well-established products for dengue control (4)

- Environmental management: empty/clean scrub weekly, recycle and disposal of water
- Improvement of water supply and water-storage systems
- Mosquito- proofing of water-storage containers (tight lids)
- Solid waste management (refers to non-biodegradable items of household and industrial waste)
- Street cleansing – removes discarded water-bearing containers and cleans drains
- Building structures – urban renewal schemes for reducing larval habitats
- Mosquito larvicides
- Insecticides for space spraying
- Repellents: IR3535 and KBR3023

Some products, designed for malaria, have collateral effects on dengue:

- Long-lasting insecticidal nets (LNs)
- Insecticides for indoor residual spraying (IRS)

However, these vectors can escape from all these vector control measures due to their capacity of adaptation. They can lay eggs in unconventional sites like septic tanks (Puerto Rico) and rooftop gutters (Singapore) (43)

Another big concern is the growing resistance to insecticides. Research data shows that the resistance to organophosphates (temephos) and pyrethroids is widespread in the specie Aedes aegypti and to some extent Aedes albopictus (43)

There are some innovative vector control tools under research which might play a significant role in long-term dengue prevention. These new developments are:

a. Microbial control of human pathogens in adult vectors

Wolbachia-based bio control

Symbiotic Wolbachia spp. Bacteria is introduced into *Aedes* mosquito populations to reduce their ability to transmit dengue viruses to humans. Laboratory research data has shown that Wolbachia infection reduces the replication of the virus within the mosquitoes and eliminates or considerably delays the appearance of dengue virus in mosquito saliva, thus making the mosquito an incompetent vector for transmission of dengue viruses (57)

If this approach works as pictured, it is predicted that it will provide a solution to dengue transmission control, capable of propagating itself without the need for reapplication or human behavioural change (58)

b. Spatial repellents

The spatial repellent releases metofluthrin or transfluthrin in the air at low vapour concentrations. This product will be effective for at least 2 weeks (57).

c. Reducing vector populations through genetic manipulation

Prototype: OX513A TRANSGENIC AEDES AEGYTPI

OX513A is a transgenic strain of *Ae. aegypti* plotted to carry a dominant lethal gene that suppresses *Ae. aegypti* mosquito populations. After releasing transgenic males, these mate with wild females *aegypti* causing lethal offspring, either "female specific" (female offspring do not survive to adulthood) or "bisex" (neither sexes survive to adulthood). This lethal is a dominant gene; any larvae carrying one or more copies of it will develop normally but die before functional adulthood. The lethal gene is repressible by tetracycline (or analogues). A fluorescent marker gene named DsRed2 allows to track the genetic material introduced in the mosquito larvae (58)

It is important to highlight that dengue is not only transmitted by vectors, but also through other novel means such as blood transfusion, organ transplantation and needlestick.

4. Vaccine Implementation

Research is being done for safe and cost-effective vaccines. There are some candidates that have reached clinical phases. One of them is the Sanofi Pasteur tetravalent dengue vaccine (TDV). "*It is composed of four recombinant, live, attenuated vaccines (CYD-1-4) based on yellow fever vaccine 17D (YFV 17D) backbone, each expressing the pre-membrane and envelope genes of one of the four dengue virus serotypes. This vaccine is genetically and phenotypically stable, non-hepatotropic, less neurovirulent than YFV 17D, and does not infect mosquitoes by the oral route"* (60). More than 6000 adults and children have been administered this vaccine and no issues were reported on safety concerns. The regimen consists in a three-dose vaccination which induces an immune response against all four serotypes in the vast majority of vaccines. Preexisting immunity for flavivirus favours higher and quicker immune response to CYD TDV, without adversely effecting clinical safety or increasing vaccine viremia (59).

This vaccine has been evaluated in multiple clinical trials. No significant safety concerns have been found. The Phase 3 trials met their endpoints in terms of overall reduction of confirmed dengue fever, and, most importantly a significant reduction in severe dengue and hospitalization due to dengue. However, based on results that have been published so far, efficacy in preventing serotype 2 infections is less than that for the other three serotypes. In the development of these chimeric vaccines, an important series of comparative studies of safety and efficacy were made using the parental YF 17D vaccine virus as a benchmark (60)

Many more candidates are being tried in clinical trials, and results are yet to be declared.

5, Research

Many aspects need deep research. Effective tools are needed to empower prevention and control programmes as well as new diagnostic and vector control tools. The core areas of research are (43):

- Correct management of severe cases with accurate diagnosis. Research is needed for alternate methods on fluid management, better guidance for blood products, and for dealing with dengue in pregnant women and comorbidities
- Quality improvement of current rapid diagnostic tests
- Improving preparedness for early epidemic response
- Assess dengue risk factors/indicators as early warning signs
- Research on transmission patterns and the impact on dengue due to climatic variables
- Enhance the implementation of cost-effective strategies
- Strategies to manage insecticide resistance
- Development of new non-insecticidal effective tools to prevent outbreaks
- Develop and evaluate safe vaccines

4. Discussion of the challenges of the global strategy

The aims of the global strategy, as mentioned before, are reducing mortality and morbidity rates as well as estimating the true burden of disease. Mortality rates are being claimed of having declined in the past years due to correct application of the case management, yet, the number of cases is increasing each year. The mortality rates could be lower if the World used the same case management method and all the health care providers had knowledge on dengue, plus, diagnostic tests available worldwide. As far as dengue morbidity is concerned, it seems to be increasing too due to lack of sustainable vector control, thus, it all depends on how sustainable the new vector control tools are and how much public acceptance there is. Apart from being sustainable, these tools should be cost-effective and easy to apply. Research is being made and some new tools are in the pipeline. Vaccines are also being developed but due to genetical modifications of dengue mosquitoes, their effectiveness is still doubted.

It would be ideal if the targets could be achieved. Nonetheless, estimating the global burden of disease can be difficult until a systematic worldwide surveillance program is not implemented. Every country should have a notification system so that all cases are documented. This target will be accomplished only after a correct diagnosis. Rapid diagnostic tests should be available in every endemic and non-endemic country so that not even one case remains undiagnosed. Moreover, more investment should be done on education on dengue and its clinical symptoms. Dengue knowledge is the key element for achieving any of the goals.

5. Review and comparison of the criteria for both case classifications.

1997, original case definition:

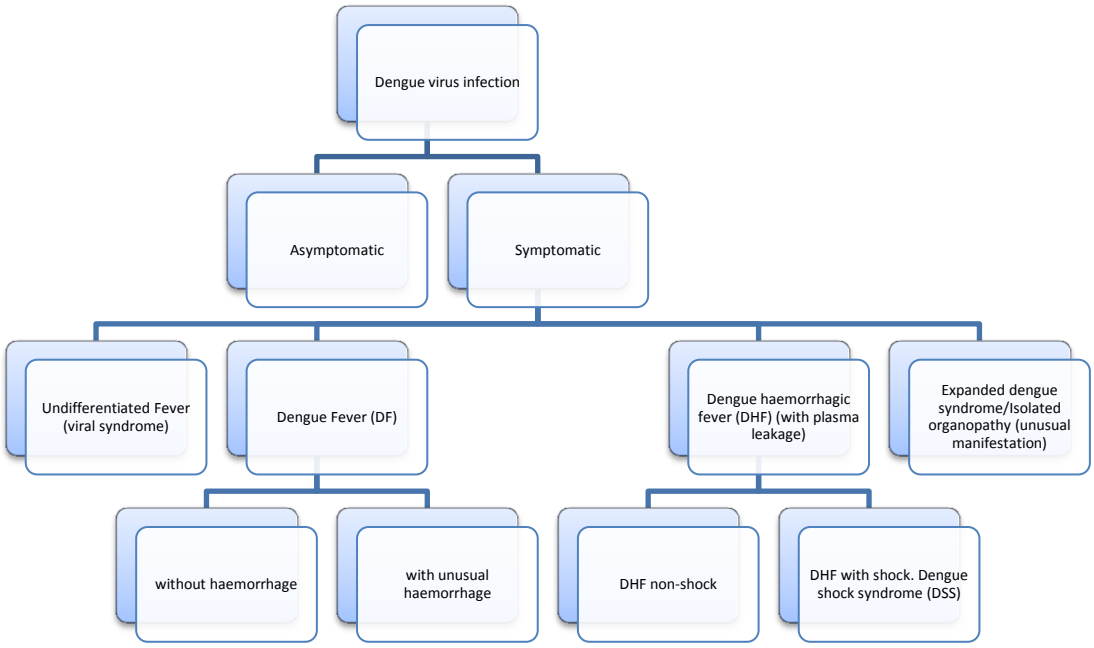
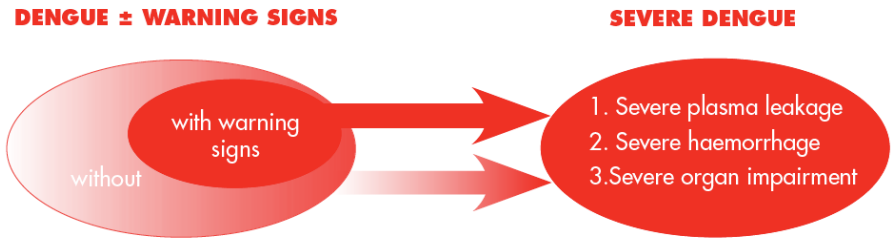


Figure 2 Criteria for the old case classification (45)

2009, revised case definition:

Figure 1.4 Suggested dengue case classification and levels of severity



CRITERIA FOR DENGUE ± WARNING SIGNS

Probable dengue
live in /travel to dengue endemic area.
Fever and 2 of the following criteria:
- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Leukopenia
- Any warning sign

Laboratory-confirmed dengue
(Important when no sign of plasma leakage)

Warning signs*
- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargment >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

*(requiring strict observation and medical intervention)

CRITERIA FOR SEVERE DENGUE

Severe plasma leakage
leading to:
- Shock (DSS)
- Fluid accumulation with respiratory distress

Severe bleeding
as evaluated by clinician

Severe organ involvement
- Liver: AST or ALT >=1000
- CNS: Impaired consciousness
- Heart and other organs

Figure 3 Criteria for the new case classification (4)

WHO- regional guidelines, SEARO, New Delhi (2011). (a revision of the 1997)	WHO headquarters guidelines, Geneva (2009)	WHO-Organización Panamericana de la Salud (2014). Revision of 2009
I Undifferentiated fever DENV primary infection. Self-limiting, recovery. Mixed with other arboviral, bacterial and parasitic infections	***I Undifferentiated fever*** Not taken into account	***I Undifferentiated fever*** Not taken into account
IIA Dengue fever without haemorrhage **Age group:** ▪ Older children <15 yrs ▪ Adolescents ▪ Adults **Constitutional symptoms:** ▪ Acute fever, febrile ▪ Sometimes biphasic ▪ Severe headache ▪ Myalgia/arthralgia ▪ Rash ▪ Leucopaenia ▪ Bone/joint pains (adults) ▪ Retro-orbital pain ▪ Anorexia ▪ Colicky pain/ abdominal tenderness	***IIA Non-severe dengue fever (Probable)*** **Age group:** not taken into account **Constitutional symptoms:** ▪ Living in DF-endemic areas or travellers ▪ Acute fever (with 2 of the following): o Nausea/vomiting o Rash o Aches/pain o Positive tourniquet test o Leukopenia o Any warning sign **Laboratory-confirmed dengue** ▪ Important when sign of plasma leakage	***IIA Dengue without warning signs*** Any person that has travelled in the las 14 days to an endemic area and presents an acute fever onset usually from 2-7 days of evolution and is accompanied by 2 or more of the following symptoms: ▪ Nausea/vomit ▪ Exanthema ▪ Headache/ retro-orbital pain ▪ Myalgia/arthralgia ▪ Petechiae or positive tourniquet test ▪ Leukopenia Also, consider as a potential case, any child resident or coming from a dengue endemic area with acute fever usually between 2-7 days of evolution and with no apparent focus.
IIB DF with unusual haemorrhage ▪ All signs as DF (IIA) ▪ Skin haemorrhage as positive tourniquet test and/or petechiae ▪ Massive epitaxis ▪ Thrombocytopenia ▪ Occasionally gastrointestinal	***II B Dengue with warning signs**** ▪ Abdominal pain/tenderness ▪ Persistent vomiting ▪ Clinical fluid accumulation ▪ Mucosal bleed ▪ Lethargy/restlessness ▪ Liver enlargement > 2cm ▪ Laboratory increase in HCT concurrent with decrease in platelet count	***IIB Dengue with warning signs*** Any case of dengue posing near and preferably at the fall of the fever with 1 or more of the following signs (most of them are a result of an increased capillary permeability-start of critical

bleeding, hyper Menorrhea.	***** requires strict observation and medical intervention	phase): - Severe abdominal pain or during abdominal palpation due to peritoneal extravasation (51) - Persistent vomiting 3 or more/h or 4 in 6h). Related to severity of disease*. OR=3.04; IC 95%: 1.05 to 8.80 - Fluid accumulation (Ascites*, pleural/ pericardial effusion) : VPP 90% - Mucosal bleeding* - Lethargy; irritability (Glasgow <15). Due to cerebral hypoxia - Postural hypotension (lipothymy) - Hepatomegaly > 2cms - Progressive increase in haematocrit (at least in two consecutive measurements) *This stage requires strict medical observation and immediate intervention* *- any of these if accompanied by haemodynamic alteration will be considered as a severe dengue*
III DHF (with plasma leakage) ***IIIA DHF- Non-shock*** **Age group:** More common in children <15 years (incidence in adults is increasing) ▪ Acute febrile illness with all signs of DF in early febrile phase	***III Severe disease*** **1. Severe plasma leakage leading to:** ▪ Shock (DSS) ▪ Fluid accommodation with respiratory distress **2. Severe bleeding** ▪ As evaluated by clinician	***III Severe Dengue*** Any case of dengue that has ONE more of the following manifestations: (Usually, when fever reduces, if the vascular permeability increases and the hypovolemia is not treated promptly, the patient may

- Positive tourniquet test
- Petechial, easy bruising and/or
- GI haemorrhage
- Marked thrombocytopenia
- Generalized abdominal pain
- A rising haematocrit, e.g 10% to 15% above baseline

3. Severe organ involvement

- Liver AST or ALT >= 1000
- CNS impaired consciousness
- Heart and other organs

In different words, severe dengue should be considered if the patient is from an area of dengue risk presenting fever of 2-7 days plus any of the following:

- High or progressive rising of haematocrit
- Pleural effusions or ascites
- Circulatory compromise or shock /tachycardia, cold and clammy extremities, capillary refill time > 3 seconds, weak or undetectable pulse, narrow pulse pressure or, in late shock, unreportable blood pressure)
- Significant bleeding
- Altered level of consciousness (lethargy or restlessness, coma or convulsions)
- Severe gastrointestinal involvement (persistent vomiting, increasing or intense abdominal pain, jaundice)
- Severe orgain impairment (acute liver failure, acute renal failure, encephalopathy or encephalitis, cardiomyopathy or any other unusual manifestations)

progress to dengue shock)

- **<u>Shock or difficulty in breathing</u>** due to severe plasma leakage. The shock is evidenced by: weak or undetectable pulse, tachycardia, cold extremities and capillary refill > 2 seconds, pulse pressure <= 20mmHg: Hypotension in late stage.
- **<u>Severe bleeding:</u>** assessed by the physician (e.g: hematemesis, melena, or nervous system bleeding)
- **<u>Severe organ impairment</u>** such as liver damage (AST or ALT >= 1000 IU), nervous system (impaired consciousness, encephalitis), heart (myocarditis), hepatitis or other organs.

[shock and prolonged hypoxia can lead to metabolic acidosis and multiple organ failure]

This stage requires strict medical observation and immediate intervention

IIIB DHF (with shock)

- Towards end of febrile phase
- Hyporolemic shock
- Pleural effusion
- Gall bladder oedema
- Acute abdominal pain

IV DF with expanded dengue syndrome ▪ Unusual manifestations ▪ Involvement of liver, kidney, brain or heart are increasingly being reported in DHF ▪ Occurred in DF cases also with no evidence of plasma leakage ▪ May be due to co-morbidities

Table 3 **Comparison of the two main dengue case definitions**

The current classification of the World Health Organization (WHO) 2009 considers two categories: Dengue and severe dengue (4). This classification arose from many criticisms and differences with the previous version of Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) with four grades of severity, because it did not allow classifying a large number of cases confirmed by dengue laboratory. This was a limitation for surveillance because the name itself mistakenly provoked to think that the severity of illness was related to bleeding and not with the plasma extravasation, as actually happens. Also because it was difficult or impossible to apply the classification in all the scenarios as it required a laboratory support that did not exist in most of the units, especially in primary care, where most of the fever cases were served during an outbreak. Furthermore, sometimes, the case could only be classified at the end when all criteria were met, so the diagnosis was made when complications arouse.

In 2009, the Program on Dengue PAHO / WHO with the support of a group of experts from the Region of the Americas, made own this classification and joined the Care Guidelines for dengue patients in the Region of the Americas. After five years of being recommended, this classification of dengue has been incorporated into the Guidelines on Dengue in most of the countries of Latin America and the Caribbean and has proven to be superior for clinical management of patients, particularly for the early identification of severe cases and those who require special attention because they could evolve to gravity (warning signs), so that diagnosis and treatment can be done at an early stage without dependence of laboratory confirmation as required in the classification of 1997.

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Grey literature

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7. Background

Dengue is a wide spread vector-borne disease affecting almost 50 million citizens annually (1). Dengue was predominantly a paediatric disease but it now affects all age groups (4, 9, 13, 22, and 26). The understanding on dengue's pathophysiology has also changed and the disease is now considered to be a plasma-leakage related rather than haemorrhagic. (4)

From a dengue case classification point of view, the world has been divided into two groups, both using two different dengue case classifications. South East Asian countries continues to use the original WHO case definition developed in 1975 and revised on 1997, while the American Region and few countries in other regions have adopted the newly developed revised 2009 guidelines, now further revised by PAHO in 2014.

The 1997 guideline classifies patients in three categories: dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). DF is defined as an acute febrile stage, mainly seen in older children and adults, and is accompanied by symptoms such as headache, myalgia or sometimes even unusual haemorrhage (skin petechiae or positive tourniquet test) and above all, a positive laboratory confirmation of DENV infection. DHF was considered when patients manifest fever, thrombocytopenia, haemorrhagic tendency and signs of plasma leakage. If the DHF is accompanied by signs of shock, this is then classified as DSS (3).

Whereas, the 2009 case classification is based on warning signs and easily distinguishes between severe and non-severe dengue (1, 3). The non-severe group is divided into two sub categories: with and without warning signs. Some of these warning signs could be listed as abdominal tenderness/pain, clinically manifestation of fluid accumulation, persistent vomiting, mucosal bleeding, lethargy, hepatomegaly, restlessness and increase in haematocrit with a drop in platelet count. Meanwhile, severe dengue is defined by the appearance of leakage of plasma and/or fluid accumulation leading to shock or respiratory distress; and/or severe bleeding; and/or organ impairment.

Clinically, the dengue infection can be divided into three phases: Febrile, Critical and the Recovery phase. Warning signs can be present in the febrile phase which might lead to a severe disease. The critical phase starts round the defervescence period, when the fever drops. The patient then can rather enter a recovery phase or present severe signs such as progressive leukopenia along with a drop in the platelet count and followed by haemorrhage or plasma leakage. This clinical course has no endpoints unlike both case classifications. (3)

Since a decade, the accuracy of the 1997 World Health Organization dengue case classification has been a topic of debate for having a strict definition, misclassifying some severe cases as non-severe. (3.5.6.7.8) The spread of dengue and the incidence increase in older age groups also contributes to the limited applicability of the older version, as this one is merely based on the symptoms of Thai children (paediatric patients) (4, 9, 13, 22 and 26).

Another drawback pointed was the difficulty in applying the method in resource-limited countries. (13,22) A prospective clinical dengue study (DENCO) in 7 countries of South Asia and Latin America, formed the basis of the revised 2009 case definition (2,26).

Most of the papers revised mentioned the shortcomings of the 1997 original case classification, which are listed below:

- Less user friendly. Complexity level high for having 5 categories (DF, DFI, DFII, DFIII, DHFIV) (22)
- Misclassification of severe cases/ poorly related to disease severity (13, 22 and 25)
- More importance is given to the haemorrhagic manifestations rather than the plasma leakage which can mislead a clinician (9, 22)
- Not helpful in patient triage during outbreaks (25)
- Difficult to use for laboratory confirmation criteria (25)
- The tourniquet test does not differentiate DF from DHF neither dengue from other febrile illness (5, 9, 20, 26 and 27)
- Overlap in the three identities DF, DHF and DSS (9)
- All four essential elements for the WHO definition of DHF (fever, haemorrhage, thrombocytopenia and signs of plasma leakage) might not always be present or achieved (9)
- The DHF/DSS classification excludes severe dengue disease associated with unusual manifestations
- A study found a sensitivity of 64% and a specificity of 57% (10, 11).

However, some positive aspects of the WHO 1997 old case classification were also described:

- More emphasis is given to haemorrhage, *"because of the term haemorrhagic dengue fever and/or thrombocytopenia, as opposed to signs of capillary extravasation, which are responsible for the severe form of disease. This could lead to early discharge of patients not showing signs of haemorrhaging and/or thrombocytopenia and who are seeking medical care for the first time, resulting in the return of these patients presenting symptoms of severe dengue."* (12)
- Showed better specificity in one study: 93.4% $P<0.001$ for the detection of severe forms of dengue and a high positive predictive value (82.6% $P<0.001$) (24). Another study found a sensitivity 62% and specificity 92% for WHO case definition of DHF (16).
- 99% of the non-dengue cases are said to be excluded without the need for laboratory tests with the DHF case definition (13, 16)
- Disease severity may be a result of delays in treatment of dehydration and metabolic disturbances rather than dengue case definitions. (20)
- *"Thrombocytopenia is also a marker of severity in dengue patients who did not fulfil the WHO case definition of DHF". (20).*

"Studies have shown that platelet counts inversely correlated with plasma viral load, which has been shown to correlate with the extent of plasma leakage" (21)

Albeit, the new case classification (2009) was also accused:

- Wide range of warning signs/symptoms defined as being vague and less specific (could be also related to other diseases) (4, 13)
- Less strict criteria, leaving room for interpretation by clinician. Loose case definition. (3) "*A patient presenting with abdominal pain and vomiting in an area of endemicity during the dengue season will qualify as a probable dengue case"* (13)
- May lead to more cases classified as dengue / more hospital admissions (3)
- The unnecessary observation/admission will increase the workload in the hospitals in most resource-poor dengue endemic countries (14). Its seems to create more than 2 times the work load on health care personnel and has increased the number of suspected dengue patients for monitoring
- Plasma leakage is a key element in the new WHO case definition and helps with the clinical triage but it needs to be modified for being more simpler and user friendly
- Organ failure is mainly caused by delayed detection and treatment of plasma leakage is listed as severe manifestations. "*By listing severe organ involvement as a criterion for severity separate from plasma leakage, the revised classification places emphasis on isolated organ failure as common and significant cause of dengue severity. With the exception of liver failure, which has been reported to occur in <1% in moderate to severe adult dengue cases"* (13, 14, 15). The frequencies of severe organ impairment in the absence of plasma leakage have not been well documented
- The new case definition has been designed primarily as a case management tool; thus, less importance is given to the pathophysiology. (13)
- Severe dengue category in the revised classification includes cases the mechanism of which is potentially unrelated to dengue. For instance, patients with high levels of liver enzymes could easily be classified as severe dengue regardless of the presence of other clinical findings (eg haemorrhage or plasma leakage) (13)
- Shock and severe organ impairment can be either part of the manifestations of the disease, complications of an inappropriate or delayed treatment, or even an adverse effect of medication. An example would be fluid accumulation with respiratory distress. It is listed as a severe criterion in the revised classification, but in reality is often a complication of overtreatment with intravenous fluids. (13, 23)
- Clinicians from Thailand predict that the usage of the new case definition could result in over admission of patients to hospitals during epidemics which might also possibly reduce the efficiency of the triage and affect adversely the quality of the case management. (23)

Positive aspects of which are (4):

- Useful for classification and triage of patients (26). Better categorization (25)
- Simplifies case management
- Leads to adequate treatment
- More practical/ user friendly
- No laboratory data required for the diagnosis
- Saves resources and contributes to reduction of dengue mortality
- Prasad et al concluded the new case definition is highly sensitive for detecting severe dengue cases and has easy application. (17, 18)
- Useful for grouping of the patient for further management (17,19)
- The revised scheme has better sensitivity (86.8% $P<0.001$) for the detection of severe forms of dengue (24). But, lower positive predictive value (61.6% $P<0.001$) when compared to the traditional scheme (82.6% $P<0.001$) (24)

However, the guidelines should be adapted to the local variations found in the warning signs to be more effective. (26)

8. Objectives

The main purpose of this study is to show how effective the new case classification has been regarding mortality rates.

9. Hypothesis

The new dengue case classification aids dengue case management to reduce mortality at national level

10. Methods

A literature review was carried out to update the pros and cons and the efficacy of both methods for the *background*. Reporting items for systematic reviews and meta-analyses (PRISMA) were followed (see annex 1 and 2). Eligibility criteria included (1) research on dengue and case classification, (2) any study design and (3) no date limit was imposed. All languages were included, although the search was conducted in English. Studies with no full text articles available were excluded. The literature search was carried out on 12th October 2015. The search terms used were ***(dengue[MeSH Terms]) AND case classification NOT vaccines NOT serotypes*** with 136 initial hits of which only 20 were used and ***(Kalayanarooj, Siripen[Author]) AND dengue*** with a total of 62 articles from which only 6 were extracted. Siripen is a known author who defends the original 1997 WHO case classification, thus, due to time limit, we narrowed our search at the beginning. Owing to the short time, the search strategy was only applied to PubMed Database.

This is a cross-sectional retrospective study where WHO published national surveillance data was used to do the statistical analysis. Data from five countries from each SEAR and PAHO regions were collected for the comparative analysis. These five countries were selected for having the highest suspected cases, thus baring 90% of all the region`s burden. Data on suspected cases was entered and 3 axis graphs were created with Microsoft excel. Suspected cases are defined as cases of dengue and DHF for (2005-2009) and dengue and severe dengue for (2010-2014).

The case fatality ratio (CFR) was calculated by dividing Deaths with Suspected cases [CFR= Deaths/Suspected * 100]. Chi square was practiced with Epistat to interpret the findings and know whether they were statistically significant using 0.05 as a benchmark for P value. This test will be used to show if the drops/rises in the deaths between the two year groups (2005-2009) old case classification and (2010-2014) new case classification have been significant or not. The chi square was calculated again comparing the groups (2005-2010) and (2011-2014) to avoid false results. We supposed that the new case classification was published on 2009 but was implemented by all PAHO countries since 2010. Later, a linear regression continued by a Poisson regression was carried out for CFR, suspected and deaths by using Stata 10 (see annex 3 and 6). Expert advice was sought for the statistical analysis. A Spearman correlation for CFR and year was also carried out separately for the two regions (see annex 4). Each statistical test was used for different purpose. The chi square allows knowing whether there is any difference between the two groups we compare and if that difference is statistically significant. The Fisher's test was used when the numbers were below 5. The Linear regression was used for seeing the trends; meanwhile the Poisson regression quantified that trend. Poisson regressions can only be used when the variables are counts. The Spearman correlation was used to determine the relationship/association between the independent variable (CFR) and the year.

11. Data Analysis and Results

We first looked at the trends for suspected cases in both SEAR and PAHO regions to view the progress or any change in the number of suspected cases after the introduction of the new case classification. Linear regression graphs are used (*Figure A and B)*

SUSPECTED CASES

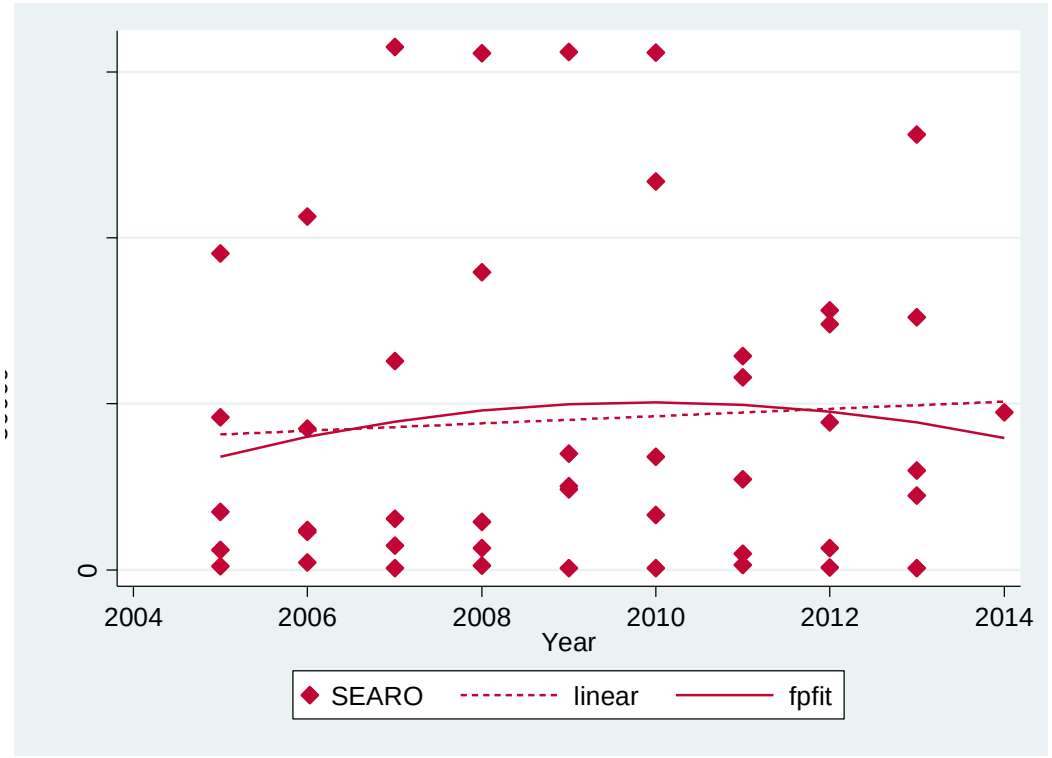

Figure A. Linear Regression graph for suspected cases in SEAR

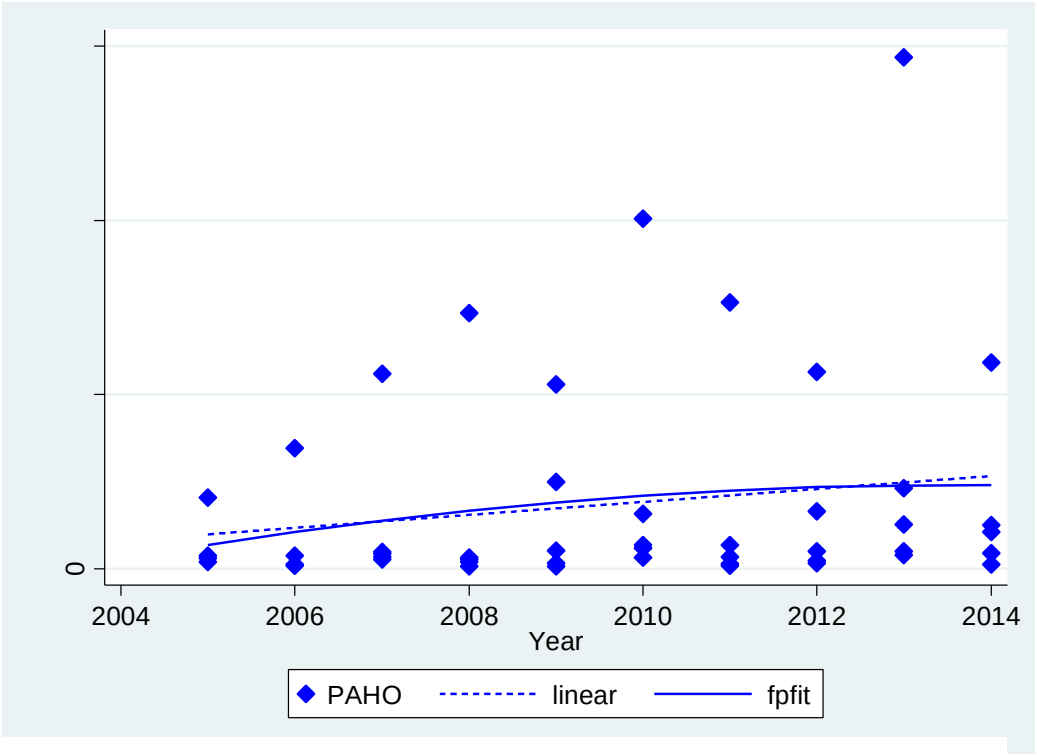

Figure B. Linear Regression graph for suspected cases in PAHO

Visually a certain increase in number of suspected cases for both Regions can be seen. The trend seems to be linear and positive. To quantify the increase, a Poisson regression test was used.

SUSPECTED CASES -Poisson regression statistical calculation:

SEAR

```
Poisson regression                              Number of obs   =         46
                                                LR chi2(1)      =    8647.41
                                                Prob > chi2     =     0.0000
Log likelihood = -1138206.1                     Pseudo R2       =     0.0038
```

suspected	IRR	Std. Err.	z	P>|z|	[95% Conf.	Interval]
year2	1.024563	.0002675	92.96	0.000	1.024039	1.025088

PAHO

```
Poisson regression                              Number of obs   =         48
                                                LR chi2(1)      =  717331.48
                                                Prob > chi2     =     0.0000
Log likelihood = -7562787.8                     Pseudo R2       =     0.0453
```

suspected	IRR	Std. Err.	z	P>|z|	[95% Conf.	Interval]
year2	1.108335	.0001366	834.73	0.000	1.108067	1.108603

The Poisson test shows the Incidence Rate Ratio (IRR) for both Regions. SEAR IRR: 1.024563, which is statistically significant, being the number bigger than 1. There is a positive relationship for SEAR (increase in the slope). Each year there is an increment of 1.024563 in the graph.

Meanwhile, PAHO has an IRR of 1.108335, which is also statistically significant. The increment is bigger than the SEAR graph for suspected cases.

Subsequently, data was collected on the number of Dengue Haemorrhagic Fever (DHF) cases in the PAHO region.

PAHO	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
total suspected	290,905	8024	711,565	818371	918295	1318102	887308	817615	1916729	875975
total DHF	6,586	6,715	18,601	21,685	32,648	35,647	17,136	26,888	33,562	14,315
total CFR (D/DHF)	15.20	11.60	13.71	3.27	6.92	9.21	8.44	11.80	14.47	66.94
total CFR (D/Susp)	0.21	0.16	0.17	0.20	0.27	0.36	0.24	0.27	0.28	0.30

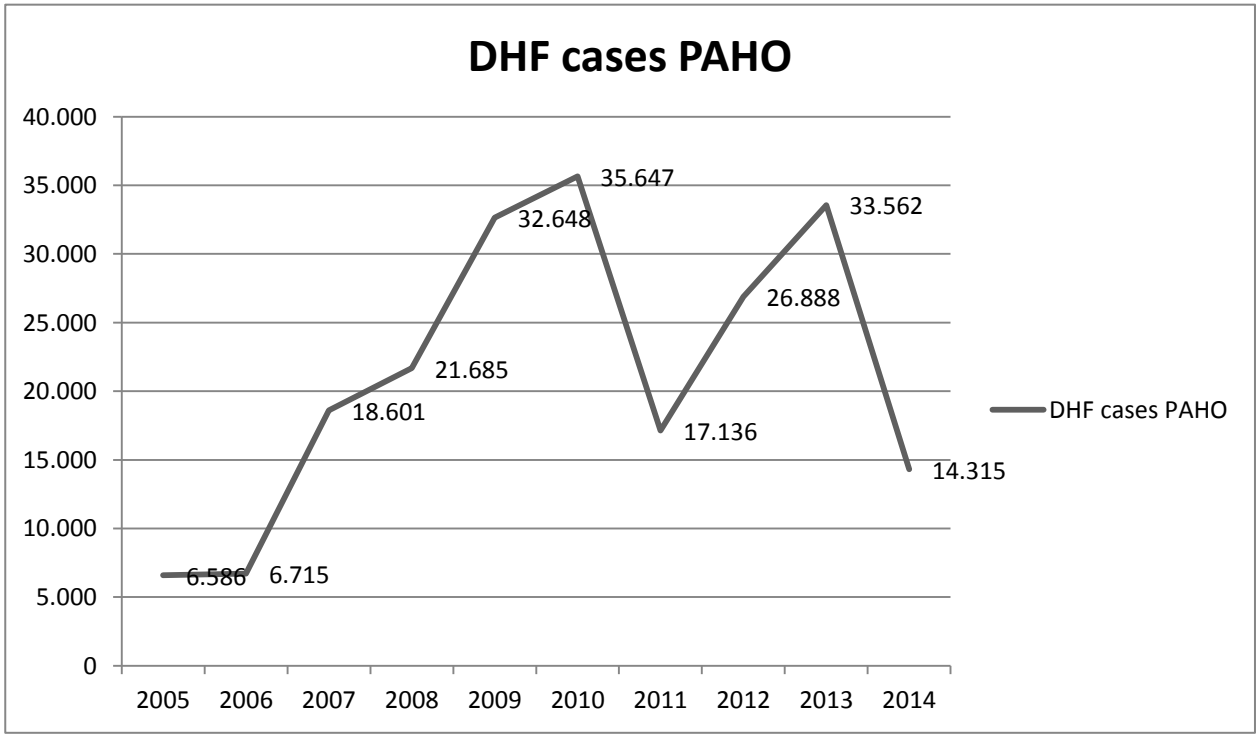

Figure C. Graph on Dengue Haemorrhagic cases for PAHO region

The Graph above (Figure C) shows how the severe dengue cases (dengue haemorrhagic cases) have evolved during the past decade. The increase here is evident.

Consequently, surveillance data was collected from the five top countries that had the highest number of cases. Graphs were made for each country comparing the suspected cases with the case fatality ratio (CFR) along the years.

Table 4. **SEAR (SOUTH EAST ASIA REGION)**

Country	Year	Suspected	Deaths	CFR (Deaths/Suspected)* 100
Thailand	2005	45,893	71	0.15
	2006	42,456	59	0.14
	2007	62,949	67	0.11
	2008	89,626	102	0.11
	2009	25,194	2	0.01
	2010	116,947	139	0.12
	2011	64,374	58	0.09
	2012	78,063	80	0.10
	2013	131,090	128	0.10
Bangladesh	2005	1,048	4	0.38
	2006	2,198	11	0.50
	2007	466	1	0.21
	2008	1,181	0	0.00
	2009	474	0	0.00
	2010	409	0	0.00
	2011	1,362	6	0.44
	2012	671	1	0.15
	2013	457	0	0.00
Indonesia	2005	95,279	1298	1.36
	2006	106,425	1096	1.03
	2007	157,442	1446	0.92
	2008	155,607	940	0.60
	2009	156,052	1396	0.89
	2010	155,777	1358	0.87
	2011	58,065	504	0.87
	2012	74,062	646	0.87
	2013	76,090	487	0.64
Myanmar	2005	17,454	169	0.97
	2006	11,383	128	1.12
	2007	15,285	171	1.12
	2008	14,480	100	0.69
	2009	24,287	181	0.75
	2010	16,529	117	0.71
	2011	4,738	16	0.34
	2012	6,433	26	0.40
	2013	22,335	55	0.25

Sri Lanka	2005	5,994	27	0.45
	2006	11,980	44	0.37
	2007	7,314	25	0.34
	2008	6,555	19	0.29
	2009	35,010	346	0.99
	2010	34,105	246	0.72
	2011	27162	173	0.64
	2012	44456	220	0.49
	2013	29878	85	0.28
	2014	47502	95	0.20

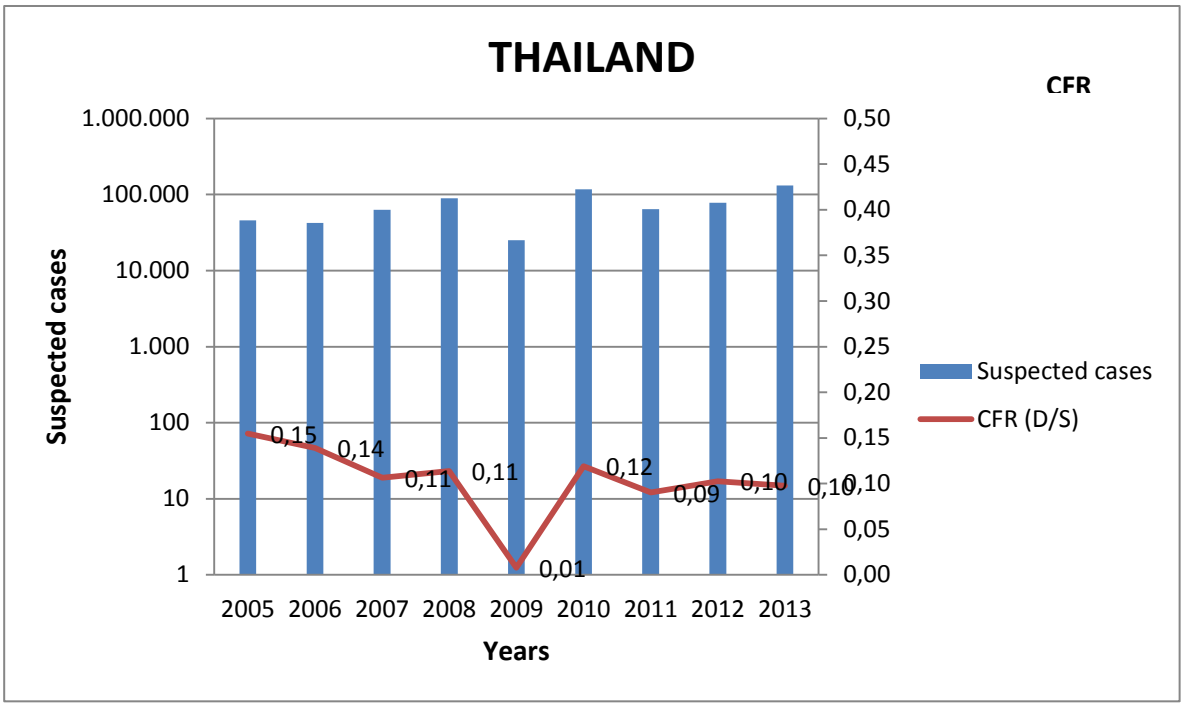

Figure D. Graph on CFR and Suspected cases for Thailand.

The CFR ratios in Thailand show that there has been a slight decrease in mortality ratio over the past years. Yet, the suspected cases and deaths have been growing. *(Table 4 and Figure D)*

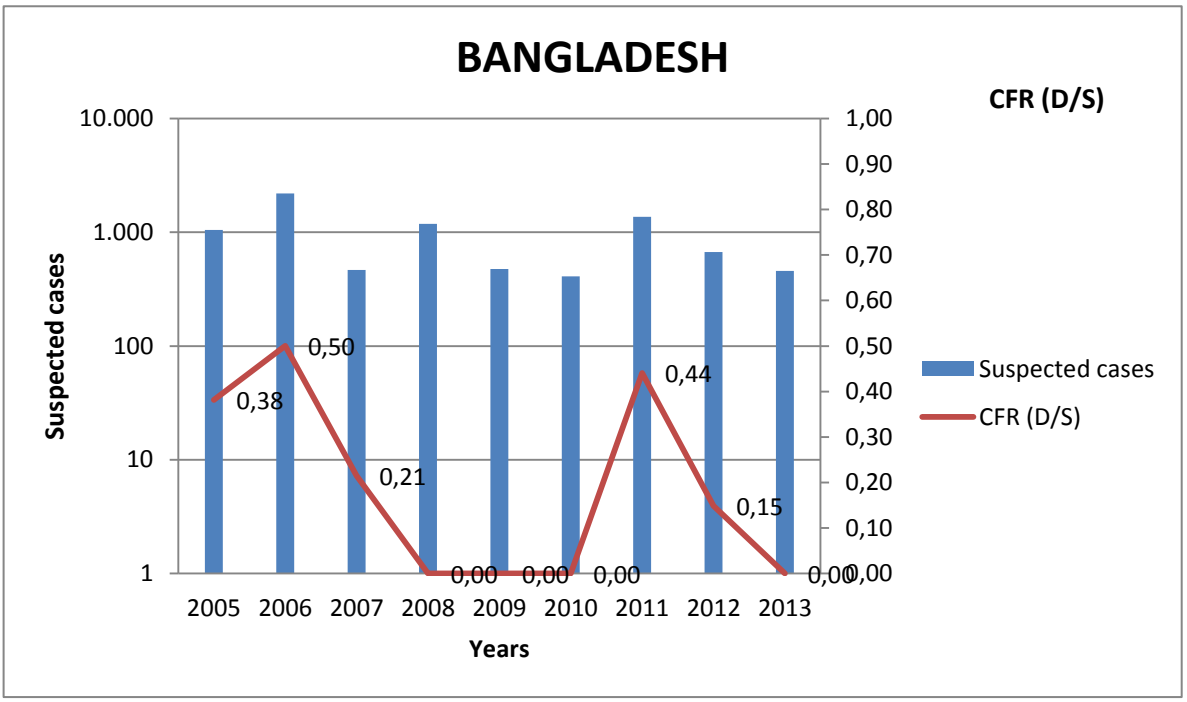

Figure E. Graph on CFR and Suspected cases for Bangladesh

The CFR had a peak in 2011 and then decreased till 2013. However, less suspected cases and less death have been reported in the past few years. The national program attributes this decline in mortality to better case management after training health workers. *(Table 4 and Figure E)*

Figure F. Graph on CFR and Suspected cases for Indonesia

The CFR in Indonesia has overall decreased since 2005. The number of cases and deaths have also been lower compared to early 2000. *(Table 4 and Figure F)*

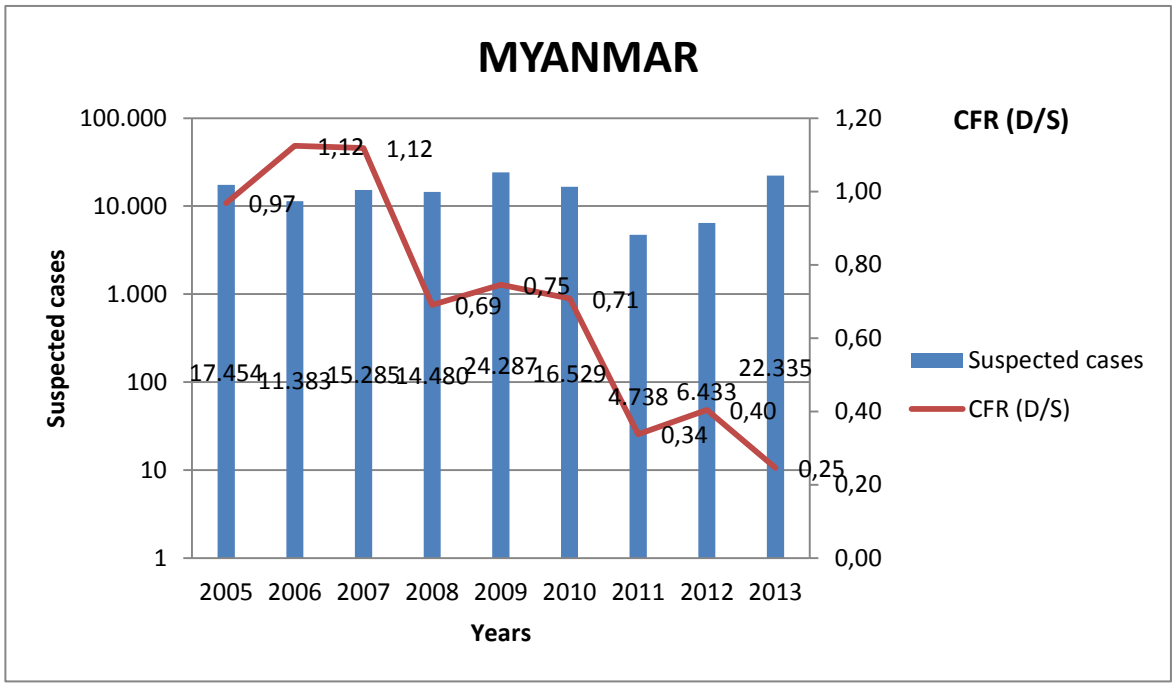

Figure G. Graph on CFR and Suspected cases for Myanmar

Myanmar has showed great decrease in its mortality rates. The suspected cases have been almost stable since 2005, although fewer deaths have occurred in the past years. *(Figure G)*

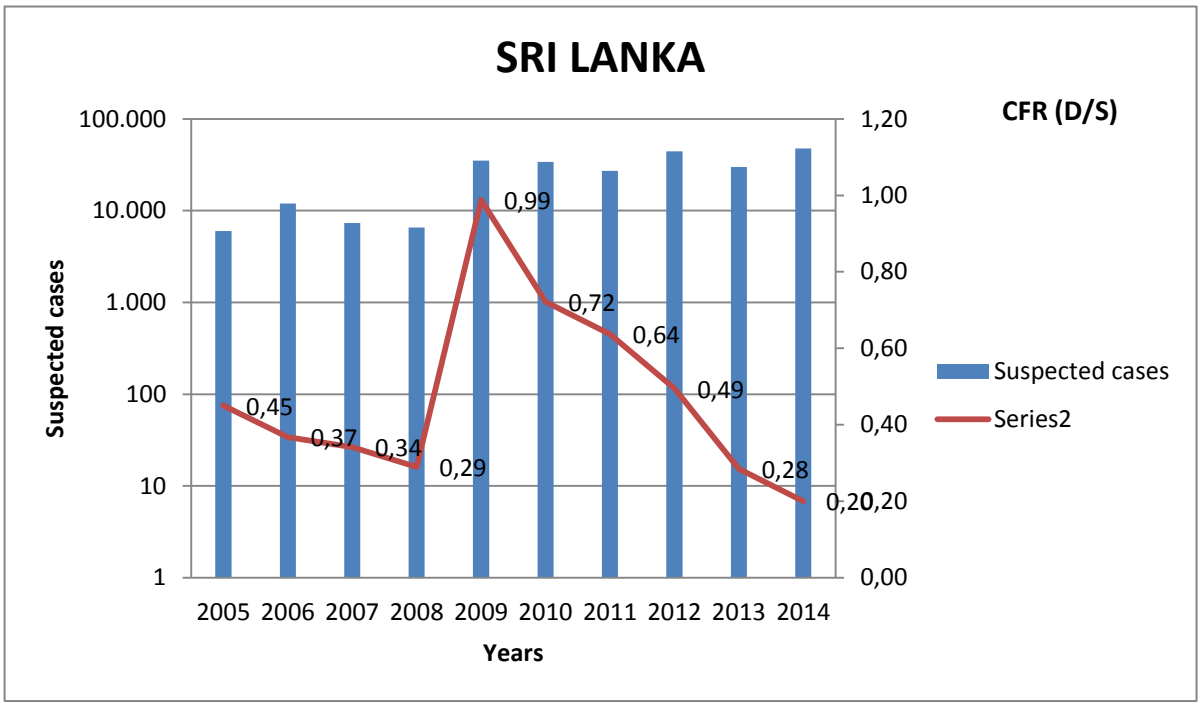

Figure H. Graph on CFR and Suspected cases for Sri Lanka

Sri Lanka also shows a negative slope for the CFR. It had a peak of suspected cases and deaths between 2009-2010 but afterwards, the numbers have decreased. *(Table 4 and Figure H)*

Table 5. **PAHO**

Country	Year	Suspected	Deaths	CFR (Deaths/Suspected)* 100
Brazil	2005	203,789	43	0.02
	2006	346,550	67	0.02
	2007	559,954	158	0.03
	2008	734,384	212	0.03
	2009	528,883	298	0.06
	2010	1,004,392	673	0.07
	2011	764,032	482	0.06
	2012	565,510	284	0.05
	2013	1,468,873	545	0.04
	2014	591,080	410	0.07
		6,767,447		
Colombia	2005	30,475	47	0.15
	2006	36,471	50	0.14
	2007	43,227	20	0.05
	2008	26,732	12	0.04
	2009	51,543	44	0.09
	2010	157,152	217	0.14
	2011	33,207	42	0.13
	2012	49,361	51	0.10
	2013	127,219	161	0.13
	2014	105,356	166	0.16
Costa Rica	2005	37,798	2	0.01
	2006	12,124	0	0.00
	2007	26,440	8	0.03
	2008	7,160	0	0.00
	2009	6,946	0	0.00
	2010	31,773	0	0.00
	2011	13,854	0	0.00
	2012	22,243	0	0.00
	2013	49,868	1	0.00
	2014	11,140	0	0.00
Honduras	2005	18,843	6	0.03
	2006	8,436	0	0.00
	2007	33,508	16	0.05
	2008	18,941	9	0.05
	2009	15,291	14	0.09
	2010	66,814	83	0.12

	2011	8,297	0	0.00
	2012	15,554	4	0.03
	2013	39,271	29	0.07
	2014	43,456	5	0.01
Mexico	*2005*	*16,862*	*NA*	*NA*
	2006	*27,287*	*NA*	*NA*
	2007	48,436	10	0.02
	2008	31,154	24	0.08
	2009	249,763	96	0.04
	2010	57,971	20	0.03
	2011	67,918	36	0.05
	2012	164,947	153	0.09
	2013	231,498	104	0.04
	2014	124,943	76	0.06

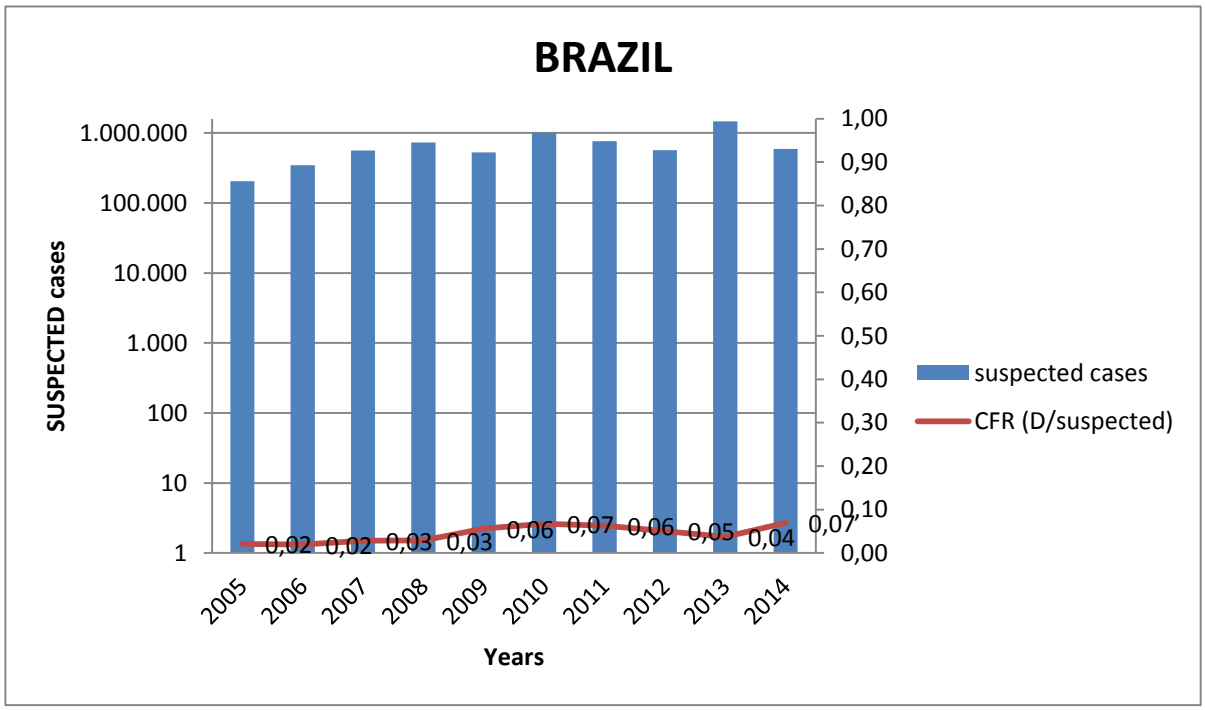

Figure I. Graph on CFR and Suspected cases for Brazil

Brazil had a sudden rise in suspected cases around 2014 (FIFA world cup that was held in this region). The deaths have increased since 2009 and the CFR shows a slight growth. *(Table 5 and Figure I)*

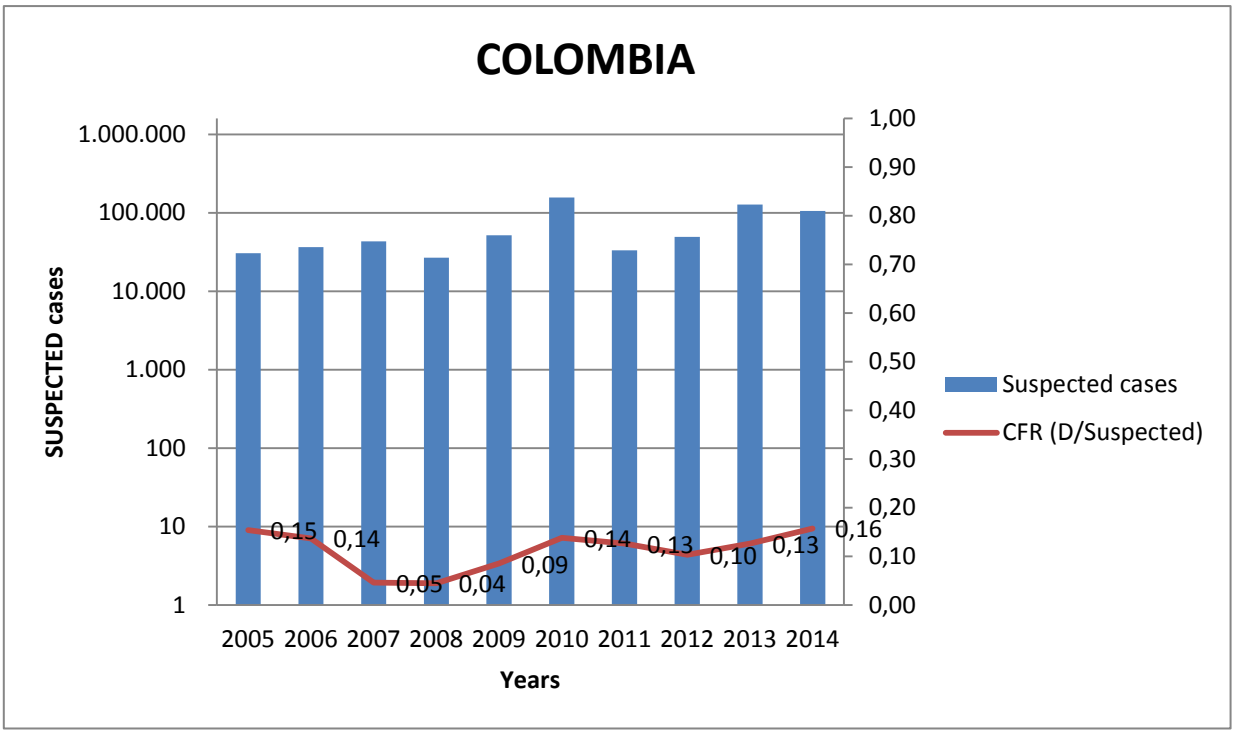

Figure J. Graph on CFR and Suspected cases for Colombia

There have been minor changes in CFR. Suspected and deaths rates are increasing year by year.

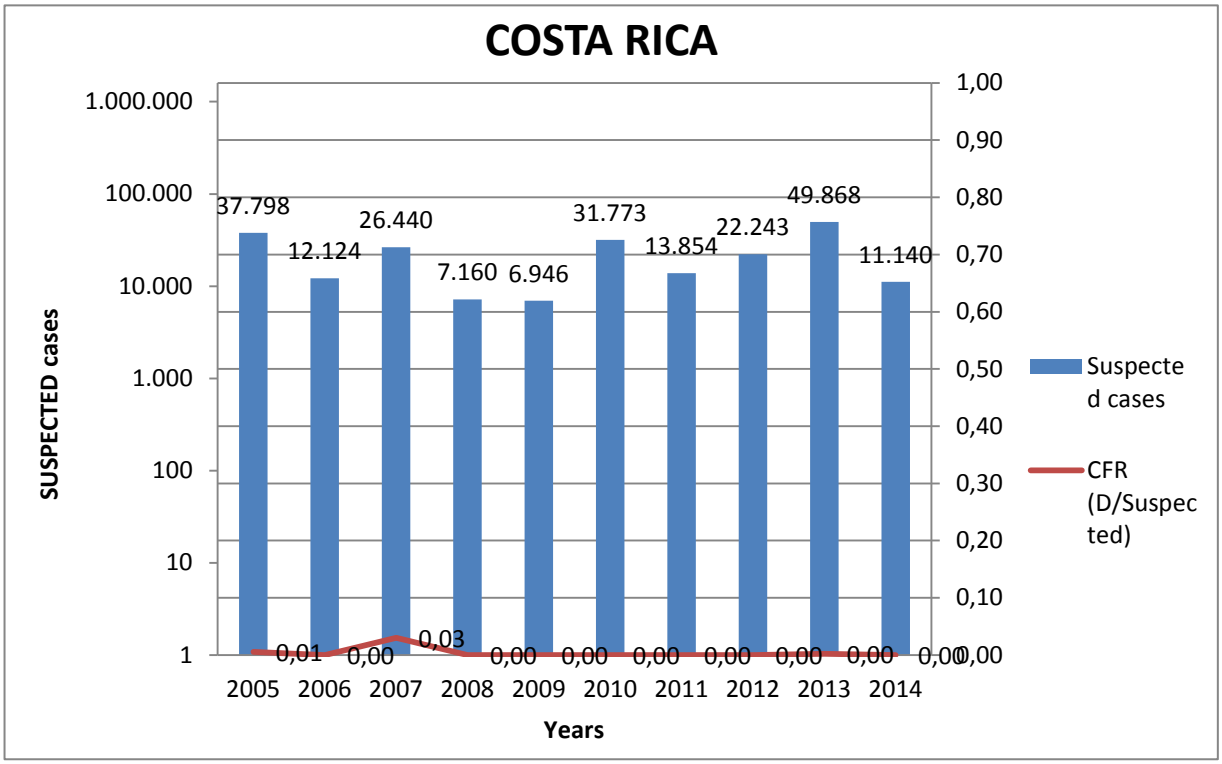

Figure K. Graph on CFR and Suspected cases for Costa Rica

CFR on the border line. Number of suspected cases is high but deaths are low. *(Figure K)*

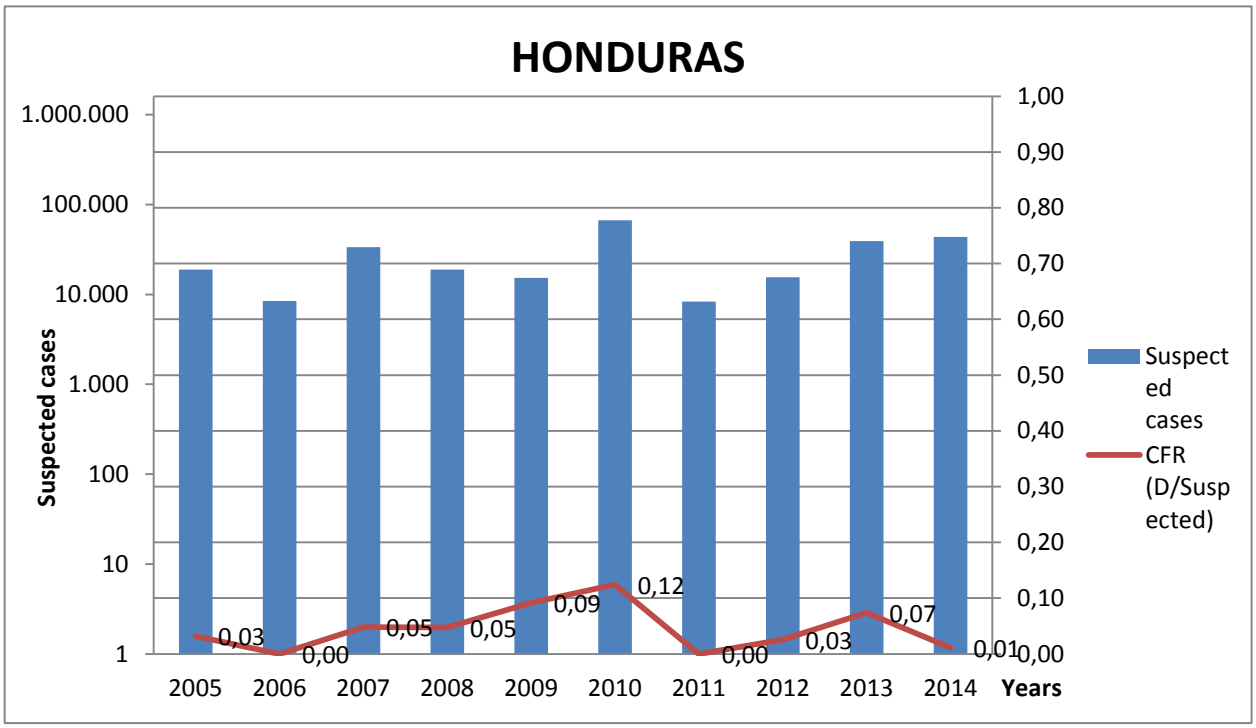

Figure L. Graph on CFR and Suspected cases for Honduras

CFR ratios stable since 2005, with no major changes. The number of suspected cases till remains high and the deaths are variable. *(Figure L)*

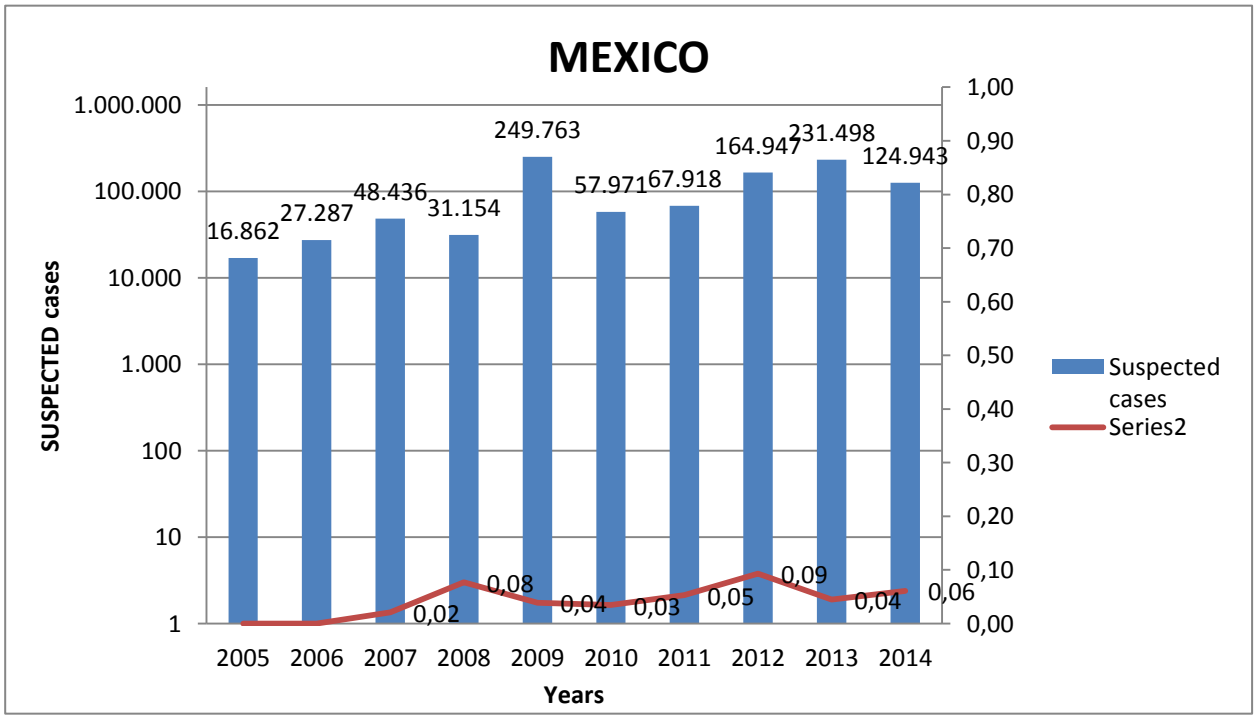

Figure M. Graph on CFR and Suspected cases for Mexico

CFR has been quite stable, with no major swings. Deaths and suspected cases have risen.

Overall, the CFR rations for the different countries of SEAR have shown to decrease in the past years. However, these drops have been minor. On the other hand, the CFR for PAHO countries seem to be quite constant with a slight increase. Nevertheless, the mortality rates can be influenced by many factors. Outbreaks are an important reason for high mortality. The elements to consider are the weather, serotype switches (ones are more virulent than others) and the rise of secondary infections which also may lead to the variations in the CFR. These countries bear the heaviest burden among all SEARO.

Regional Graphs (see annex 5)

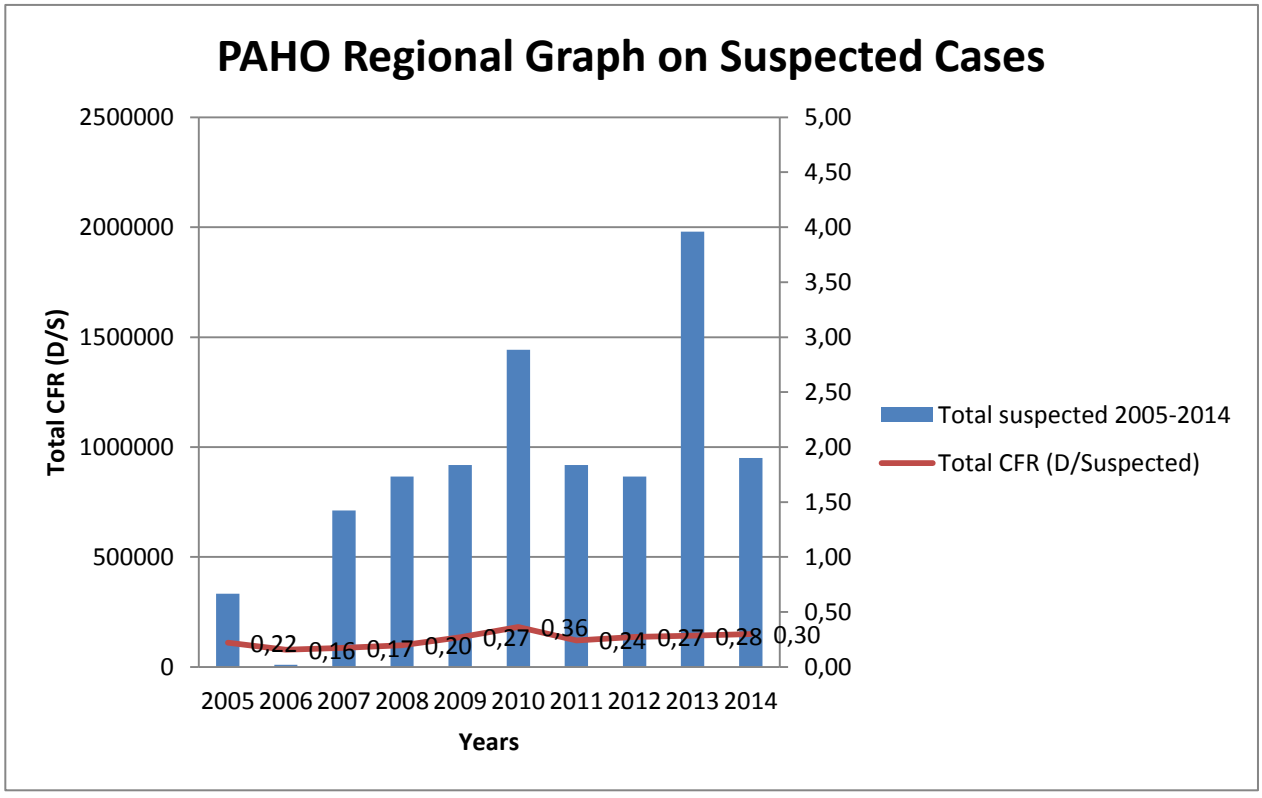

Figure N. Regional graph on suspected cases for PAHO

When the data was combined for all countries of the PAHO region, the CFR did not vary along the past years when taking the suspected cases into account. *(Figure N)*

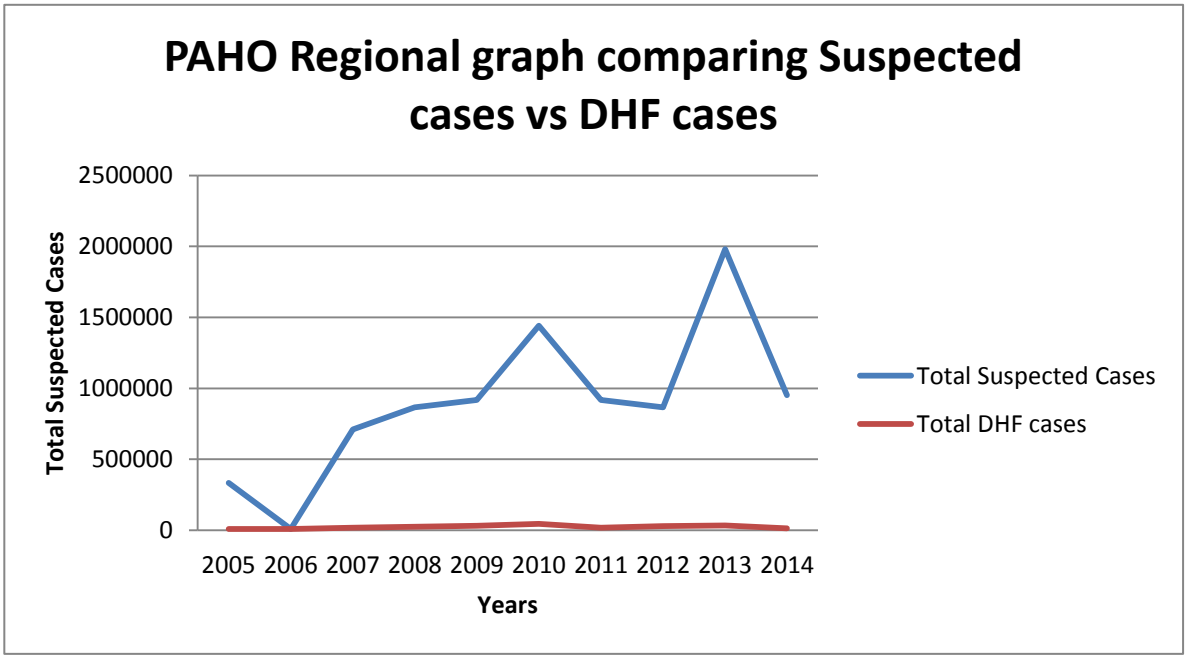

Figure O. Graph comparing suspected cases vs DHF cases for PAHO region

The number of suspected cases in the PAHO region is high and has shown a significant growth compared to early 2000's. The number of suspected cases remains low, as the majority recover after the dengue fever. *(Figure O)*

Chi square calculation

Ho: The number of deaths is independent of the case definition system used (defined by years)

Ha: The number of deaths is associated with the case definition system (defined by years)

(2005-2009)/ (2005-2010) = old case definition for PAHO and SEARO

(2010-2014)/ (2011-2014) = new case classification for PAHO and old case definition for SEARO

CHI SQUARE FOR PAHO REGION						
Brazil		**Suspected cases ALIVE**	**Deaths**	**TOTAL Suspected**		*Significance level of 0.05*
	2005-2009	2,372,782	778	2,373,560		The Chi-square statistic is 502038705451.141. The P value is 0. This result is significant at p < 0.05.
	2010-2014	4,391,493	2,394	4,393,887		
	2005-2010	3,376,501	1,451	3,377,952		The Chi-square statistic is 535718823571.574. The P value is 3E-06. This result is significant at p < 0.05.
	2011-2014	3,387,774	1,721	3,389,495		
Colombia						
	2005-2009	188275	173	188448		The Chi-square statistic is -203423990.0913. The P value is 6E-06. This result is significant at p < 0.05.
	2010-2014	471658	637	472295		
	2005-2010	345210	390	345600		

	2011-2014	314723	420	315143		The Chi-square statistic is 213873666.8117. The P value is 0.01779. This result is significant at $p < 0.05$.
Costa Rica						
	2005-2009	90458	10	90468		The Chi-square statistic is -3581054.2785. The P value is 0.000819. This result is significant at $p < 0.05$. The Fisher exact test statistic value is 0.000979. The result is significant at $p < 0.05$.
	2010-2014	128877	1	128878		
	2005-2010	122231	10	122241		The Chi-square statistic is -9020161.6184. The P value is 0.018821. This result is significant at $p < 0.05$. The Fisher exact test statistic value is 0.028737. The result is significant at $p < 0.05$.
	2011-2014	97104	1	97105		
As one value is less than 5, we need to do the Fisher exact test.						

Honduras						
	2005-2009	94,974	45	95,019		The Chi-square statistic is -20135249.9905. The P value is 0.025432. This result is significant at p < 0.05.
	2010-2014	173,271	121	173,392		
	2005-2010	161,705	128	161,833		The Chi-square statistic is 12612614.4024. The P value is 9E-06. This result is significant at p < 0.05.
	2011-2014	106,540	38	106,578		
°2007 STARTS						
Mexico						
	2005-2009	373,372	130	373,502		The Chi-square statistic is -535400080.0718. The P value is 0. This result is significant at p < 0.05.
	2010-2014	646,888	389	647,277		
	2005-2010	431,323	150	431,473		The Chi-square statistic is -6982297742.8325. The P value is 0. This result is significant at p < 0.05.
	2011-2014	588,937	369	589,306		

CHI SQUARE FOR SEAR REGION					
Thailand		**Suspected cases ALIVE**	**Deaths**	**TOTAL Suspected**	
					The Chi-square statistic is -94005351.761. The P value is 0.254488. This result is *not* significant at p < 0.05.
	2005 - 2009	265,817	301	266,118	
	2010 - 2013	390,069	405	390,474	
	2005 - 2010	382,625	440	383,065	The Chi-square statistic is -6087429.9314. The P value is 0.031788. This result is significant at p < 0.05.
	2011 - 2013	273,261	266	273,527	
Bangladesh					**The Chi-square statistic is 0.2177. The P value is 0.640764. This result is *not* significant at p < 0.05.**
	2005 - 2009	5,351	16	5,367	
	2010 - 2013	2,892	7	2,899	
	2005 - 2010	5,760	16	5,776	**The Chi-square statistic is 0.0011. The P value is 0.973998. This result is *not* significant at p < 0.05.**
	2011 - 2013	2,483	7	2,490	

Indonesia					
	2005 - 2009	664,629	6176	670,805	The Chi-square statistic is 171201525.9708. The P value is 0. This result is significant at $p < 0.05$.
	2010 - 2013	360,999	2995	363,994	
	2005 - 2010	819,048	7534	826,582	The Chi-square statistic is 379844827.8117. The P value is 0. This result is significant at $p < 0.05$.
	2011 - 2013	206,580	1637	208,217	
Myanmar					
	2005 - 2009	82,140	749	82,889	The Chi-square statistic is -920120.0542. The P value is 0. This result is significant at $p < 0.05$.
	2010 - 2013	49,821	214	50,035	
	2005 - 2010	98,552	866	99,418	The Chi-square statistic is 6419442.6199. The P value is 0. This result is significant at $p < 0.05$.
	2011 - 2013	33,409	97	33,506	
Sri Lanka					
	2005 - 2009	66,392	461	66,853	The Chi-square statistic is -7344297.1429. The P value is 0. This result is significant at $p < 0.05$.

	2010 - 2014	182,284	819	183,103	
	2005 - 2010	100,251	707	100,958	The Chi-square statistic is -7726033.9924. The P value is 0. This result is significant at $p < 0.05$.
	2011 - 2014	148425	573	148998	

Chi square was calculated for every country in PAHO and SEAR regions. The criteria used to analyse results is:

- If $P < 0.05$ = we reject the null hypothesis in favour of the alternative hypothesis (Ha). We refer as a significant diference

- If $P > 0.05$ = we fail to reject the null hypothesis. There is no statistical significance in the number of deaths.

<u>Results obtained from PAHO and SEARO:</u>

Al of the countries from PAHO showed a significant difference in the number of deaths when compared the two year groups, which represent the old and the new case definitions respectively.

Similarly for SEARO, Indonesia, Myanmar, Sri Lanka and (2005-2010/20011-2014) calculation for Thailand showed significant differences, meanwhile Bangladesh and (2005-2009/2010-2014) calculation for Thailand failed to show statistical differences regarding number of deaths.

Thus, it can be concluded that the majority of the countries analysed had significant changes in the number of deaths when changed from one case definition to another one. The only drawback of Chi square calculation is that the direction of the difference cannot be said. It only tells if the difference is significant or not.

Looking at raw data, our supposition says there has been a positive difference in the number of deaths regarding PAHO region, meaning more deaths have occurred during the implementation of the new case definition.

Regression trend models

DEATHS

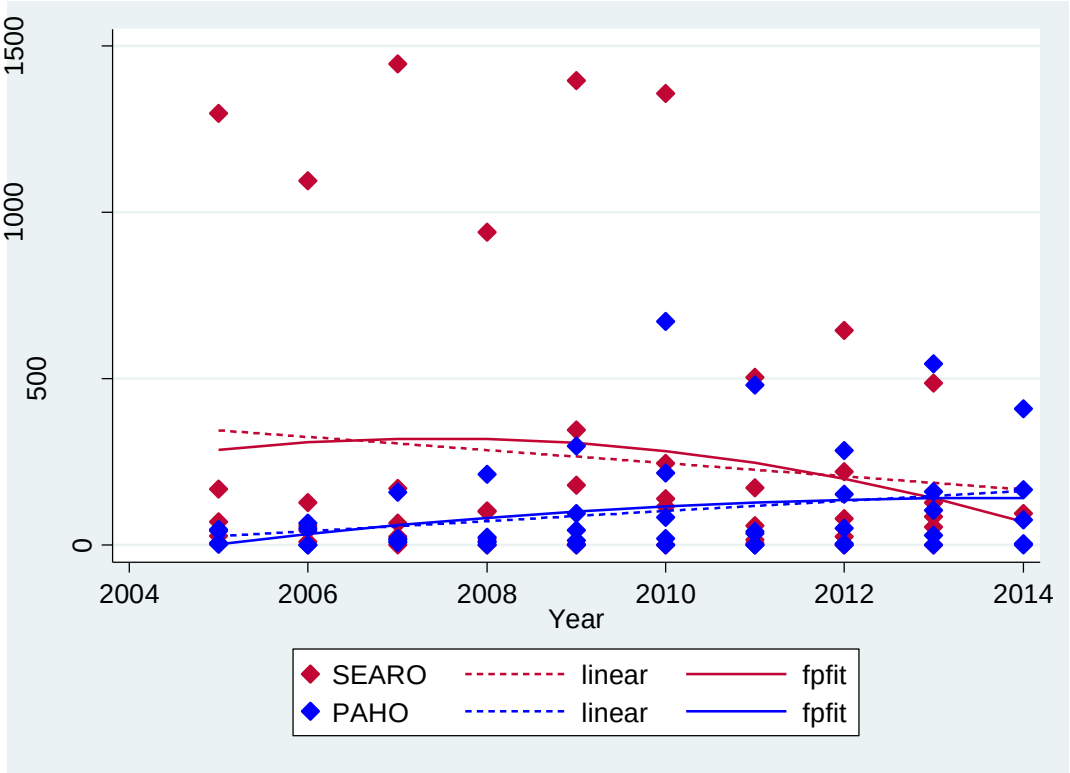

Figure P. Linear Regression graph for Deaths in SEAR and PAHO

CFR

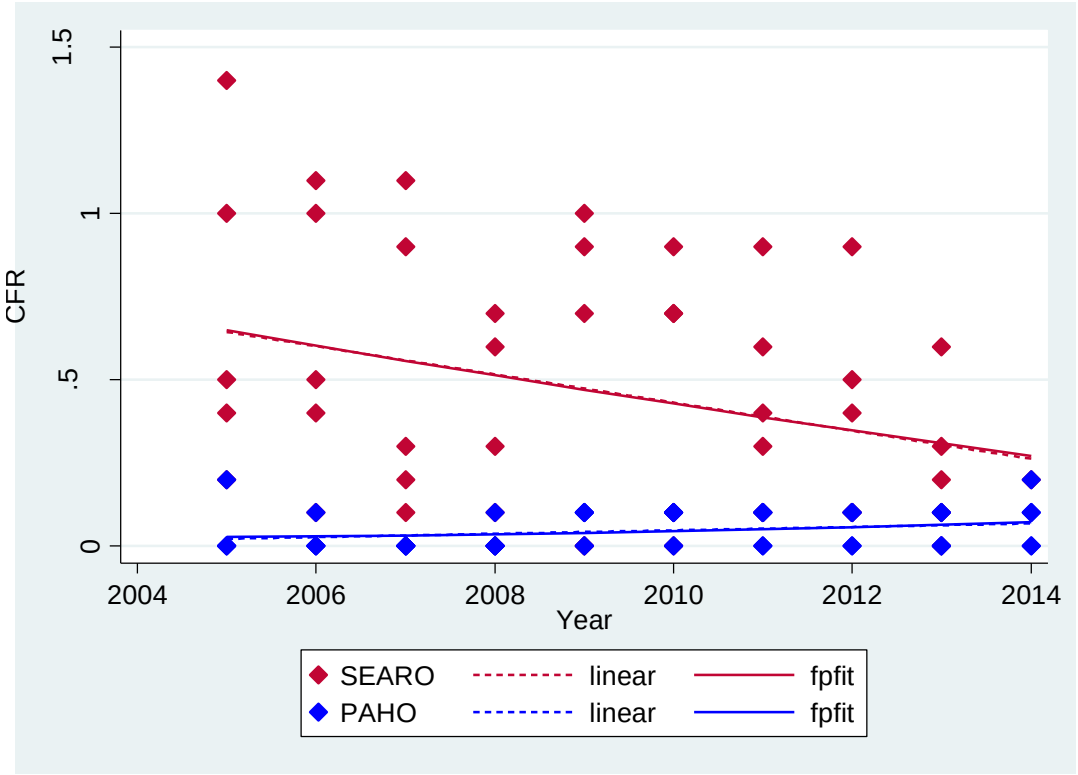

Figure Q. Linear Regression graph for CFR in SEAR and PAHO regions

Overall there is a decrease in deaths in SEAR and increase in PAHO. Clear drop in CFR in SEAR region and a minor rise in the PAHO region. Positive relationship for PAHO (rise in slope) and negative relationship for SEAR region (decrease in slope). *(Figures P and Q)*

SEAR

```
Poisson regression                              Number of obs   =         46
                                                LR chi2(1)      =     927.10
                                                Prob > chi2     =     0.0000
Log likelihood = -2858.1562                     Pseudo R2       =     0.1396
```

deaths	IRR	Std. Err.	z	P>|z|	[95% Conf.	Interval]
year2 suspected	.8956831 (exposure)	.0032769	-30.11	0.000	.8892835	.9021287

AMERICAS

```
Poisson regression                              Number of obs   =         48
                                                LR chi2(1)      =     132.45
                                                Prob > chi2     =     0.0000
Log likelihood = -715.96988                     Pseudo R2       =     0.0847
```

deaths	IRR	Std. Err.	z	P>|z|	[95% Conf.	Interval]
year2 suspected	1.069998 (exposure)	.0063818	11.34	0.000	1.057562	1.082579

Both results are statistically significant (do not include number 1). SEAR shows a negative relationship (negative slope for CFR) meanwhile PAHO shows an increase in CFR (positive relationship), thus CFR has decreased widely in the Region where the old case definition is used (SEAR) meanwhile the CFR in PAHO (where the new case definition is implemented) has increased.

Spearman test for SEAR and PAHO

1. **Spearman CFR Year if Region=="SEARO"**

Number of obs = 46

Spearman's rho = -0.2842

Test of Ho: CFR and Year are independent

Prob > |t| = 0.0556

2. **pearman CFR Year if Region=="PAHO"**

Number of obs = 48

Spearman's rho = 0.1628

Test of Ho: CFR and Year are independent

Prob > |t| = 0.2689

The results will be interpreted by the following table (30):

Size of Correlation	**Interpretation**
.90 to 1.00 (−.90 to −1.00)	Very high positive (negative) correlation
.70 to .90 (−.70 to −.90)	High positive (negative) correlation
.50 to .70 (−.50 to −.70)	Moderate positive (negative) correlation
.30 to .50 (−.30 to −.50)	Low positive (negative) correlation
.00 to .30 (.00 to −.30)	negligible correlation

Our spearman results show a P value of 0.0556 for SEAR and a P value of 0.2689 for PAHO. The correlation is very low and not significant. However, it is important to highlight that the P value for SEAR is close to 0.05, therefore, there might be a visible trend in the future for CFR and year.

12. Discussion

Dengue is a widespread disease causing many deaths being really important to arrive to a consensus on how to best diagnose and treat it. The literature review gave an overall view on the different pros and cons of each method. It was evident that the old 1997 case classification was criticised for having a strict definition and only based on paediatric cases. On the other hand, the new 2009 case classification is being challenged for its loose case definition and for giving room to subjective interpretation of the warning signs. This motivated this study to collect all the national data since 2005 for both regions and analyse the case fatality ratios. For SEAR we selected Thailand, Bangladesh, Indonesia, Myanmar and Sri Lanka. For PAHO region we listed Brazil, Colombia, Costa Rica, Honduras and México.

The parting hypothesis was that the new 2009 case classification is better and effective in reducing mortality compared to the old 1997 original case definition. The data analysis clearly showed that our assumption not proven. Numbers proved that SEARO's death ratio has declined in the past years using the old 1997 case definitions whereas the PAHO region, who implemented the new 2009 case definition, has had an increase in its case fatality ratio. However, the number of suspected cases has been rising in both regions. Overall deaths in SEAR show a decline and PAHO graphs show a slight rise. Nevertheless, it is too early to conclude that the new case classification is not adequate knowing that there are many variables in between which have not been studied and should be taken into account. It should also be noted that PAHO has adopted the new case classification in phases and based on the initial lessons learnt they have finalised a new revised document for case management (2015). Many factors could have influenced the increase in the CFR in the PAHO region. In the past recent years, the surveillance system has significantly improved in many countries and probably leads to more deaths reported. Several genotype switch has occurred in PAHO in recent years (within the four dengue serotypes) are also a major problem that needs to be tackled. A more virulent form of dengue could be the reason of increasing mortality rates in this region along with more secondary infections while SEAR has recorded dengue since 1953 and all serotypes are in circulation.

In conclusion, looking at the national data available, till date the new dengue case classification has not aided in the reduction of the mortality. A more comprehensive study needs to be made after 2-3 years in order to assess the full implementation of the 2015 guidelines in PAHO. There could also be the possibility to merger both old and new case classification, taking the positive points of both.

13. Limitations

This project has some time limitations. The literature review was only carried out from one database. The articles were not reviewed by another co-worker, thus no Cohen's kappa could be calculated (a statistical test to assess the agreement between reviewers). Also, the main obstacle for obtaining true data is the poor surveillance system that leads to underreporting. Another drawback is the lack of information on the deaths. Age, sex and comorbidities within the death group are unknown. Deep research should be also done on the different genotypes within the serotypes living in both SEAR and PAHO regions as well as the virulence level. This could explain the differences in mortality rates.

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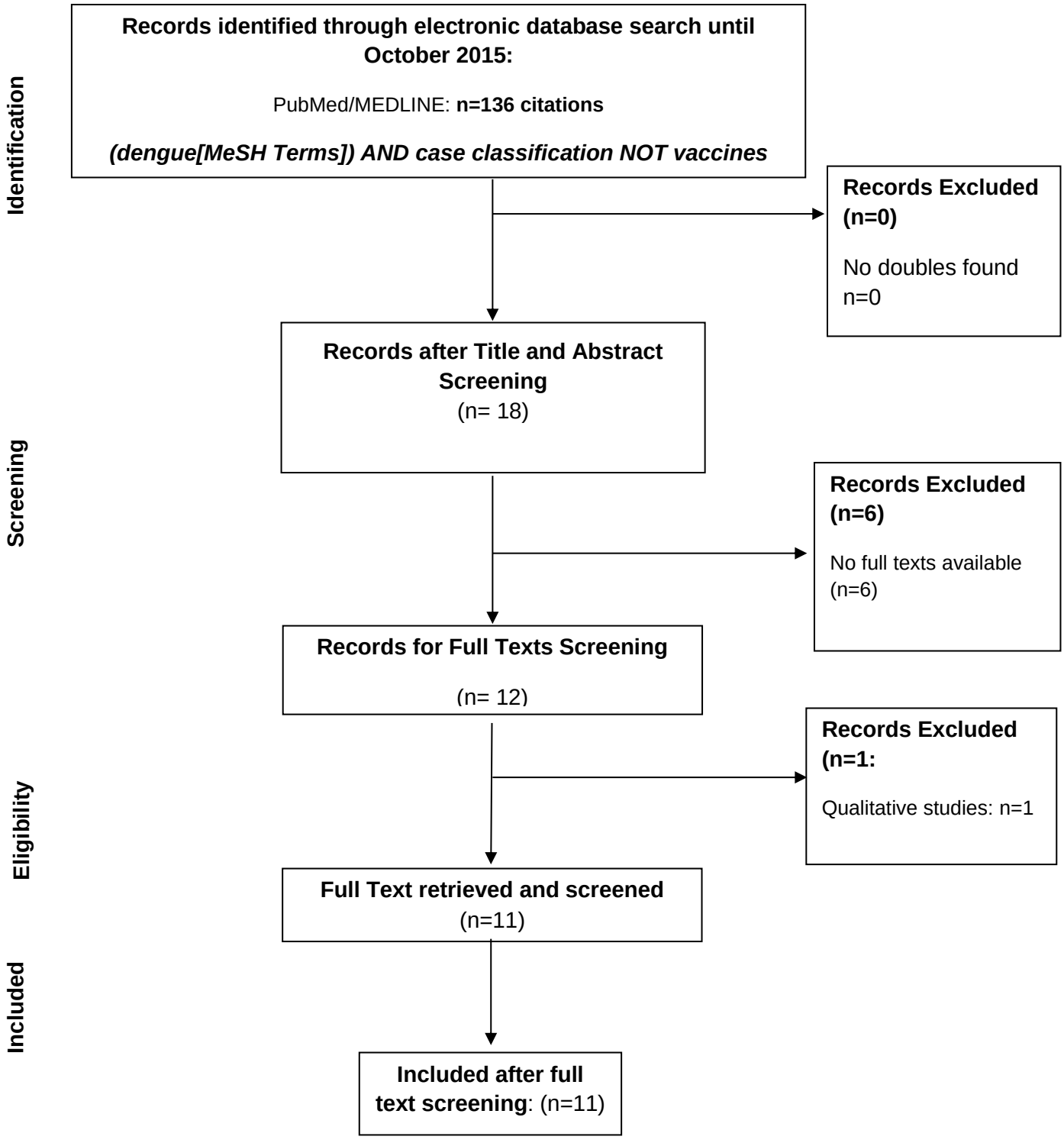
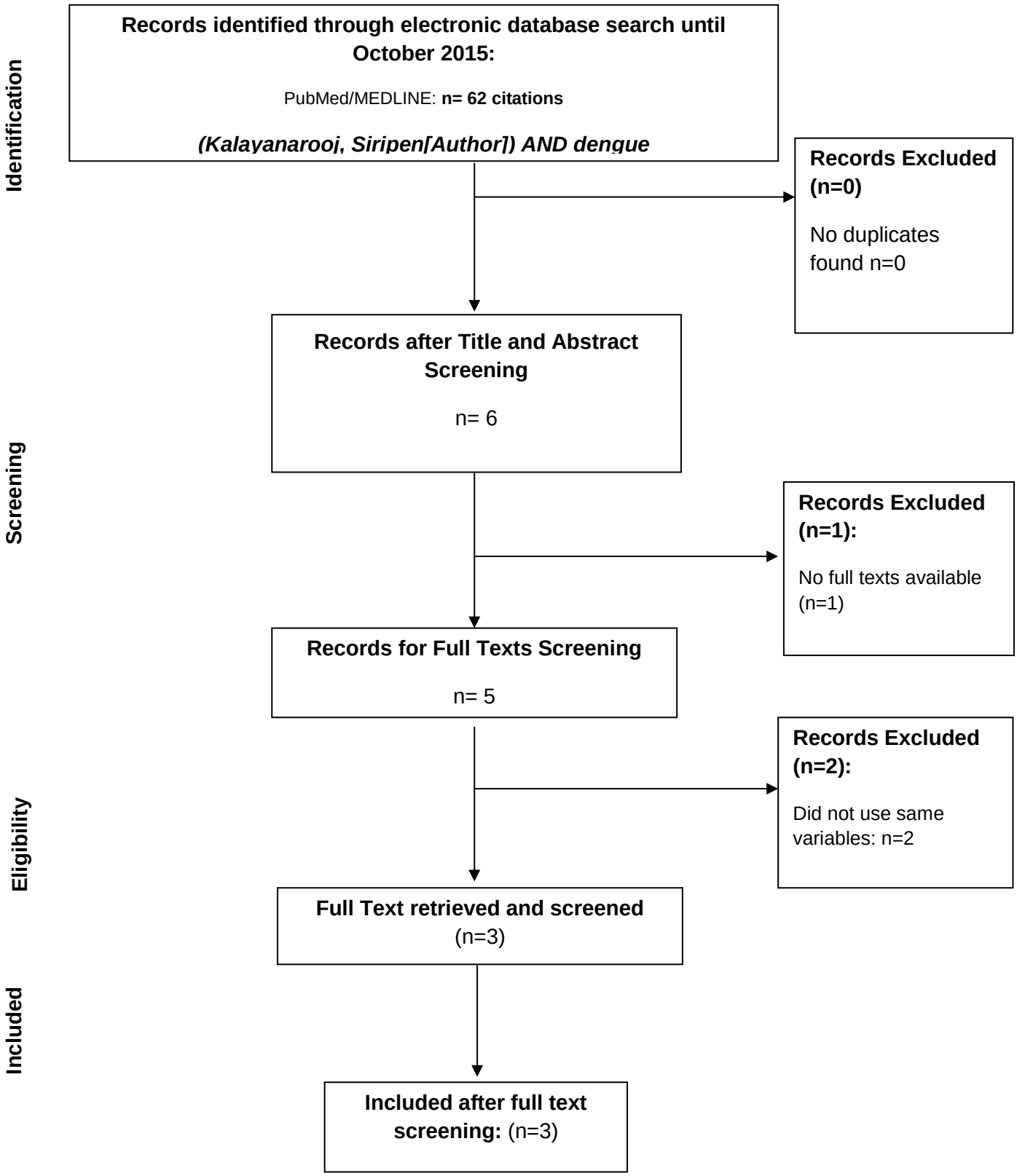
15. Annexes

Annex 1 – PRISMA flowchart for the selection of articles



Annex 2 – PRISMA flowchart for the selection of articles



Annex 3. STATA 10 – CODES FOR LINEAR AND POISSON REGRESSION

```
import excel "D:\owncloud\SERVER\Dengue\Data.xlsx", sheet("Tabelle1") firstrow clear
histogram cfr

***CFR

gen cfr100=cfr*100

twoway scatter cfr100 year if region=="SEARO", msymbol(diamond) mcolor(cranberry)  || ///
     lfit cfr100 year if region=="SEARO", lcolor(cranberry) lp(shortdash) || ///
     fpfit cfr100 year if region=="SEARO", lcolor(cranberry)  || ///
          scatter cfr100 year if region=="PAHO", msymbol(diamond) mcolor(blue)  || ///
     lfit cfr100 year if region=="PAHO", lcolor(blue) lp(shortdash) || ///
          fpfit cfr100 year if region=="PAHO", lcolor(blue)  ytitle(CFR) ///
         legend(rows(2) lab(1 "SEARO") lab(2 "linear") lab(3 "fpfit") lab(4 "PAHO") lab(5 "linear")
lab(6 "fpfit") )

gen year2= year-2005

poisson deaths year2 if r_americas==0 , exposure(suspected)
poisson deaths year2 if r_americas==0 , exposure(suspected) irr
poisson deaths year2 if r_americas==1 , exposure(suspected)
poisson deaths year2 if r_americas==1 , exposure(suspected) irr

mfp: poisson deaths year2 if r_americas==0 , exposure(Suspected)
poisson deaths year2 if r_americas==0 , exposure(suspected) irr
mfp, poisson deaths year2 if r_americas==1 , exposure(Suspected)

regress cfr100 year2 if r_americas==0
regress cfr100 year2 if r_americas==1


*** Number of cases (suspected)


/// need population denomiator

twoway scatter suspected year if region=="SEARO", msymbol(diamond) mcolor(cranberry)  ||
///
     lfit suspected year if region=="SEARO", lcolor(cranberry) lp(shortdash) || ///
     fpfit suspected year if region=="SEARO", lcolor(cranberry) ytitle(Suspected) ///
          legend(rows(1) lab(1 "SEARO") lab(2 "linear") lab(3 "fpfit")  )
```

```
twoway scatter suspected year if region=="PAHO", msymbol(diamond) mcolor(blue)  || ///
    lfit suspected year if region=="PAHO", lcolor(blue) lp(shortdash) || ///
        fpfit suspected year if region=="PAHO", lcolor(blue)  ytitle(Suspected)  ///
        legend(rows(1) lab(1 "PAHO") lab(2 "linear") lab(3 "fpfit") )

poisson suspected year2 if r_americas==0, irr
poisson suspected year2 if r_americas==1, irr
```

***** Number of deaths**

```
twoway scatter deaths year if region=="SEARO", msymbol(diamond) mcolor(cranberry)  || ///
    lfit deaths year if region=="SEARO", lcolor(cranberry) lp(shortdash) || ///
    fpfit deaths year if region=="SEARO", lcolor(cranberry)  || ///
        scatter deaths year if region=="PAHO", msymbol(diamond) mcolor(blue)  || ///
    lfit deaths year if region=="PAHO", lcolor(blue) lp(shortdash) || ///
        fpfit deaths year if region=="PAHO", lcolor(blue)  ytitle(Deaths)  ///
       legend(rows(2) lab(1 "SEARO") lab(2 "linear") lab(3 "fpfit") lab(4 "PAHO") lab(5 "linear")
lab(6 "fpfit") )
```

Annex 4. SPEARMAN CODE ON STATA 10

```
spearman Year CFR if Region=="SEARO"

spearman Year CFR if Region=="PAHO"
```

Annex 5. Reorganized Data for creating the PAHO regional graphs

Country	Year	Suspected	DHF/DSS	Deaths	CFR (Deaths/DHF)* 100	CFR (Deaths/Suspected)* 100
Brazil	2005	203,789	433	43	9.93	0.02
Colombia	2005	30,475	4,306	47	1.09	0.15
Costa rica	2005	37,798	52	2	3.85	0.01
Honduras	2005	18,843	1,795	6	0.33	0.03
Brazil	2006	346,550	628	67	10.67	0.02
Colombia	2006	36,471	5,379	50	0.93	0.14
Costa rica	2006	12,124	72	0	0.00	0.00
Honduras	2006	8,436	636	0	0.00	0.00
Brazil	2007	559,954	1,541	158	10.25	0.03
Colombia	2007	43,227	4,665	20	0.43	0.05
Costa rica	2007	26,440	318	8	2.52	0.03
Honduras	2007	33,508	4,180	16	0.38	0.05
Mexico	2007	48,436	7,897	10	0.13	0.02
Brazil	2008	734,384	9,957	212	2.13	0.03
Colombia	2008	26,732	3,081	12	0.39	0.04
Costa rica	2008	7,160	52	0	0.00	0.00
Honduras	2008	18,941	2,481	9	0.36	0.05
Mexico	2008	31,154	6,114	24	0.39	0.08
Brazil	2009	528,883	8,223	298	3.62	0.06
Colombia	2009	51,543	7,131	44	0.62	0.09
Costa rica	2009	6,946	8	0	0.00	0.00
Honduras	2009	15,291	763	14	1.83	0.09
Mexico	2009	249,763	11,374	96	0.84	0.04
Venezuela	2009	65,869	5,149	0	0.00	0.00
Brazil	2010	1,004,392	16,540	673	4.07	0.07
Colombia	2010	157,152	9,482	217	2.29	0.14
Costa rica	2010	31,773	21	0	0.00	0.00
Honduras	2010	66,814	3,268	83	2.54	0.12
Mexico	2010	57,971	6,336	20	0.32	0.03
Brazil	2011	764,032	10,545	482	4.57	0.06
Colombia	2011	33,207	1,388	42	3.03	0.13
Costa rica	2011	13,854	28	0	0.00	0.00
Honduras	2011	8,297	885	0	0.00	0.00
Mexico	2011	67,918	4,290	36	0.84	0.05
Brazil	2012	565,510	4,055	284	7.00	0.05
Colombia	2012	49,361	1,329	51	3.84	0.10
Costa rica	2012	22,243	54	0	0.00	0.00
Honduras	2012	15,554	2,730	4	0.15	0.03
Mexico	2012	164,947	18,720	153	0.82	0.09

Brazil	2013	1,468,873	6,969	545	7.82	0.04
Colombia	2013	127,219	3,377	161	4.77	0.13
Costa rica	2013	49,868	151	1	0.66	0.00
Honduras	2013	39,271	4,398	29	0.66	0.07
Mexico	2013	231,498	18,667	104	0.56	0.04
Brazil	2014	591,080	689	410	59.51	0.07
Colombia	2014	105,356	2,619	166	6.34	0.16
Costa rica	2014	11,140	4	0	0.00	0.00
Honduras	2014	43,456	2,335	5	0.21	0.01
Mexico	2014	124,943	8,668	76	0.88	0.06

Annex 6. Data used for the Regression model

Region	r_americas	Country	Year	y_2010	Suspected	Deaths	CFR
SEARO	0	Thailand	2005	0	45,893	71	0.002
SEARO	0	Bangladesh	2005	0	1,048	4	0.004
SEARO	0	Indonesia	2005	0	95,279	1298	0.014
SEARO	0	Myanmar	2005	0	17,454	169	0.010
SEARO	0	Sri Lanka	2005	0	5,994	27	0.005
PAHO	1	Brazil	2005	0	203,789	43	0.000
PAHO	1	Colombia	2005	0	30,475	47	0.002
PAHO	1	Costa Rica	2005	0	37,798	2	0.000
PAHO	1	Honduras	2005	0	18,843	6	0.000
SEARO	0	Thailand	2006	0	42,456	59	0.001
SEARO	0	Bangladesh	2006	0	2,198	11	0.005
SEARO	0	Indonesia	2006	0	106,425	1096	0.010
SEARO	0	Myanmar	2006	0	11,383	128	0.011
SEARO	0	Sri Lanka	2006	0	11,980	44	0.004
PAHO	1	Brazil	2006	0	346,550	67	0.000
PAHO	1	Colombia	2006	0	36,471	50	0.001
PAHO	1	Costa Rica	2006	0	12,124	0	0.000
PAHO	1	Honduras	2006	0	8,436	0	0.000
SEARO	0	Thailand	2007	0	62,949	67	0.001
SEARO	0	Bangladesh	2007	0	466	1	0.002
SEARO	0	Indonesia	2007	0	157,442	1446	0.009
SEARO	0	Myanmar	2007	0	15,285	171	0.011
SEARO	0	Sri Lanka	2007	0	7,314	25	0.003
PAHO	1	Brazil	2007	0	559,954	158	0.000
PAHO	1	Colombia	2007	0	43,227	20	0.000
PAHO	1	Costa Rica	2007	0	26,440	8	0.000
PAHO	1	Honduras	2007	0	33,508	16	0.000
PAHO	1	Mexico	2007	0	48,436	10	0.000
SEARO	0	Thailand	2008	0	89,626	102	0.001
SEARO	0	Bangladesh	2008	0	1,181	0	0.000
SEARO	0	Indonesia	2008	0	155,607	940	0.006
SEARO	0	Myanmar	2008	0	14,480	100	0.007
SEARO	0	Sri Lanka	2008	0	6,555	19	0.003
PAHO	1	Brazil	2008	0	734,384	212	0.000
PAHO	1	Colombia	2008	0	26,732	12	0.000
PAHO	1	Costa Rica	2008	0	7,160	0	0.000
PAHO	1	Honduras	2008	0	18,941	9	0.000
PAHO	1	Mexico	2008	0	31,154	24	0.001
SEARO	0	Thailand	2009	1	25,194	2	0.000
SEARO	0	Bangladesh	2009	1	474	0	0.000

SEARO	0	Indonesia	2009	1	156,052	1396	0.009
SEARO	0	Myanmar	2009	1	24,287	181	0.007
SEARO	0	Sri Lanka	2009	1	35,010	346	0.010
PAHO	1	Brazil	2009	1	528,883	298	0.001
PAHO	1	Colombia	2009	1	51,543	44	0.001
PAHO	1	Costa Rica	2009	1	6,946	0	0.000
PAHO	1	Honduras	2009	1	15,291	14	0.001
PAHO	1	Mexico	2009	1	249,763	96	0.000
SEARO	0	Thailand	2010	1	116,947	139	0.001
SEARO	0	Bangladesh	2010	1	409	0	0.000
SEARO	0	Indonesia	2010	1	155,777	1358	0.009
SEARO	0	Myanmar	2010	1	16,529	117	0.007
SEARO	0	Sri Lanka	2010	1	34,105	246	0.007
PAHO	1	Brazil	2010	1	1,004,392	673	0.001
PAHO	1	Colombia	2010	1	157,152	217	0.001
PAHO	1	Costa Rica	2010	1	31,773	0	0.000
PAHO	1	Honduras	2010	1	66,814	83	0.001
PAHO	1	Mexico	2010	1	57,971	20	0.000
SEARO	0	Thailand	2011	1	64,374	58	0.001
SEARO	0	Bangladesh	2011	1	1,362	6	0.004
SEARO	0	Indonesia	2011	1	58,065	504	0.009
SEARO	0	Myanmar	2011	1	4,738	16	0.003
SEARO	0	Sri Lanka	2011	1	27162	173	0.006
PAHO	1	Brazil	2011	1	764,032	482	0.001
PAHO	1	Colombia	2011	1	33,207	42	0.001
PAHO	1	Costa Rica	2011	1	13,854	0	0.000
PAHO	1	Honduras	2011	1	8,297	0	0.000
PAHO	1	Mexico	2011	1	67,918	36	0.001
SEARO	0	Thailand	2012	1	78,063	80	0.001
SEARO	0	Bangladesh	2012	1	671	1	0.001
SEARO	0	Indonesia	2012	1	74,062	646	0.009
SEARO	0	Myanmar	2012	1	6,433	26	0.004
SEARO	0	Sri Lanka	2012	1	44456	220	0.005
PAHO	1	Brazil	2012	1	565,510	284	0.001
PAHO	1	Colombia	2012	1	49,361	51	0.001
PAHO	1	Costa Rica	2012	1	22,243	0	0.000
PAHO	1	Honduras	2012	1	15,554	4	0.000
PAHO	1	Mexico	2012	1	164,947	153	0.001
SEARO	0	Thailand	2013	1	131,090	128	0.001
SEARO	0	Bangladesh	2013	1	457	0	0.000
SEARO	0	Indonesia	2013	1	76,090	487	0.006

SEARO	0	Myanmar	2013	1	22,335	55	0.002
SEARO	0	Sri Lanka	2013	1	29878	85	0.003
PAHO	1	Brazil	2013	1	1,468,873	545	0.000
PAHO	1	Colombia	2013	1	127,219	161	0.001
PAHO	1	Costa Rica	2013	1	49,868	1	0.000
PAHO	1	Honduras	2013	1	39,271	29	0.001
PAHO	1	Mexico	2013	1	231,498	104	0.000
SEARO	0	Sri Lanka	2014	1	47502	95	0.002
PAHO	1	Brazil	2014	1	591,080	410	0.001
PAHO	1	Colombia	2014	1	105,356	166	0.002
PAHO	1	Costa Rica	2014	1	11,140	0	0.000
PAHO	1	Honduras	2014	1	43,456	5	0.000
PAHO	1	Mexico	2014	1	124,943	76	0.001