# ANTECEDENTS OF DENGUE VIRUS DURING THE CLINICAL INVESTIGATIONS IN FAISALABAD

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**Abstract:**
*Background: Dengue is the most important viral mutagenic disease in public health, caused by one of the four serotypes (DEN-1, 2, 3 and 4) of the dengue virus, a positive chain RNA virus of the Flaviviridae family, which produces a spectrum of illness ranging from dengue fever to dengue hemorrhagic fever / shock syndrome (shock) due to dengue, the latter a serious infection with vascular and haemostatic abnormality that can lead to death.*
*Objectives: To identify by reverse transcription-polymerase chain reaction (RT-PCR) and specific restriction sites - polymerase chain reaction (RSS-PCR) to the causative agent of the epidemic outbreak presented in the district of Faisalabad in April 2017.*
*Materials and methods: twenty serum samples collected during the dengue outbreak were processed by RT-PCR to determine the serotype; this technique was performed in one step. The RSS-PCR technique was then applied to identify the circulating genotype and the results were subsequently corroborated with viral isolation and sequencing.*
*Results: The analysis of the RTPCR of the RNA extracted from the samples presented an amplified product of 290pb corresponding to the dengue serotype. The analysis of the RSS-PCR products of RNA extracted from dengue isolates corresponded to pattern C, included in genotype III. The isolations of the dengue virus in C6 / 36 cell lines, typed by IFI and the genetic sequencing confirmed the results obtained by the tests previously described. Conclusion: During the classic dengue epidemic outbreak in Lima, genotype III of the dengue virus circulated.*
**Keywords**: *Classic dengue outbreak; dengue virus; molecular typing; RT-PCR; RSS-PCR.*

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*Please cite this article in press Faiqua Nayyer et al., **Antecedents of Dengue Virus during the Clinical Investigations in Faisalabad,** Indo Am. J. P. Sci, 2018; 05(04).*

## INTRODUCTION:

Dengue is the most important viral mutagenic disease in public health, caused by one of the four serotypes (DEN-1, 2, 3 and 4) of the dengue virus, a positive chain RNA virus of the Flaviviridae family, which produces a spectrum of illness ranging from dengue fever to dengue hemorrhagic fever / shock syndrome (shock) due to dengue, the latter a serious infection with vascular and haemostatic abnormality that can lead to death1. The serological surveillance of dengue is mainly based on the detection of specific IgM antibodies, while the detection of circulating serotypes is traditionally carried out by viral isolation and its identification [1-4].

A few years ago, the reverse transcription-polymerase chain reaction (RT-PCR) test was started, which allows the identification of serotypes in serum samples and supernatants of infected cells with clinical samples2-5. The RTPCR is useful for obtaining rapid information on circulating dengue serotypes; however, it is very important to isolate the virus to confirm your identity and conduct more detailed studies [4-8].

Each serotype of the virus is subdivided into subtypes based on the genetic diversity of the envelope gene (E) 6. These subtypes are studied for a better understanding of the origin and evolution of virus strains and the existing correlation between subtypes and degree of disease severity present in epidemic outbreaks[3, 9, 10]. To determine the subtypes, traditionally, sequencing is performed to generate the phylogenetic tree by laborious methods and sophisticated7,8. Recently, a new method for the identification of subtypes was developed based on a simple PCR called Specific Sites of Restriction - Polymerase Chain Reaction (RSS-PCR) [11, 12]

The presence of indigenous cases of dengue in Lima had not been documented in the last 100 years; however, the presence was notified for the first time in March 2000 in five localities of Lima10; by April 2004 there were already 44 localities infested with the vector, which included the district of Comas11; In addition to the presence of imported cases of dengue12, the risk of an outbreak in Lima was high. [3, 13]

For this reason, a vector and febrile surveillance system was established for the areas at risk, in order to detect the first cases of dengue in a timely manner and carry out control actions. In this context, on April 14, 2017, febrile cases with symptoms compatible with dengue were reported in the Comas area; the active search for febrile cases was carried out in the community, and 75 patients were found who were taken serum samples to diagnose the cause of the outbreak .[3, 14]. The objective of the study was to quickly identify, using molecular techniques, one-step RT-PCR and RSS-PCR, to the causal agent of the epidemic dengue outbreak in Faisalabad, which occurred in April 2017.

## MATERIALS AND METHODS:

Of the first 75 cases of April 14, a group of 20 samples with a disease time of less than five days at the time of sampling was randomly selected. We proceeded to perform the RT-PCR, simultaneously, the samples were inoculated in C6 / 36 cell culture for viral isolation and analyzed by RSS-PCR for the determination of genetic subtypes.

The RNA was extracted from the serum samples using: viral RNA Mini Kit cat. according to the protocol established by the manufacturer. The procedures described by Harris et al9 were followed. This rapid typing system consists of a reverse transcription and amplification using four primers corresponding to regions obtained by the use of restriction enzymes of the envelope gene (E). This method is easy to perform, fast, requires minimal laboratory equipment and widely distributed reagents are used.

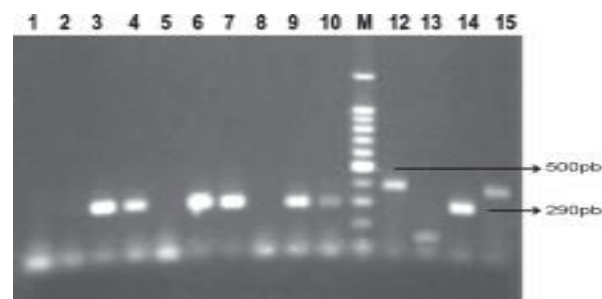Viral Isolation and Identification for Immunofluorescence.



**Figure 1. Analysis of the RT-.**

## RESULTS:

After six hours of delivering the serum samples, analysis of the RT-PCR product by electrophoresis in 1.5% l of the RNA extracted from the samples showed an amplified product of corresponding to DENGUE (Figure 1). The analysis of RSS-PCR products by agarose gel electrophoresis 1.5% of RNA extracted from DENGUE isolates corresponded to pattern C, included in genotype III or Asian genotype, and a homology of 98 was found. , 5% with the sequence of DEN3 isolated in Nicaragua in 1996 (Figure 2). The samples inoculated in cell line C6 / 36 presented syncytial effect between seven to

ten days after the inoculation, the IFI typing applying monoclonal antibodies resulted in DENGUE, confirming the results obtained by the tests described above. Molecular typing of the dengue outbreak in Faisalabad

### DISCUSSION:

The first outbreak of epidemic dengue documented in the town of Comas in Lima was caused by subtype III or Asian genotype of DENGUE virus, diagnosed with molecular RT-PCR techniques six hours after receiving the news of the presence of fevers in the area and subtype by RSS-PCR, subsequently confirmed by viral isolation and immune fluorescence (IFI).

The etiological diagnosis of dengue is made by viral isolation in the cell line and the identification of the serotypes by IF, a procedure that requires between 7-15 days to obtain the result, in addition to the high cost, application of laborious techniques, obtaining, storage and transport of the sample as factors that guarantee the success of the diagnosis. This procedure has been carried out in our laboratory since 1990, when the first outbreak of DEN 1 occurred in the tropical zone of Loreto. Currently only 10% of the samples that come for this diagnosis are being worked on, mainly due to the high cost of their execution.

Alternatively, the RT-PCR technique that we used to define the Comas outbreak in a short time, previously described9, is being used in other countries as a surveillance method only at the start of outbreaks.
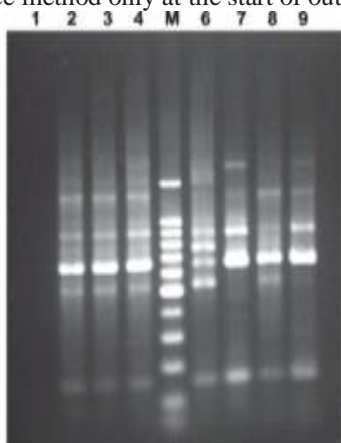


**Figure 2. Analysis of the product of DENGUE amplification by RSS-PCR by agarose gel electrophoresis 1.5% of the samples of patients with dengue from the Comas outbreak, Faisalabad-2017.**

Routine diagnosis, previously validated against the isolation that is the gold test14, which allows to simplify the response time and reduce the diagnostic

costs. This is an important option, taking into account that 90% of serum samples from febrile patients with probable dengue symptoms are within the first five days of the disease and that viral isolation represents a high cost and response time. The determination of the genotype of circulating dengue viruses is valuable information when an outbreak occurs, it is known that some Asian genotypes are associated with serious dengue symptoms15,16. The RSS-PCR9 typing system is an easy-to-carry procedure similar to a PCR method and much more accessible than a sequence and phylogenetic analysis, which makes it an important tool in molecular epidemiology for laboratories that do not have sequencing systems. The results demonstrated the circulation of subtype III of the DENGUE virus, this subtype is a member of the Sri Lanka subtype, of Asian origin and has been associated with dengue hemorrhagic fever. Due to the history of previous outbreaks of DENGUE in endemic areas of Peru such as the eastern region and the northern coast17,18, only genotype III has been observed so far and this has been associated with clinical cases of dengue hemorrhagic fever, observed in the outbreak of Comas to date are clinical pictures of classic dengue. The rapid and timely diagnosis allowed the authorities of the health sector to act immediately to carry out the control measures of the outbreak. The rapid and timely diagnosis allowed the authorities of the health sector to act immediately to carry out the control measures of the outbreak hybridization.

### CONCLUSION:

The analysis of the RTPCR of the RNA extracted from the samples presented an amplified product of 290pb corresponding to the dengue serotype. The analysis of the RSS-PCR products of RNA extracted from dengue isolates corresponded to pattern C, included in genotype III. The isolations of the dengue virus in C6 / 36 cell lines, typed by IFI and the genetic sequencing confirmed the results obtained by the tests previously described. Conclusion: During the classic dengue epidemic outbreak in Lima, genotype III of the dengue virus circulated.

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