

# Outbreak Investigations



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# Outbreak Investigations

## 1. Outbreak Investigations

### 1.1 Bird flu

#### 1.1.1 Navapur, Nandurbar district, Maharashtra

An outbreak of Avian Influenza with a high mortality in the poultry farms situated in Navapur taluka of Nandurbar district, Maharashtra state was reported during Jan-Feb. 2006. The NIV teams carried out field investigations at Navapur in February and March 2006 to explore the possible involvement of humans in this outbreak. NIV team played various roles and assisted in creating the isolation facility in rural hospital setting, training the staff in use of protective devices, established the bird flu OPD, monitored the suspected avian influenza patients admitted in Isolation ward, established the quarantine procedures, initiated community surveillance, monitored culler's health, investigated the egret deaths, surveyed the water reservoir sites and collected migratory birds specimens for establishing the chain of transmission. Throat and nasal swabs were collected from 12 suspected cases. Serum samples were also collected from suspected cases, contacts, poultry workers and staff members of state health department involved in the supervision and performance of culling operations. All the investigators, health workers involved in surveillance, cullers, family contacts of suspected patients were given prophylactic Tamiflu tablets for a week. Preliminary tests carried out on the serum samples from suspected cases were negative for H5N1 virus. The serum samples from poultry workers and health staff were also tested and found negative for H5N1 virus.

Navapur has the highest density of poultry farms (52) bearing about ten thousand to one lakh birds in an individual farm. Mortality of birds was observed since last week of January 2006. Poultry specimens were tested in High Security Animal Disease Laboratory, Bhopal. Avian influenza virus H5N1 was detected in poultry birds at various poultry farms in Navapur taluka. Mass culling process of the poultry was started on 19/2/2006. Initially birds were given pentobarbitone tablets through water but this method was found not suitable, finally the culling was done by manual decapitation method. Lakhs of birds were culled and buried on the same location. Observation of mortality in backyard poultry led to culling of these birds. The local egret deaths were reported from the locality. NIV team investigated the role of egrets in the transmission. There were two dams about 12-15 kms from Navapur having the intermixing ecology among the migratory birds, wild birds, backyard poultry and human. About twenty species of migratory birds were observed. Faeces of migratory birds, dead egrets in the locality, contaminated water specimens were collected from different locations. None of the specimens was positive for Avian influenza.

#### 1.1.2 Jalgaon, Maharashtra

In March 2006, following poultry deaths in Jalgaon district of Maharashtra a team from NIV visited the area. Reports received by the state health authorities indicated that poultry samples from 4 villages had tested Influenza H5 positive. Three of the above villages were visited, and families whose birds had tested positive were interviewed, examined and blood samples of 12 such persons were taken. One 5 year, male child was febrile for 2 preceding days, with flu-like symptoms; his throat swab was taken. Another 11-year male was admitted for investigation in the rural hospital with a 3 day history of unresolving, low grade fever. He belonged to one of the three above families. His blood, throat & nasal swab were taken. 84 blood samples were taken from villagers who had handled dead poultry with bare hands or had such birds in the household. One person

presented with low-grade fever, his throat swab was collected. Regarding the human issues, separate cell was created to examine the suspected case of bird flu, including isolation ward. Some blood, throat and nasal samples were collected for lab diagnosis. No human case was found to be positive for avian influenza. Jalgaon area has few organized poultry farms, but mostly affected birds were from the backyard. About 400 villages were affected. The birds were culled and buried.

### 1.1.3. Burhanapur, M.P.

Backyard Poultry deaths were reported from district of Burhanpur in MP in April 2006. The investigations carried out in this area did not reveal any suspected human cases of avian influenza.

#### Laboratory testing

Seventy four TS/NS and 20 environmental samples from Navapur and 35 TS/NS/environmental samples from Jalgaon were tested by RT-PCR; 44 samples in ABI, 19 samples Corbett by Real time PCR; 33 samples by NASBA: and 29 samples by Virus isolation. H5N1 was not detected in any of the human samples tested.

### 1.2 Acute Haemorrhagic fever in Kalyan, 2005

An outbreak of acute hemorrhagic fever from Kalyan area was investigated in August 2005. Adolescent and adults cases with short history of fever, bloody vomit and death were reported. Post mortem was done in 3 cases. PM findings showed grossly haemorrhagic lungs, kidneys and necrotic liver. Cardiomyopathy was also noted in one patient. Deaths were within short duration. There were no epidemiological linkages between the cases. Common exposure history was the exposure to stagnant water for long time and rigorous efforts by these persons to save others. Family contacts were spared. During these investigations, abstracting of the details of death cases from postmortem reports was done. The inpatient records were abstracted for clinical presentation and blood samples from 29 in-patients were collected. Eleven postmortem blood samples and 29 organs from 5 death cases were collected. Initial inoculations in suckling mice and cell culture did not yield any virus. Sera were tested for Dengue, Leptospira and Hantan. Only 1 of 51 sera samples was positive for Dengue. None of the sera from 12 dead patients were positive for Dengue or Hantan. 3 were positive for Leptospira by MAT. PCR for Hantan using PM organs were negative. 12 cases with fever collected from contacts of dead patients showed IgM antibodies for Hantan by commercial kits (Focus Diagnostics, USA). Leptospirosis PCR was also negative. No conclusion could be drawn for the etiology.

In addition, entomological studies were conducted in the affected areas to assess the post-flood mosquito-genic conditions and the rodent fauna in the noso-areas where cases were reported. Neither adult mosquitos nor their breeding was noted in the affected areas. During the second visit ten days later rodents were trapped in these areas as follows- *Suncus murinus* (shrew 17), *Mus musculus* (mice 9), *Rattus (Rattus) rufescens* (7), *.Rattus (Rattus) novregicus* 3, *Bandicota bengalensis* 1. In all a total of 34 rodent blood samples containing sera and clots, and 147 rodent samples of different organs viz. spleen, liver, kidney and lung have been brought to the laboratory on LN2. These will be processed for the presence of virus or detection of virus antibody.

### 1.3 Fever with arthralgia/arthritis in Andhra Pradesh (Chikungunya)

An outbreak of fever with arthralgia/arthritis in eight districts of Andhra Pradesh was investigated in February 2006. Surveillance and reporting was lacking in most areas thereby making it difficult to assess the magnitude of the disease. Overall attack rates ranged between 15 and 45 %. A total of 334 cases were examined and sampled for viral etiology. The cases presented with acute onset of moderate to high-grade fever with chills, which lasted for 3 to 5 days and joint pains/swelling lasting for weeks to months. Transient rash was reported in a few patients. Headache and body ache were also reported in few patients. The cases were reported predominantly from rural areas with focal distribution in the affected areas. Familial clustering was observed. There was no major sex difference. Human sera were tested for IgM antibodies to CHIK. Virus isolation from acute sera was carried out in C6/36 cell line, isolates were confirmed by IFA and PCR. Sequences of PCR product were matched with earlier strains. IgM positivity in sera from Kurnool, Kadappa, Chittoor, Anantapur, Nalgonda, Hyderabad, Prakasam and Guntur indicated Chikungunya outbreak.

Majority (300/334, 90%) of the cases were above 15 years of age. Out of 334 cases, 325 cases sera were tested for IgM antibodies against CHIK and DEN. Seropositivity of IgM antibodies to CHIK in females (87/174, 50%) was slightly higher than males (63/151, 42%). Also, seropositivity of IgM antibodies to CHIK in >15 years of age (144/293, 49%) was significantly higher ( $p < 0.05$ ) than in <15 years of age (6/32, 19%). Seropositivity to CHIK IgM antibodies in affected localities (150/325, 40%) was significantly higher ( $p < 0.05$ ) than in unaffected localities (9/52, 17%). CHIK virus isolations were done in 4 cases in Kalkada, Mungilipatlu and Devalammanagaram villages. In 24 localities of 6 districts, 460 households were screened. Average House index (AHI) and Breteau index (BI) ranges were 10-60 and 13-75 respectively. CHIK IFA positivity in headsquashes of *Ae. Aegypti* was detected in Kadapa, Chittoor, Ananthapur and Nalgonda districts.

### 1.4 Encephalitis outbreaks

#### 1.4.1 Encephalitis in Muzzafarpur, Bihar

During May- June 2005, 101 encephalitis cases with 42 deaths (CFR-41.5%) were reported from various blocks in Muzzafarpur district of Bihar. The last case was reported on the 23rd of June. A team from NIV visited the affected areas in the last week of June when no acute case was available for examination. Seven children who survived were examined and their blood samples were taken for etiological diagnosis. Further, visits were made to case-villages and 39 contacts (siblings and neighbors) in the pediatric age group were bled. Similarly, non-case villages were visited and blood samples from 41 children were collected after filling a questionnaire. All the sera when tested for IgM antibodies against Chandipura virus were negative.

Entomological studies were carried out in the area. In all 14 affected villages from 4 PHCs were surveyed. The overall affected population belonged to low socio-economic strata. Most of the households were single storeyed with 2-3 rooms. The walls of the houses were made up of bamboos covered with dry grass. Their walls were plastered about 12" to 18" from the floor with mud and dung. DDT was sprayed in most of the areas about a fortnight prior to the survey. These houses were amidst either agricultural fields or mango or leechi plantations. Occasionally cattle sheds were present among the human dwellings. The area witnessed occasional showers during the study. Overall the adult mosquito catch inside the house was low, and was

represented by *Culex quinquefasciatus* (40F) and *Armigeres subalbatus* (1F). The sand flies were abundant despite insecticide spray. *Sergentomyia* sp., *Phlebotomus* sp., *Phlebotomus papatasi*, *Phlebotomus argentipes* were collected. It was evident that DDT spraying was not effective against the sandfly population. During a period of '4' days of arthropod collection visits and 16 Man-hour period, a total of 377 sandfly species were collected. These were transported to Pune laboratory on wet ice. 32 pools of sandfly and 2 pools of mosquito have been prepared and *being* processed for virus isolations..

#### 1.4.2 Saharanpur, Uttar Pradesh

An outbreak of suspected viral encephalitis in children in Saharanpur, UP was investigated by NIV. From 15<sup>th</sup> September to mid- November 2005, 170 encephalitis cases with 126 deaths (CFR 74 %) were reported. There was no geographic clustering. All cases were below 15 years of age with slight female preponderance. The cases presented with history of 0-3 days fever with altered sensorium. Vomiting and loose motions were reported in a few cases. There were no signs of meningeal involvement. The cases recovering from illness had no major neurological sequelae. Routine CSF evaluation showed raised protein level, normal sugar and 0-10 cells, mostly mononuclear lymphocytes. During investigations, 15 acute samples comprising 4 acute sera, 4 CSF, 4 throat swabs and 3 rectal swabs were collected from 4 cases. Two convalescent and 8 contact sera were also collected. Four throat swabs, 3 rectal swabs and 2 acute sera inoculated in Vero cells did not show any CPE. No virus was isolated. Two acute sera, 3 CSF and two convalescent sera and 8 contact sera were negative for JE IgM by MAC-ELISA. Etiology could not be confirmed.

#### 1.4.3. Gorakhpur, Uttar Pradesh

A large outbreak of Japanese encephalitis was reported from Uttar Pradesh during July to October 2005. A total of 6061 cases including 448 cases from Bihar and 31 from Nepal were reported. Mortality rate was about 25% (1500 out of 6061). Majority of cases were from Gorakhpur region in the state. Children in the age group of 3 months to 15 years were mainly affected. IgM ELISA was carried out on representative 65 CSF specimens. IgM antibodies to JE virus could be detected in 42 out of 65 cases. In pediatric cases 33/50 showed anti-JE IgM antibodies while 9/15 cases were positive in adults confirming that the epidemic was due to JE virus. JE isolation was attempted from 11 blood clots. RBCs from blood clots were lysed with ammonium chloride and WBCs were co-cultured with phytohemagglutinin stimulated normal human PBMC. After three days of incubation three co-cultures showed changes. Culture supernatant from these cultures were tested for presence of JE antigen by antigen capture ELISA using JE specific MAb. Two samples showed presence of JE antigen. These samples were inoculated in one day old infant mice for isolation of JE virus. Mice inoculated with sample 057434 showed sickness on 3rd day. Virus pool from passage two has been prepared. Sickness in mice is stabilized and virus has been adapted in Vero cell. Virus Titre was  $2.5 \times 10^8$  PFU/ml.

During this period few cases with fever of 4-10 days and swelling all over body were also reported from pediatric group. Altered sensorium and/ or convulsions, loose motions, Tachypnea, Tachycardia, muffled heart sounds and low voltage ECGs were the clinical features. Eight fecal samples and 21 throat swabs were negative for Enteroviruses by PCR. Ten rectal swabs were processed. No virus was isolated.

#### 1.4.4 Rae Bareilly, Uttar Pradesh

Outbreak of acute encephalitis was investigated from Rae Bareilly, UP in August, 2005. Majority cases were children but few adults were also infected. 83% of sera collected from 32 patients were positive for JE by MAC ELISA (22/28 sera and 10/13 CSF). No virus could be isolated. Water stagnation and dry areas were interspersed in the region. This is the first report of JE from Rae Bareilly.

#### 1.4.5 Warangal, Andhra Pradesh

Ninety cases with 49 deaths (CFR 54.4%) of acute viral encephalitis in children reported between 9th May 2005 and 22nd March 2006 were admitted in MGM HOSPITAL, WARANGAL. 52 cases of these could be labeled as suspect cases as per case definition. Majority of the cases were from Warangal (42), followed by Karimnagar (8), Khammam (1) and Adilabad (1) Clustering was not observed. The ages of the patients ranged between 9 months and 13 years with more cases in 0-4 years (35/52, 70%). Male to female ratio was 1:2. Case fatality rate was high (25/52, 48.1%); majority of deaths (19/25, 76%) occurred within 48 hours of hospitalization. CHP viral RNA was detected in 20 (45.4%) of 44 cases and CHP virus specific IgM was detected in 4 (7.7%) of 52 cases. 2 cases seroconverted. Thus CHP viral etiology was detected in 26 cases reporting up to mid- September 2005, which is the period of late summer and early monsoon. JE virus specific IgM was detected in 4 /37 (10.8%). One more case (case no. 10) seroconverted to JE virus specific IgM antibodies in convalescent phase. All JE cases were reported in later period after mid-September, which is late monsoon season. In summary, among 52 cases, 26 (50%) cases were detected with CHP viral etiology and 5 (9.6%) cases with JEV etiology. All these cases had no major neurological sequelae at follow-up in field. No illness in contacts was reported.

During focal serosurvey in contacts 188 sera were collected from 10 rural and 4 urban localities in Warangal district. Twenty-five of 188 (13.3%) contact sera were positive for CHP-IgM. Four CHP-IgM positive contacts reported febrile illness (16%). Thirtytwo serum samples from districts of Karimnagar, Khammam and Adilabad were tested for Chandipura. Three sera were IgM positive and four sera were PCR positive. All were were JE negative.

#### 1.4.6 Kurnool Andhra Pradesh

Suspected viral encephalitis in children started reporting in June 2005. A total of 52 cases with 7 deaths (CFR 14 %) were reported till 24<sup>th</sup> November 2005. All cases were below 15 years of age. The cases were not clustered. No illness in contacts was observed. The cases presented with a history of fever of 4-7 days with altered sensorium and/or convulsions. Headache was present in few cases. Hypertonia of extremities with neck stiffness was observed. CSF protein was minimally raised; sugar was normal and total cells 10-50, mostly lymphocytes. Five of 12 acute sera, 2/2 CSF and one of 4 convalescent sera showed IgM positivity for JE by MAC-ELISA. All these samples were negative for CHP-IgM by MAC-ELISA. The epidemiological and laboratory investigation showed the endemicity of JE virus in Kurnool.

#### 1.4.7 Encephalitis in Bhandara & Nagpur, Maharashtra

An outbreak of suspected acute viral encephalitis in children in Bhandara and Nagpur districts, Maharashtra was investigated during July 2005. A total of 72 cases were reported during the period between June and August 2005. Virological investigations were done in subset of 21 cases. Male to Female ratio was 1:1.1. There were 11 cases in 1-4 year's age group, 7 cases in 5-9 years and 3 cases in 10-14 years. The clinical presentations (n=21) include Altered sensorium (14), unconsciousness (5), convulsions (4), coma (4) cases, vomiting (2) cases, loose motions (3) and headache in 3 cases. Twenty-one acute sera, three early convalescent sera and five CSF samples were tested from 21 suspected cases. Seven of 21 cases were confirmed as Chandipura (CHP) virus infection. Of these seven cases, 1 case was PCR positive for CHP viral RNA, 2 cases were CHP IgM positive and 4 cases were positive for both i.e. PCR CHP viral RNA and CHP IgM antibodies. All these cases were negative for JEV IgM.

Fifty-nine serum samples from contacts of CHP confirmed cases and 42 serum samples from one control village were collected during the investigation. Four of 59 contacts of confirmed cases were CHP IgM positive by MAC ELISA as compared to none of 42 sera collected from control village. Thirty-one of the 59 contacts of confirmed cases showed evidence of N antibodies to CHP virus as compared to 1 of the 42 sera collected from control village. Chandipura virus was found to be the etiological agent associated with the cases of viral encephalitis among children in Bhandara and Nagpur district of Maharashtra state

A total of eight localities in the districts of Nagpur (7) and Bhandara (1) were visited. Despite insecticide applications by the State Health Authorities sandfly population was abundant. *Sergentomyia* spp., *Phlebotomus papatasi* and *Ph.argentipes* were collected during the survey.

### 1.5 Viral hepatitis:

#### 1.5.1 Maharashtra state

A total of 558 serum samples representing 17 epidemics of viral hepatitis from various areas in the state of Maharashtra were referred by the State Government for etiological diagnosis. Sixteen epidemics were attributed to HEV while one was caused by HAV.

#### 1.5.2 Baramati, Maharashtra

An outbreak of viral hepatitis with 1291 cases of viral hepatitis was reported from Baramati town, District Pune between 22nd March and 7th June, 2005. Four fatal cases included one pregnant woman. The most affected age groups were between 15 and 40 years (73.6%) and male to female ratio was 2.8: 1. Nine hundred and thirty two patients resided in Baramati town whereas, 359 lived in the surrounding rural areas and frequently traveled to the town. Water supply was found to have fecal contamination and leakages were found in water pipes which lay close to drains. Ten samples of patients which were initially referred by the local health authorities were IgM HEV positive. 415 serum samples and 82 stool samples of asymptomatic persons were taken to assess magnitude of subclinical infections. Of these, 16 people had elevated ALT and IgM anti HEV positivity. After 2 months blood samples of 32 people were available for follow up, and one person developed overt hepatitis, the remaining 15 had subclinical hepatitis E.

### 1.5.3 Ahmedabad, Gujarat

An outbreak of hepatitis was investigated in April 2005 as part of a team constituted by the Directorate General of Health Services (Emergency Medical Relief). Retrospective record analysis indicated 1848 viral hepatitis cases between the 1<sup>st</sup> January and 5<sup>th</sup> April 2005, the incidence was significantly higher than in the previous 2 years. There were 2 deaths. Although cases were noted in all parts of the city, 1485 patients resided in 7 maximally affected areas. The age group affected most was 15-40 years (63%) and male to female ratio was 2.3: 1. Patients presenting with symptoms of viral hepatitis in the OPD of the isolation hospital were examined. Blood samples of 43 patients (31 adults and 12 children) were collected. Detailed questionnaire was completed. Serological tests at NIV showed that 29/31 adults were IgM-anti HEV positive indicating recent hepatitis E. Ten of the 12 children tested positive for IgM-anti HAV, which was indicative of recent hepatitis A, all were IgM anti HEV negative. It can be concluded that the outbreak in adults was caused by hepatitis E virus and hepatitis A virus was mainly responsible for hepatitis in children.

### 1.5.4 Hyderabad, Andhra Pradesh

An epidemic of hepatitis was reported in the old city of Hyderabad, A.P. during April 2005. This was investigated as a part of central team. During the investigation, 40 acute serum samples from cases of hepatitis were collected. Twenty-eight of the 40 acute human serum samples were positive for anti-HEV IgM antibodies and 2 were positive for anti-HAV IgM antibodies.

### 1.6 Hand Foot and Mouth Disease (HFMD) in Calicut, Kerala

A total of 24 sera (12 acute and 12 convalescent) of patients of HFMD were received from Malabar institute of Medical sciences, Calicut, Kerala. All serum samples were analysed by RT-PCR for the detection of enteroviruses by using enterovirus common primers, CA-16 and EV-71 specific primers selected from VP-1 region. Only one sample showed positivity for CA-16 (251bp). None of the samples were found positive for EV-71 (VP-1). The preliminary phylogenetic analysis of CA-16 (VP1) positive sample sequence revealed 92% sequence homology with that of China and Taiwan strains. None of the convalescent phase serum samples were found to be positive for enteroviruses.

### 1.7 Conjunctivitis in Panchgani, Maharashtra

An outbreak of conjunctivitis occurred in Jan-Feb 2006 in a school from Panchgani, Satara district in Maharashtra. A total of 31 serum samples were collected in early convalescent phase from conjunctivitis patients. Paired eye swabs were also collected from 8 patients. The cluster included 28 students aged 10-18 yrs and 3 staff members. PCR was carried out for the detection of Adeno, Herpes Simplex Virus (HSV) and Enteroviruses in both serum and eye swabs. Suspensions of eye swabs were inoculated in Vero and HeLa cell cultures for virus isolation. ELISA for IgM, IgG were carried out for detection of Adeno and HSV antibodies in patients serum. Out of 8 sera tested, one was found positive for Adeno IgM and seven for IgG antibodies respectively. However, out of eight sera tested for HSV, four were found positive for IgM and four for IgG antibodies. No conclusive etiology was found.

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