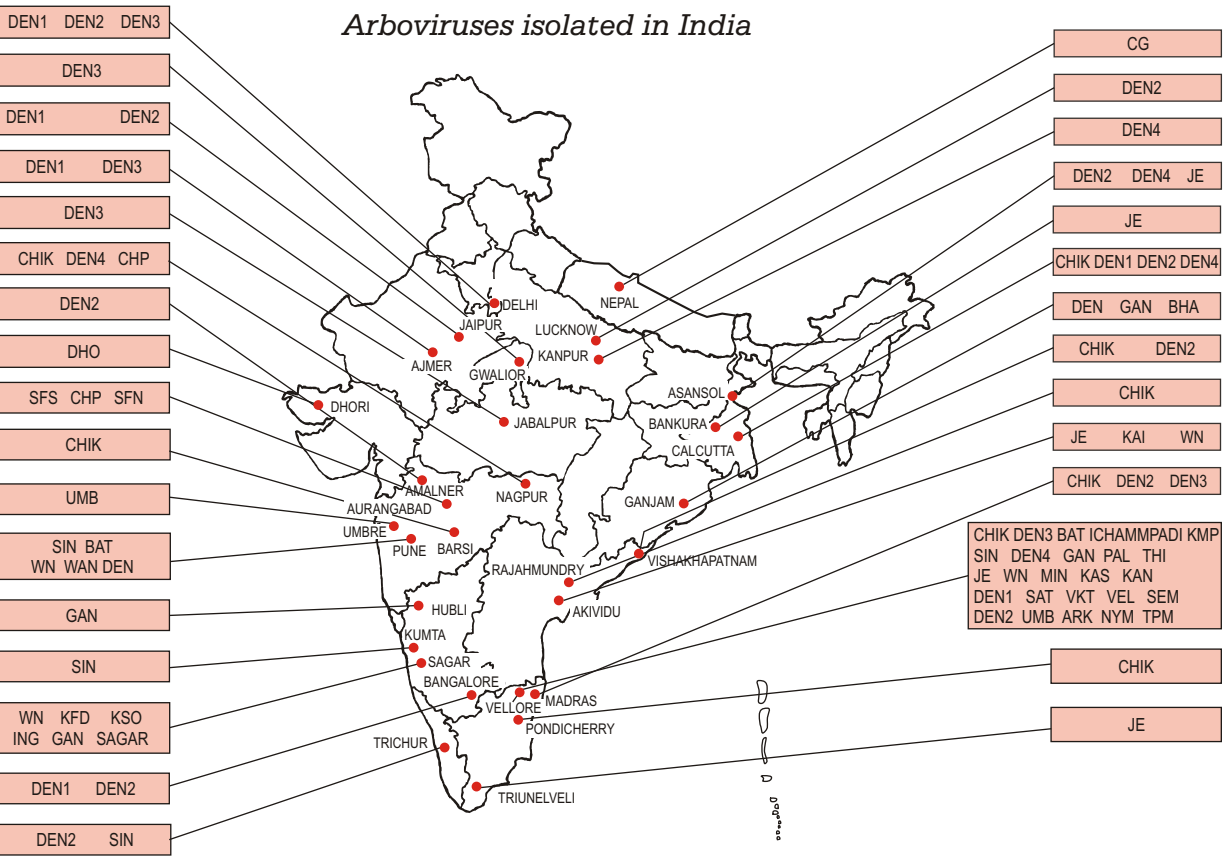


Other Viruses

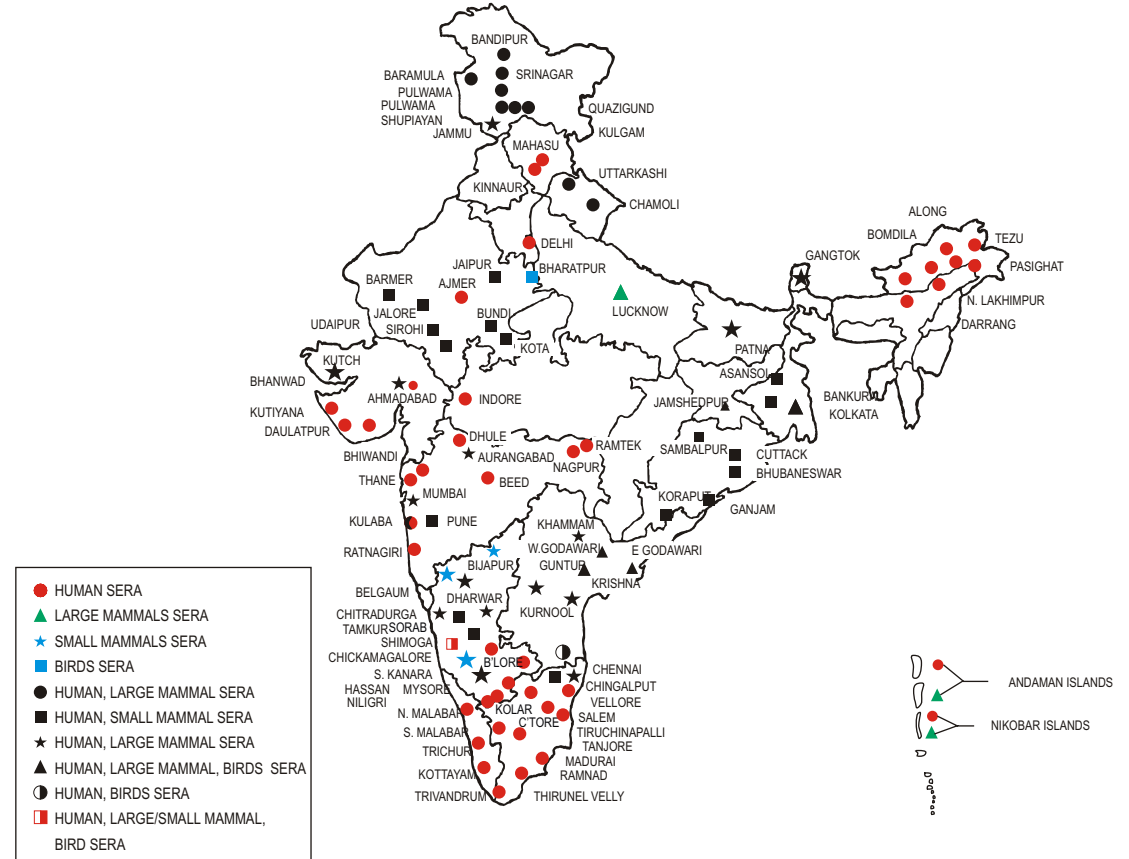
Arboviruses isolated in India



Abbreviations used

Abbreviation	Virus	Abbreviation	Virus
AHS	African Horse Sickness	KSO	Kaisodi
ARK	Arkonam	MIN	Minnal
BAT	Batai	NYM	Nyamanini
BHA	Bhanja	PAL	Palyam
CG	Chobar Gorge	Sagar	Sagar
CHIK	Chikungunya	SAT	Sathuperi
CHP	Chandipura	SEM	Sembalam
DEN-1 -4	Dengue type- 1 -4	SFN	Sandfly fever (Naples)
DHO	Dhori	SFS	Sandfly fever (Sicilian)
GAN	Ganjam	SIN	Sindbis
Ichampadi	Inchampadi	THI	Thimiri
ING	Ingwavuma	TPM	Thottapalayam
JE	Japanese encephalitis	UMB	Umbre
KAI	Kaikalur	VEL	Vellore
KAN	Kannamangalam	VKT	Venkatapuram
KAS	Kasba	WAN	Wanowrie
KFD	Kyasanur Forest Disease	WN	West Nile
KMP	Kammavanpettai		

Sero-surveys for viral diseases carried out in India



Human papilloma (HPV) and Epstein Barr (EBV) viruses

Human papilloma viruses are sexually transmitted agents, infect female genital tract and cause genital warts in external and internal genitalia. HPV type 16 and 18 are the high-risk types detected in invasive cancers and HPV 6 and 11 are the low-risk ones, generally detected in precancerous lesions of the cervix or in normal controls.

HPV has also been found to be associated in upper aero-digestive malignancies. EBV is considered as

cofactor associated with HPV in these malignancies.

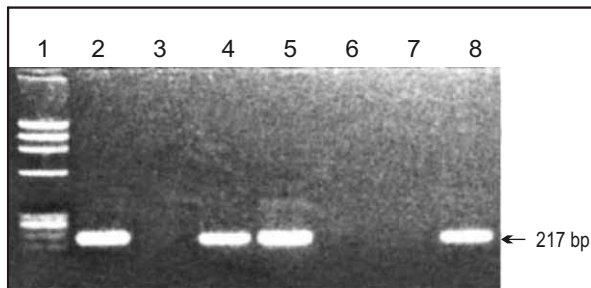
Oral cancers

Forty-five surgically-operated oral cancer tissues were obtained from local collaborating hospitals for detection and characterization of HPV and EBV DNA by PCR. A total of 40% (n=45) of samples were positive for HPV DNA. Of these, 31.2% were HPV type-16, 8.8% were HPV type-18. EBV was detected in 44.5% samples. Co-infections of HPV/EBV were also detected in 20% cases. Normal control showed 10-12% positivity for either of these viruses.

HIV-infected women with cervical abnormalities

During a study in collaboration with NARI, fifty cervical specimens collected from HIV-infected patients were subjected to detection of HPV genotypes by PCR. HPV type 16 and 18 were found in 14/50 (28%) specimens by PCR. These patients were further classified according to cervical abnormalities. HPV positivity was found in 2/14 in normal cytology group, 9/31 in inflammatory cytology group and 3/5 in abnormal cytology group.

PCR amplification of HPV-16 DNA in oral tumor specimens



Lane: 1 x DNA/Hae III
Lane: 2 positive control
Lane: 3 negative control
Lanes: 4,5,8 positive samples

Rift Valley Fever (RVF)

[RNA virus, family *Bunyaviridae*]

RVF is an important arthropod-borne zoonotic viral disease, primarily causing epizootics with high mortality in domestic animals, particularly sheep and goats. The disease is known to be prevalent in the countries of African continent and Middle East.

In India, the first evidence for the presence of this virus was obtained from a serological survey of sheep and goats,

conducted in Jodhpur, Bikaner and Barmer districts of Rajasthan state in 1990. About 10% (n=400) sera tested positive for HI antibodies to RVF virus.

In August-September 1994, an epizootic of febrile illness (morbidity 80%, mortality 20%) and abortions in sheep was reported from Veerapuram, Tamil Nadu. Based on clinical, histopathological and electron microscopic findings, a presumptive diagnosis of RVF-like illness was made. No virus could be isolated from autopsy specimens. Antibodies to RVF virus were detected in the convalescent sheep sera. Post-epizootic sero-survey of sheep, goats and humans from the affected areas also showed the presence of antibodies to RVF virus.

Wanowrie

(RNA virus, family *Bunyaviridae*)

Wanowrie virus was isolated from a tick species *Hyalomma marginatum*, collected from sheep near Pune. Another strain of this virus was isolated from mosquito *Cx.quinquefasciatus*. One strain of this virus has been isolated from brain specimen of an encephalitis case from Sri Lanka.

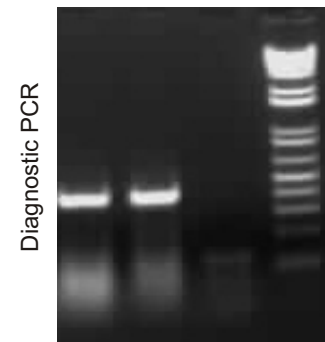
Antibodies have been shown in two percent goat sera from Orissa. About 12-15% human sera tested for complement-fixing antibodies against two strains of Wanowrie virus were positive, but all of them were negative in neutralization test.

Ganjam

[RNA virus, family *Bunyaviridae*]

Ganjam virus is antigenically similar to Nairobi sheep disease virus, which causes hemorrhagic enteritis with high mortality in sheep in Africa. Ganjam virus was isolated from *Haemaphysalis intermedia* ticks collected from goats in Orissa state, India. Another agent identical to the prototype strain of Ganjam virus was isolated from the mosquito *Cx.vishnui*.

A virus isolated from acute phase serum of a 12-year-old boy, who had fever, headache, listlessness, nausea and vomiting. This virus was found to be serologically identical to Ganjam virus. In an accidental laboratory infection with Ganjam virus, a 30-year-old technician developed fever, backache, joint pains and headache.



Antibodies have been demonstrated in human sera from Kashmir, Arunachal Pradesh and Tamil Nadu states. Sera from sheep and goats from Orissa, Gujarat, Karnataka and Kashmir tested positive for neutralising antibodies to Ganjam virus. Neutralizing antibodies were also detected in sera collected from

humans and sheep in Veerapuram, Tamil Nadu, during an epizootic of febrile illness in sheep in 1994.

Recently in 2004, six strains of the virus have been isolated from *Haemaphysalis* ticks, collected from apparently healthy cattle in Pune. The virus grows well in Vero cell line and can be identified by complement fixation and neutralization tests. PCR-based diagnosis was also standardized.

African Horse Sickness (AHS)

(RNA virus, family *Reoviridae*)

This is an insect-borne viral disease of equines with high mortality in southern and equatorial Africa. The disease is also known from North Africa, Spain, Middle Eastern countries, Pakistan and India.

In India, NIV investigated an outbreak among horses of a Cavalry unit in Jaipur, Rajasthan in 1960. The disease subsequently spread to different parts of India and over 20,000 animals died. The AHS type-8 virus was isolated. No outbreak has been reported since then.

Sandfly fever virus

(RNA Virus, family *Bunyaviridae*)

Sicilian strains of Sandfly fever virus were first isolated in India from blood samples of two patients, collected during an outbreak of febrile illness in Aurangabad, Maharashtra, in 1967. Subsequently, Naples strains were also isolated from the blood samples of four patients from the same area.

List of arboviruses of lesser importance isolated in India.

A number of them are new to science.

Viruses	Place of isolation	Source of isolation	Antibodies demonstrated
Batai	Tamil Nadu	Mosquitoes	Equines all over India
Sathuperi*	Tamil Nadu	Mosquitoes, Cattle	Humans negative
Ingwayuma	Tamil Nadu	Mosquitoes	Humans, Paddy Birds
Wad Medani	Tamil Nadu Karnataka	Ticks	-
Palyam*	Tamil Nadu	Mosquitoes	Humans negative
Kasba*	Tamil Nadu	Mosquitoes	Humans negative
Vellore*	Tamil Nadu	Mosquitoes	-
Sand fly fever Naples Type	Maharashtra	Phlebotomus spp	Humans, Cattle, Sheep
Sand fly fever Sicilian type	Maharashtra (Aurangabad)	Phlebotomus spp	Humans, from Maharashtra, Gujarat Humans, Monkeys, Equines, Cattle, Buffaloes, Sheep & Goats-All over India
Chandipura*	Maharashtra (Nagpur)	Humans and Sandflies	
Kaisodi*	Karnataka (Shimoga)	Ticks	Humans & small mammals negative
Umbre*	Maharashtra	Mosquitoes, Birds	Humans negative
Ganjam	Orissa, Karnataka, Tamil Nadu	Ticks, Humans & Mosquitoes	Sheep, Goats, and Humans from Kashmir, Orissa, Arunachal, Gujarat, Karnataka & Tamil Nadu
Barur*	Karnataka (Sagar)	Rat, Ticks	Humans
Chobar George*	Nepal	Ticks	-
Venkatapuram*	Tamil Nadu (Vellore)	Mosquitoes	-
Bhanja*	Orissa	Ticks, Man, sheep, Cattle, Rodents	Orissa, Gujarat, Karnataka, Maharashtra
Arkonam*	Tamil Nadu	Mosquitoes	-
Ichampadi*	Tamil Nadu	Mosquitoes	-
Kammav anpettai*	Tamil Nadu	Jungle Myna (Bird)	-
Kannamangalam*	Tamil Nadu	Crow (Bird)	-
Thottapalyam*	Tamil Nadu (Vellore) & Karnataka (Sagar)	Shrew	-
Dhori*	Gujarat	Ticks	Camel and Human sera positive, Sheep and Goat sera negative.
Sripur*	West Bengal	Sadfly spp.	-
Wanowrie*	Maharashtra	Ticks, Humans, Mosquitoes	Sera from Sheep and Goats from Orissa.
Thimiri*	South India	Birds	-
Kaikalur*	Andhra Pradesh	Mosquitoes	-
Nyamanini*	South India	Ticks, Birds, Rodents	Human, Goats, Monkeys
Minnal*	South India	Mosquitoes	-
Sembalam*	South India	Birds	-
Muroor*	Karnataka	Soft Ticks	-

* New viruses

Twenty more strains were isolated from *Phlebotomus* sandflies from Aurangabad district, Maharashtra. These included 11 Naples and 9 Sicilian strains.

Acute Haemorrhagic Conjunctivitis (AHC)

(RNA viruses, family *picornaviridae*)

AHC is a disease that affects the eyes and occurs in epidemic and pandemic forms. It causes occasional serious neurological complications. Two viruses enterovirus 70 (EV 70) and Coxsackie type A 24 (CA 24) cause AHC.

An epidemic of AHC occurred in Mumbai in the summer of 1971 and spread all over the country. NIV investigated this epidemic as well as the subsequent epidemics during 1975, 1979 and 1986 (CA-24); and 1981, 1992 and 1994 (EV-70). Both these viruses were isolated. Neurological complications in some cases following AHC outbreaks have been reported from Pune, Mumbai and Vellore.

Buffalopox (BP)

(DNA virus, family *Poxviridae*)

Buffalopox virus belongs to vaccinia subgroup of poxviruses. Outbreaks of BP have been reported since last 5-6 decades and continue to occur. In milch buffaloes and cows, lesions are seen on teats and udder. In milkmen, it causes fever followed by lesions on the hands

and axillary lymphadenopathy. Although the economic losses to dairy industry are considerable, no fatality in animals or humans has been reported.

During 1992-94, ten virus isolates from the skin scabs (3 human and 7 buffalo) were obtained from Jalgaon and Dhule districts of Maharashtra state.

Experiments conducted in chick embryos, albino rabbits and infant mice; and electron microscopic observations revealed a pattern of morphogenesis similar to that of vaccinia virus, except that the extracellular forms of the virus were devoid of well-defined lateral bodies. Neutralizing antibodies to BP virus were detected in all the affected individuals and in 70% of their contacts. Intra-nasal instillation of BP virus in suckling mice produced fatal disease. This model can be explored for studying pathogenesis of BP.

Buffalo pox lesions observed in animals and human contacts



Forearm of women

Teats of Buffalo

Rickettsioses

Rickettsial diseases have been known to occur in India since the early 19th century. During the Second World War

scrub typhus was a major war disease along the Indo-Burma border. It made its appearance once again during the border conflict with Pakistan in 1965.

Since 1970, NIV has carried out extensive serological surveys of humans and domestic animals in different states for several years to determine the distribution and prevalence of antibodies to various rickettsial agents.

- Prevalence of antibodies to all the species of Typhus group of Rickettsia were detected.
- Antibodies to epidemic typhus, murine typhus, Indian tick typhus, scrub typhus and Q fever were detected in 5-20 per cent of sera.
- Antibodies to Q fever were widely prevalent in humans and domestic animals, particularly cattle, sheep and goats.
- Several rickettsial species were isolated from rodents and their ectoparasites and cases of pyrexia of unknown origin.
- Experimentally-infected guinea pigs with *C.burnetii* showed loss in body weight, pyrexia and depletion of hepatic glycogen.
- Changes in serum enzyme levels of ALT, AST, LDH and creatine phosphokinase were also observed. The role of lipopolysaccharide isolated from *C.burnetii* in the causation of Q fever was demonstrated.

- *In vitro* cultivation of *C.burnetii* in established cell lines was achieved. This facilitated development of ELISA.
- Xenodiagnosis has been established for rickettsial infections.

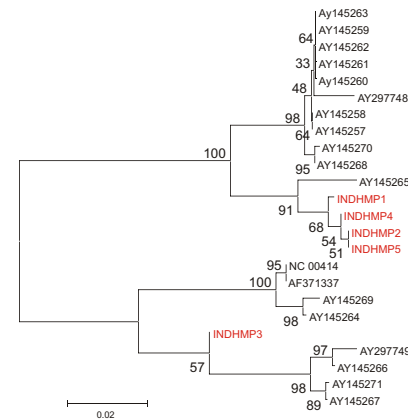
Human Metapneumo Virus (HMPV)

In 2001, the discovery of human metapneumovirus (HMPV) in respiratory patients from the Netherlands was reported. The disease spectrum ranged from mild upper respiratory tract disease to severe bronchiolitis and pneumonia.

HMPV has been recognized as a significant cause of acute respiratory illness (ARI) in infants, children and adults. Though respiratory diseases are highly prevalent in India, the role of HMPV had not been examined.

In 2003, specimens from 26 pediatric (17 severe and 9 mild ARI cases) and 18 mild adult ARI cases from Pune were screened in nested RT PCR. Among severe cases 3/17 and among mild cases 2/9 were scored positive. Importantly, 4/5 HMPV RNA positives were children below the age of one year. M gene-based phylogenetic analysis demonstrated co-circulation of distinctly different HMPV strains in Pune. The 4 isolates belonging to a lineage represented both mild and severe cases, whereas the only isolate belonging to the other lineage came from a severe case.

First detection of human metapneumo virus in India, in children with acute respiratory infection.



Phylogenetic analysis of Indian HMPV isolates

AFP surveillance for polio eradication program

NIV Field Station at Bangalore is one of the eight National Polio Laboratories in India under the WHO-SEAR Polio Lab Network of WHO's Global Eradication of Poliomyelitis Program since June 1997. It caters to surveillance of Acute Flaccid Paralysis (AFP) in Karnataka state.

Role of the National Polio Laboratories is to process stool specimens received from the program for virus isolation and to make primary identification of polio viruses, as per program guidelines and procedures. All polio isolates are sent to the Regional Reference Laboratory (ERC, Mumbai) at least 80% within 7 days of reporting for intratypic, i.e., wild or vaccine virus differentiation. Results of intratypic differentiation are also sent to the program during weekly report and any wild-type isolation is reported immediately.

All laboratories are subjected to Annual WHO-Accreditation process, since the program requires specimens to be processed in the WHO-Accredited Laboratories only. NIV Field Station, Bangalore, is fully accredited laboratory.

AFP Surveillance data is available from June 1997 to date.

AFP SURVEILLANCE AT NIV FIELD STATION, BANGALORE
Virus isolations from AFP cases

YEAR	AFP cases tested	P1 WILD	P3 WILD	POLIO vaccine	NPEV only	NEGATIVE
1997*	164	97	5	6	16	40
1998	352	74	4	10	59	205
1999	356	18	3	23	68	244
2000	333	7	1	23	73	229
2001	346	0	0	15	119	212
2002	332	0	0	16	94	223
2003	449	40	0	30	122	257

*1997-June to Dec