Vector Surveillance
Entomological & Vector Control Aspects

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VECTOR SURVEILLANCE

The last 3 decades has witnessed the emergence , resurgence or spread of vector-borne diseases like malaria, filariasis, Japanese encephalitis, Dengue/DHF, Kala-azar, plague in various parts of the country. Amongst the various reasons attributed contributing for the rising trend of vector-borne diseases, the inadequacy or lack of entomological surveillance is of paramount importance.

In view of the above it was thought worthwhile to gear up/strengthen entomological surveillance activities at various levels viz. District, Zonal, Regional, State and Central level to collect meaningful entomological data in respect of existing vector-borne diseases prevalent in the district and about the receptivity of the area for other vector-borne diseases. Some of the important characteristics of the vectors of various vector-borne diseases, sampling techniques, identification keys, techniques used for the incriminatioin of vector species for pathogens/parasites, WHO techniques to ascertain the insecticide susceptibility status of adults and immature stages and vector control measures used viz. personal prophylactic, source reduction, environmental management, biological control, chemical control and integrated measures used for the prevention and control of vector-borne diseases on long and short term basis are summarised below:

Entomological aspects

Most of vector-borne diseases prevalent in the country are transmitted by animals from 3 Classes viz. Insecta, Crustacea and Arachnida belonging to Phylum - Arthropoda (Arthros = Jointed, podos = legs). Members belonging to these Classes can easily be differentiated on the basis of following characters:

Class	Insecta	Crustacea	Arachnida
Body	Divisible into head,thorax and abdomen	Divisible into cephalothorax and abdomen	Undifferentiated
Legs	3 pairs	5 pairs	4 pairs
Antennae	1 pair	2 pairs	Absent
Wings	Present	Absent	Absent

1. Mosquitoes- Vectors of malaria, Dengue, filariasis and J.E.

Mosquitoes are worldwide in distribution. There are about 3450 species and subspecies of mosquitoes belonging to 38 genera in Family - Culicidae. In India there are about 300 species of mosquitoes.

Mosquitoes may be easily differentiated from other insects of similar shape and size on the basis of following characteristics:

i) Insects with a single pair of mesothoracic fore wings, and the hind pairs of wings are modified into halteres

ii) Presence of forwardly projecting proboscis with piercing and sucking types of mouth parts

iii) Presence of scales on the thorax, abdomen, legs and wing veins

iv) Presence of fringe scales on the posterior margin of the wings

v) Characteristic wing venation i.e. wing vein 2nd, 4th and 5th bifurcated

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1.1 Morphology of mosquito -

Mosquitoes are slender bodied , small insects measuring about 3-6 mm. in length. However, some spp. may be as small as 2 mm, while others may be as long as 19 mm (*Toxorhynchites*). The body is distinctly divided into head, thorax and abdomen. Mosquitoes possess only one pair of functional mesothoracic forewings. The hind pair of wings are represented by a pair of small knob-like structures, the halteres, which are the balancing organ while mosquito is flying.

The head bears a pair of large kidney-shaped compound eyes and a pair of filamentous and segmented antennae. In females the antennae have whorls of short hairs (Pilose) whereas in males the antennae bear a whorl of long hairs giving them feathery appearance (Plumose). Just below the antennae, head bear a pair of maxillary palpi which may be short or long and dilated or pointed at their tips depending upon the sex and whether adults are anophelines or culicines. Arising between the palpi is a single elongated structure, the proboscis, which contains the piercing and sucking types of stylets or mouth parts. The largest component of the mouth parts is a long and flexible gutter - shaped labium which terminates in a pair of small lobe like structure called labella which are sensory in nature. The labium almost encircle all the other mouth parts and serves as a protective sheath.

The upper most structure, the labrum is slender, pointed and grooved along its ventral surface. In between the labrum and labium are five needle like structures viz . a pair of toothed maxillae, a pair of serrated mandibles, a single hollow stylet, the hypopharynx.

At the time of taking blood meal, the tips of flashy labium are placed on the skin and curves backwards. This allows the paired mandibles and maxillae, labrum and hypopharynx to penetrate the host skin. Saliva is pumped into the host body through the hypopharynx. Blood is ingested by females mosquito through the pumping action of the pharynx.

The male mosquitoes are incapable of taking blood meals as the maxillae and mandibles are vestigeal and feed on plant saps or nectar.

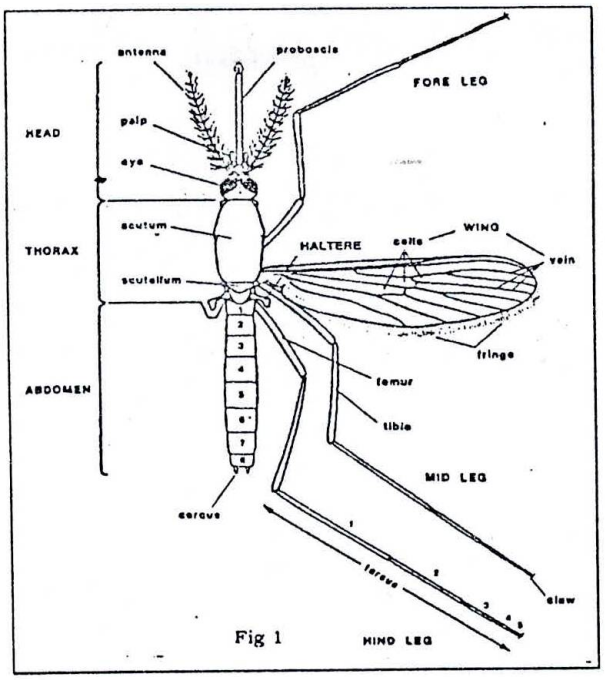
The thorax is covered dorsally and laterally with scales which may be dull or shiny, white, brown, black in colour. The arrangement of scales on the dorsal surface of the thorax helps in the identification of some species of mosquitoes (*Aedes* spp.)

The wings are long and relatively narrow, the number and arrangements of the wing veins is almost the same in all mosquito spp.The veins are covered with scales which are usually brown, black, white, creamy or yellow in colour. The shape of the scales and pattern of their distribution varies in different genera and species of mosquitoes. While sitting the wings of the mosquitoes are placed across each other over the abdomen in the form of a closed scissor. There are three pairs of tiny, elongated legs which are covered with scales. The tarsus usually terminates in a pair of toothed or simple claws. Some genera such as *Culex* have a pair of small fleshy pulvilli at the end of tarsus.

The abdomen consists of 10 segments but only the first 7 or 8 are visible.

In sub-family Culicinae, the abdomen is usually covered dorsally and ventrally with brown, black or white scales.

The last abdominal segment of the female mosquito terminates in a pair of small finger like structure called cerci, whereas in males it terminates in a pair of prominent clapers, which is a part of male gentalia. (Fig : 1)



Diagrammatic representation of a female adult mosquito

1.2 <u>Anopheline Mosquitoes</u> :- Anopheles species are mainly responsible for the transmission of malaria in various parts of the world. Out of 55 anopheline species prevalent in India , 9 are vectors of malaria viz. *Anopheles annularis, A. stephensi, A. Philippinensis, A. sundaicus, A. minimus, A. varuna, A. culicifacies, A.fluviatilis* and *A. dirus.*

i) <u>Habits</u> :- They are commonly found in large numbers in human habitations, animal shelters or in mixed dwellings. Anopheline mosquitoes have also been found resting in outdoor situations on banks of stream , under culverts, and in thick shrub, forest etc.

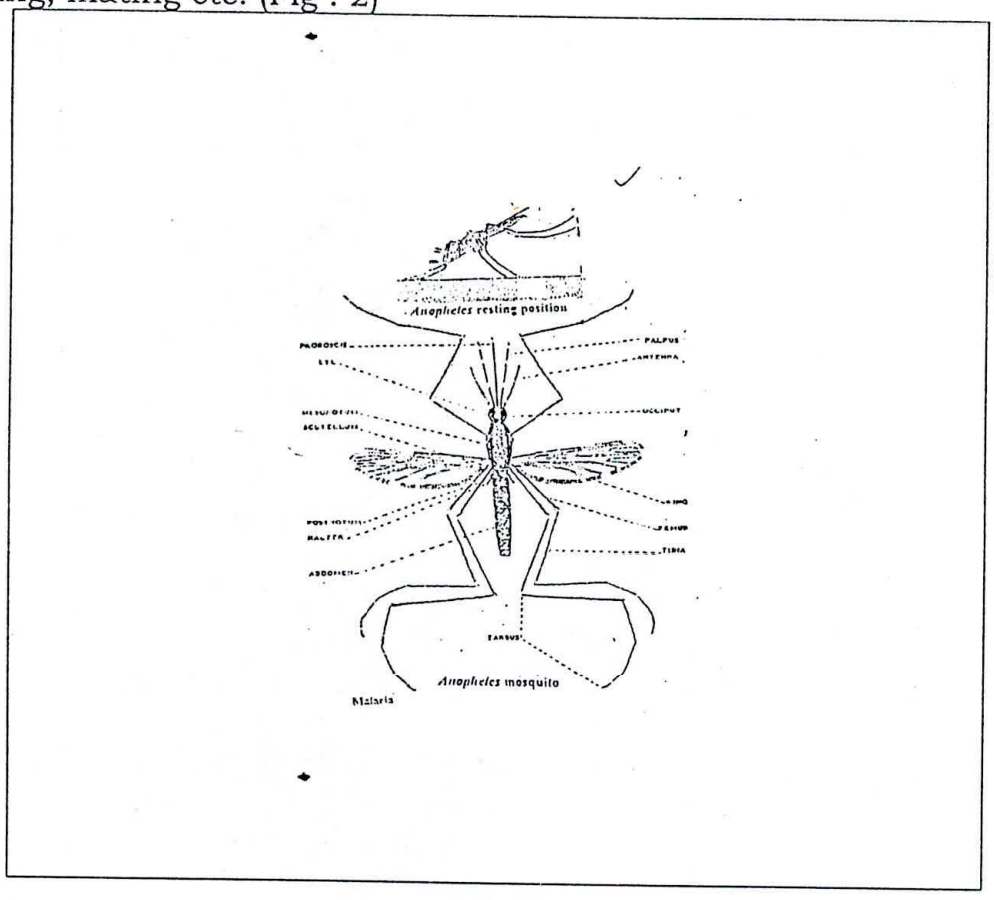
ii) <u>Breeding habits</u> :- The eggs are deposited singly and generally laid on the surface of clean and unpolluted water such as pools, rice fields, slow running streams, cisterns, overhead tanks, tree holes etc. Some species prefer standing types of water like ponds, wells, irrigation cannals, pits and also breed in various types of rain water collections.

iii) <u>Resting habits</u> :- Most of the anopheline mosquitoes are domesticated and they are found in very large number in indoor situations; in cattlesheds and human habitations. However some of the species like *A. dirus* is an outdoor rester (Exophilic). Males are usually found near the breeding places.

iv) <u>Biting and feeding habits</u> :- Male mosquitoes can not suck blood and normally feed on nectar and plant juices. Female mosquitoes are able to pierce the host skin and feed on blood. The great majority of species are zoophagic i.e. they feed on the blood of mammals, reptiles, birds, and

amphibians but some of the species like *A. fluviatilis* and *A. minimus* have got definite preference for human blood.

v) Flying habits:- Most domestic species of mosquitoes remain in close vicinity of about 1 km. of human or cattles dwellings, however, creeping movement of mosquitoes takes place on account of oviposition, feeding, swarming, mating etc. (Fig : 2)



1.3 Culicine Mosquitoes :- The important human diseases transmitted by culicine mosquitoes are **Filariasis** by *Culex quinquefasciatus* and *Mansonia* species, **Japanese encephalitis** by *Culex vishnui* group and **Dengue/Dengue haemorrhagic fever** by *Aedes* mosquitoes.

i) Habitat:- Culicine mosquitoes prefer dark places and live in human dwellings, cattlesheds and other such shelters. They may also live in outdoor situations in shrubs, grasses, forests etc.

ii) Breeding habits:- Most of the culicine mosquitoes lay their eggs in organically polluted water. The eggs are also laid in unkept drains and unused wells. *Aedes* mosquitoes generally prefer artificial breeding places

such as earthen pots, cement tanks, glass or plastic containers, tyres, coolers, in small collections of water in man made containers. *Mansonia* species breeds in water organically polluted and habouring aquatic plants like Pistia spp., water hyacinth, lemna etc. The larvae and pupae of *Mansonia* mosquitoes remain attached to the roots of these water plants through their respiratory siphon to take oxygen. Eggs are laid on the under surface of leaves of these plants.

iii) Resting habits:- *Culex* and *Aedes* are found in human dwellings or cattlesheds. *Mansonia* species mainly rest in cattlesheds and human dwellings but may also rest outdoor.

iv) Biting and feeding habits:- *Culex* mosquitoes are zoophagous and anthropophagous . *Mansonia* species prefers human blood. Only *Aedes* mosquitoes bite during day time and feed mainly on human blood.

v) Flying habit:- *Culex* and *Mansonia* species of mosquitoes can fly upto a long distance of 4 to 5 kms., while *Aedes* mosquitoes have very limited flight range of about 100 meters and remain near the host. (Fig : 3,4,5)

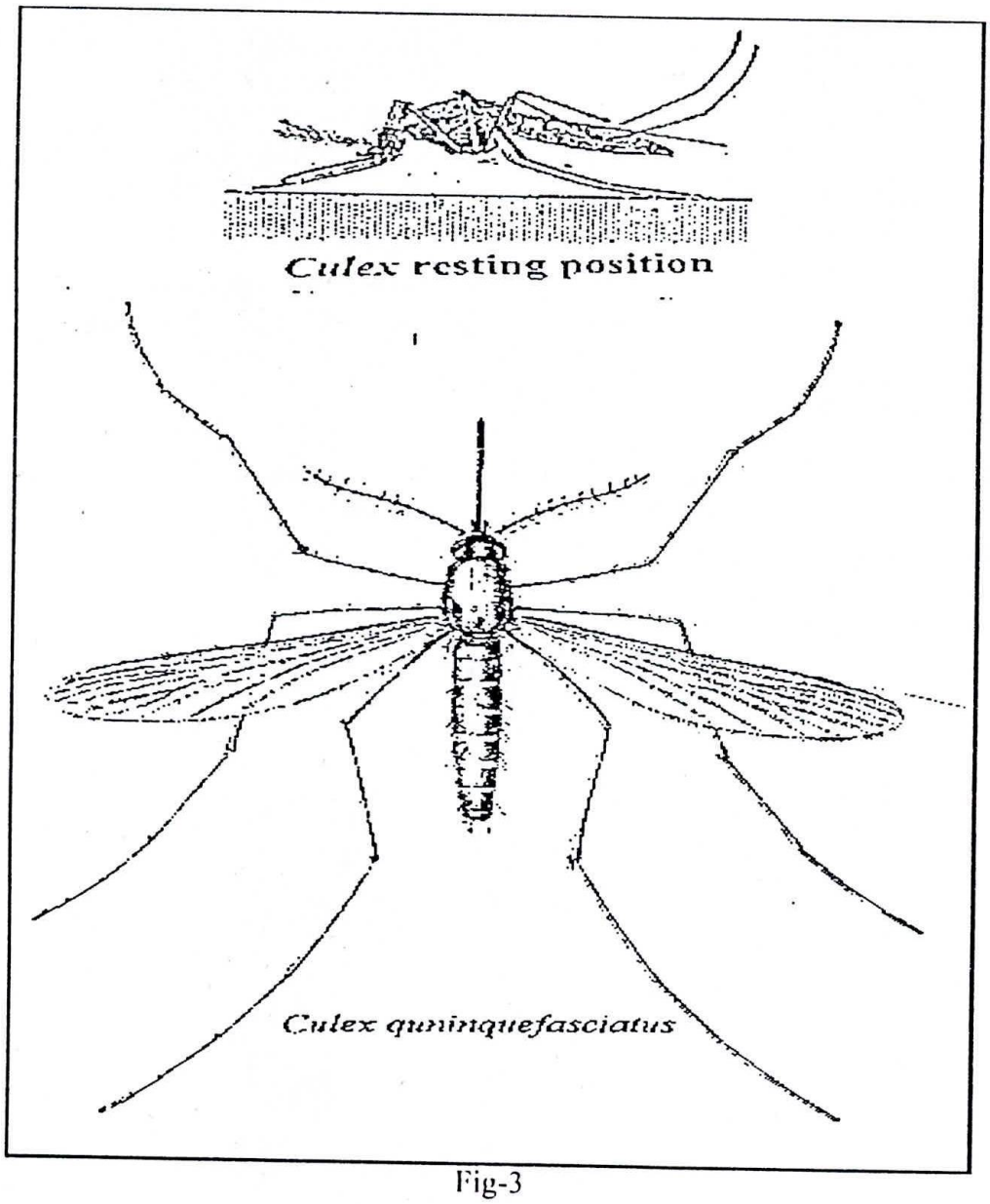


Fig-3

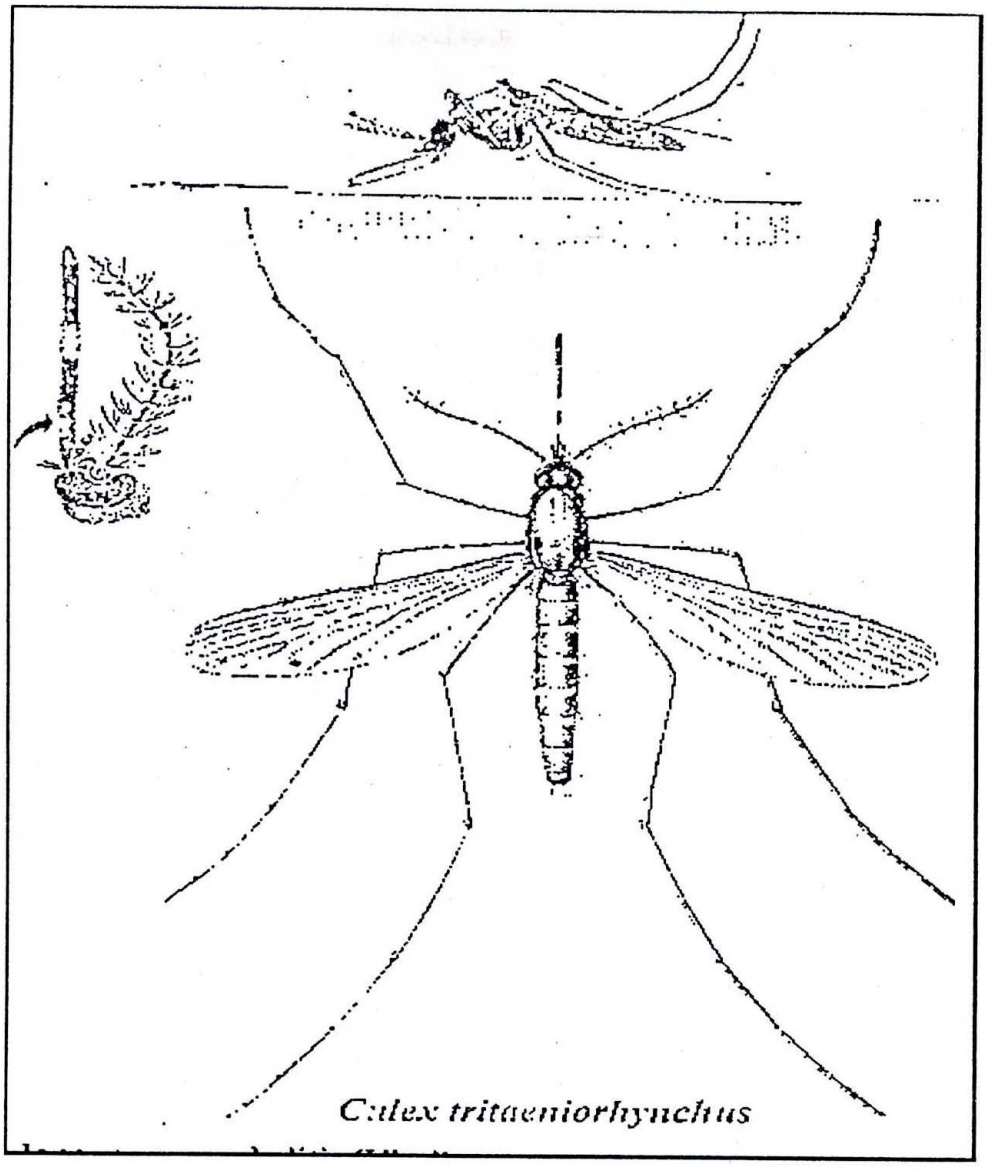
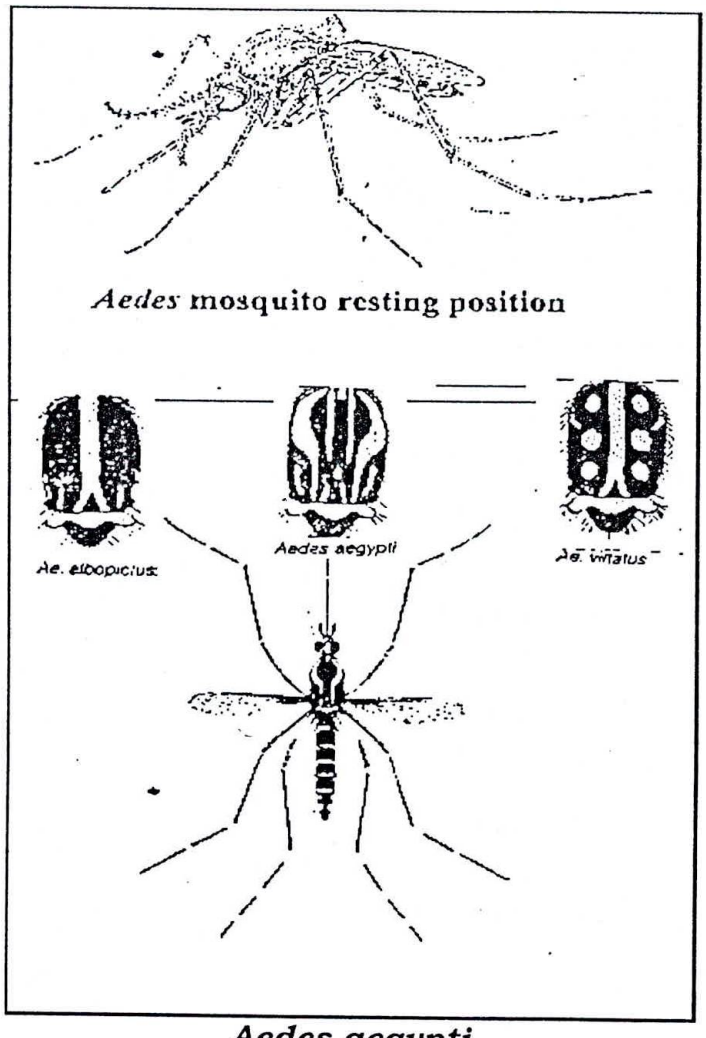


Fig-4

Japanese encephalitis (Viral)

a) Principal vectors	*Cx.tritaenirohynchus, Cx.vishuni and Cx.pseudovishnui*
b) Suspected vectors	*Cx.shitmorei, Cx.epidesmus, Cx.gelidus, A.barbirostris, A.hyrcanus, A.subpictus*



Aedes aegypti
Dengue/DHF (viral)

Fig.- 5

1.4 Life cycle

There are four stages in the life cycle of mosquitoes viz. egg, larva, pupa and adult. The first three stages are aquatic and adult stage is aerial/terrestrial

i) Egg

Anophelines generally lay their eggs singly in clean, oxygenated water. Each egg is boat-shaped in appearance and has distinct float on either side. The number of eggs laid by a single female varies from 40-150.

Culex mosquito lay eggs in the form of egg raft. Each egg raft may contain 150-400 eggs. *C. quinquefasciatus*, vector of filariasis lay eggs in organically polluted water, whereas, *Culex vishnui* group of mosquitoes, vectors of Japanese encephalitis lay egg rafts in the paddy fields, swampy and marshy areas.

The *Aedes* mosquitoes lay eggs in artificial man made containers containing fresh water. The eggs are laid singly and a female may lay 60-150 eggs in one oviposition. The eggs of *Aedes* mosquito can withstand dessication upto 1 year and hatch when containers are inundated with rain water.

The freshly laid eggs are white in colour but within half to one hour of egg laying colour changes to black. The incubation period of egg stage is about 2 days duration under favourable climatic conditions (Tem. 27 C and R.H.- 75-80%) .

ii) Larva

The larva feeds voraciously on minute algae and other plankton present in the water and grows in size. As a result of feeding and growth, the outer skin is shed and next larval stage comes out. There are four larval stages in the life cycle and after third moulting, the larva changes into pupal stage. The larval period last for 6-8 days under favourable climatic conditions.

iii) Pupa

The pupa is coma-shaped in appearance. The head and thorax are fused to form cephalothorax and the abdomen is curved. It is the resting stage in the life cycle and does not take any food. The pupa is very active and sensitive. It moves away, if disturbed. During this stage the future part of the adult mosquito is formed inside the pupa.
The pupal period last for 2 days under favourable climatic conditions.

iv) Adult

The chitinous cuticle of cephalothorax of the pupa breaks in between the respiratory trumphets and through this opening the adult mosquito emerges out. On emergence, the adult mosquito sits on the empty pupal skin or on adjoining vegetations for sometime to harden its body part after which it flies away for mating, feeding and resting.

CHARACTERISTICS OF ANOPHELINES AND CULICINES

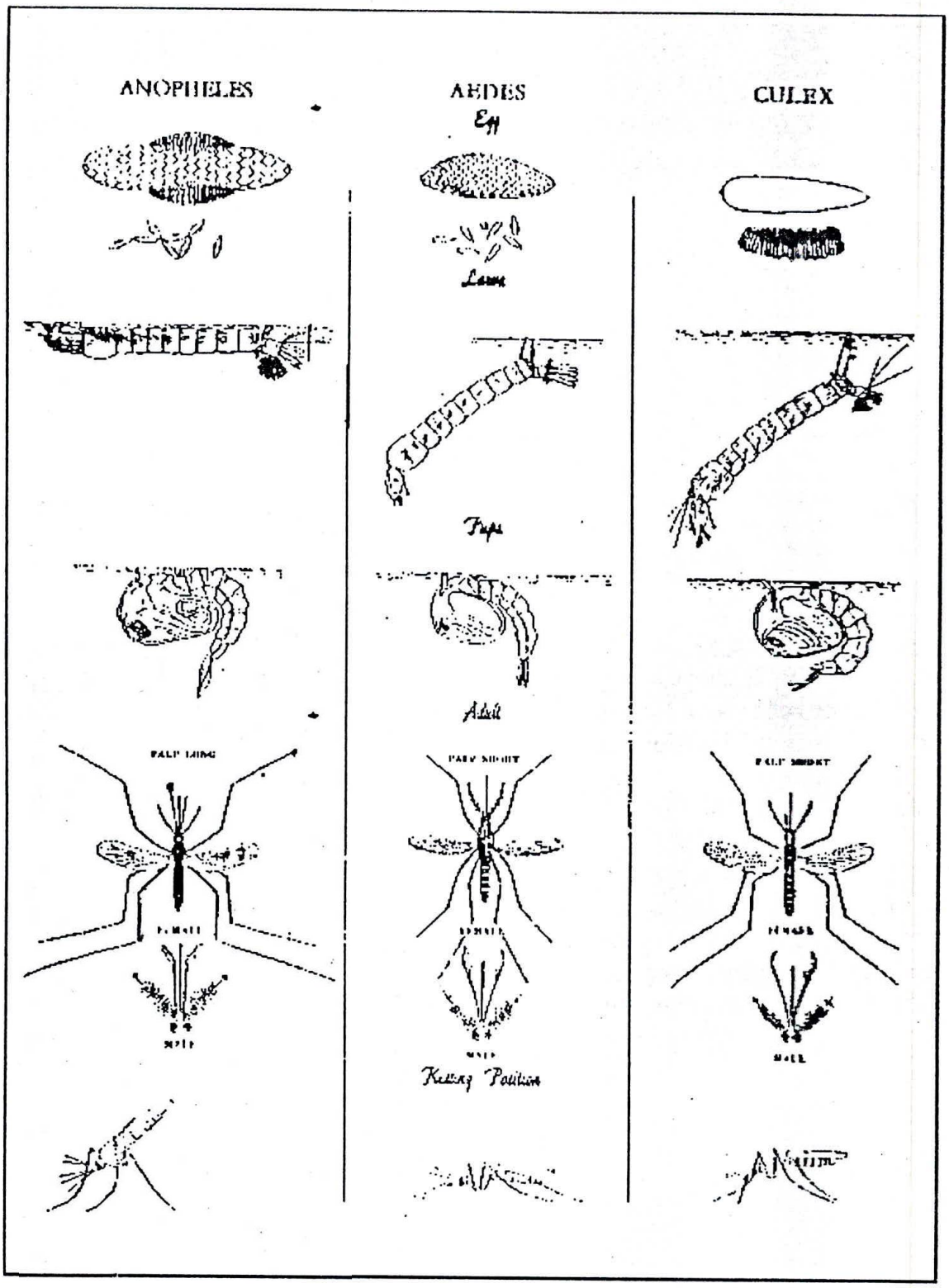


Fig. - 6

6-10 AH monitoring

Anopheles - diff Habitats

1.6 Methods for estimating the adult and larval mosquito density and for collecting information on other entomological parameters

A. Adult mosquito density

Habitat indices > 10 at risk campaign.

i) Per Man Hour Density (PMHD)

10 per man hour - Final density

$$\frac{\text{No. of mosquitoes collected}}{\text{No. of hours spent in search}}$$

(by an expert mosquito Technician)

ii) Total catch by pyrethrum space spray

Total catch by pyrethrum space spray in a unit area may be undertaken by spraying 0.1-0.2% pyrethrum extract @ 30-60 ml/1000 cubic feet.

iii) Man-mosquito contact - Mosquito biting/landing rate on human or animal bait may be determined by counting the number of mosquitoes collected while landing or biting on human or animal bait per unit time or per night.

iv) Sporozoite rate in malaria vectors

$$\frac{\text{No. of females anopheline species positive for sporozoites}}{\text{No. of mosquitoes dissected}} \times 100$$

v) Vector susceptibility to insecticides

As per standard WHO technique (Details enclosed)

vi) Parity rate

Simple detection of parous (a female which has laid eggs) females on the basis of tracheal skin or ovariole dilation.

$$\frac{\text{No. of parous females encountered}}{\text{No. of female mosquito dissected}} \times 100$$

Transovarial vertical transmission in mosquitoes

vii) Wall cage Test (Contact Bioassay test)

For studying the residual efficacy of residual insecticides under field condition.

Percent mortality obtained after 24 hours amongst mosquitoes exposed to insecticide sprayed surface for 15 minutes in plastic cones on insecticide treated surface.

viii) Collection of mosquitoes by using various traps viz. Magoon, window, light trap etc.

No. of mosquitoes collected species wise/ night/trap

ix) Anthropophilic index

Percentage of mosquito blood meal found positive for human blood

B. Larval density

a) **Collection of immature stages of mosquitoes**

For larval collections breeding habitats of the vector species may be searched at any time during the day. Mosquito larvae are collected by the following methods:

i) **Dipping** : Most frequently used method for collecting mosquito larvae from edges of swamps, ditches, rice fields and other water bodies. Equipments generally used are laddle, photo-tray, spoon etc. Larval density is calculated in terms of average number of larvae collected per dip.

ii) **Netting** : Larvae can be collected from large water bodies or streams by long handled larval net and from wells using well nets. Larval density is calculated per net.

iii) **Pipetting** : Small pipettes can be used to collect mosquito larvae from shallow breeding places like hoof prints, plant axils, tree holes etc.

b) Larval Density per dip = No. of larvae collected
(laddle, larval net, well net) No. of dips taken

For *Aedes aegypti*

i) Container index - No. of containers found positive x 100
No. of containers examined

ii) House index - No. of houses found positive x 100
No. of houses searched

iii) Breteau index - No. of containers found positive x 100
No. of houses searched

C.) Precauations to be taken while collecting mosquito larvae by dipper

1. The enemel bowl, frying pan, ladle should be immersed in the breeding places at an angle of 45^0. The surface water will flow into the cavity but care should be taken not to fill this completely as otherwise some larvae will be washed out.

2. When the surface of the water is covered with dense floating vegetation or organic debris, the water surface should be agitated to cause the larvae to sink, clear away the vegetation and then wait for 3-5 minutes for larvae to come to the surface once more.

d) Precautions to be taken while using larval net

Larvae may be collected from large stretches of water along the edge of streams, wells and other situations using a larval net of 20-25 cms. diameter. When collecting larvae, the net is held at an angle and skimmed rapidly through the surface near emerging or floating vegetation or pushed along very slowly allowing the surface water to float into the net. Alternatively, the net may be used as a ladle, a series of quick dips being made. The net is inverted and washed out in a bowl of water and the larvae are collected with a pipette.

e) Precautions to be taken while using the well net

The well net is dipped slowly into the well keeping half the border above the water. After waiting 2-3 minutes to allow the disturbed larvae to return to the water surface, the net is dragged slowly and as quietly as possible around the edge of the well keeping the net at the initial depth. When the net has been moved around the border of the well two or three times it is withdrawn and inverted in a white enamel basin containing water. Wait for 2-3 minutes then repeat. The larvae are collected with a pipette .

f) Precautions to be taken while collecting adult mosquitoes in indoor situations

Before collecting mosquitoes from human dwellings, cattle sheds, mixed dwellings etc., one should thoroughly inspect the areas for the presence of snakes, scorpions, centipedes etc. to prevent any mishappening

1.7 Susceptibililty status of vector Mosquitoes

i) Malaria vectors

An. culicifacies and *An. stephensi*, major vectors of malaria are resistant to DDT and HCH in most part of the country. In Maharashtra, Gujarat and certain parts of Haryana triple resistance against DDT, HCH and Malathion has been reported.

The other malaria vectors except *An. annularis* have been reported to be susceptible to conventional insecticides used under NMEP.

ii) Dengue/DHF Vectors

Aedes aegypti mosquito is resistant to DDT and Dieldrin but susceptible to organophosphates and synthetic pyrethroids.

iii) J.E. Vectors

The major J.E. vectors have developed resistance against organochlorine insecticides (DDT, Dieldrin) but are reported to be susceptible to organophosphates and synthetic pyrethroids.

iv) Filariasis Vectors

Cx. quinquefasciatus, vector of Bancroftian filariasis is resistant to most of the organochlorine and organophosphate compounds but susceptible to synthetic pyrethroids.

1.8 Determination of susceptibility test

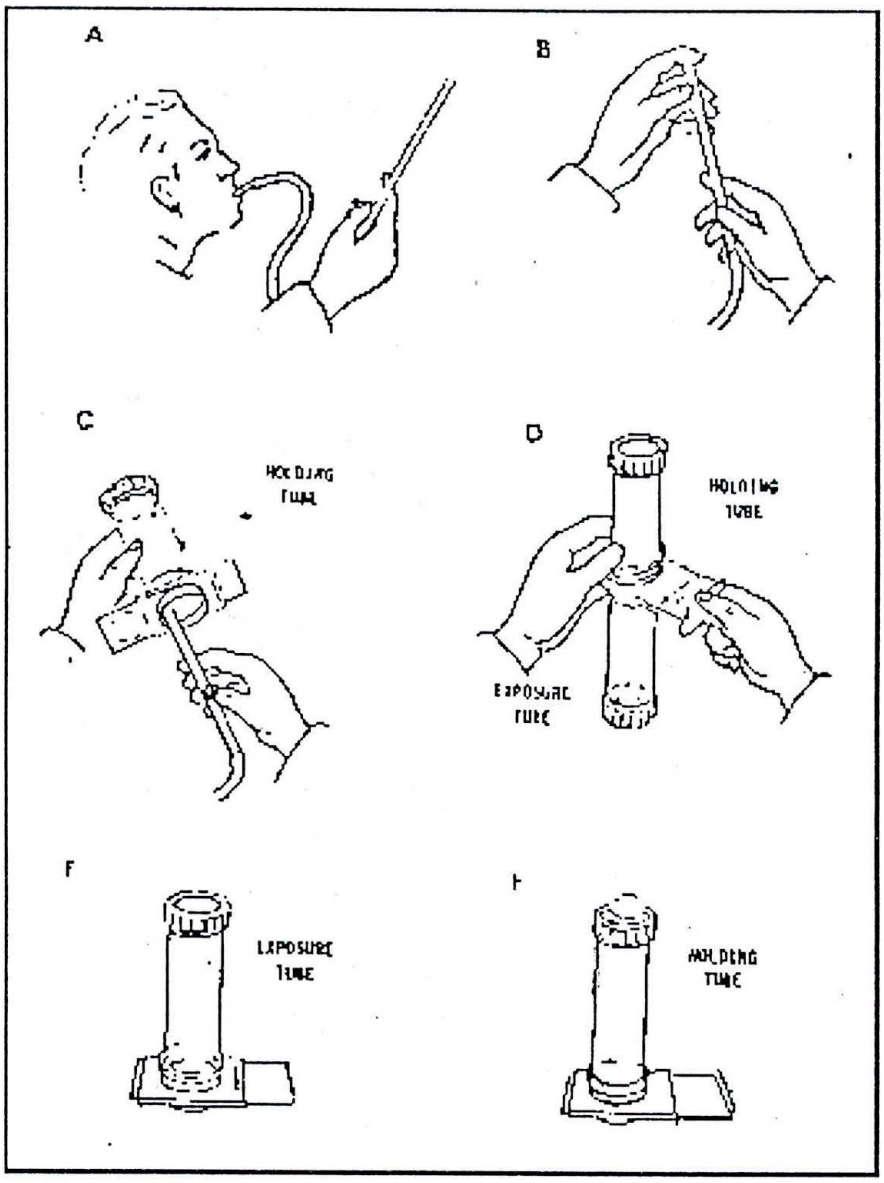
A. Adults

Susceptibility tests of adult mosquitoes are carried out at six monthly interval to ascertain the current susceptibility status of vectors against various insecticides being used under public health programme so that appropriate insecticide may be used for effective vector control.

Freshly fed female mosquitoes collected from the study area are kept under laboratory conditions and the healthy mosquitoes are exposed for a period of one hour and mortality count is made after 24 hrs. as per the WHO method.

Equipments, material and method used to determine the insecticide susceptibililty status of mosquitoes

Equipments/material required:



(Fig:7)

Composition of WHO Test Kit :

(i) - 20 plastic tubes - 125 mm length and 44 mm diameter.

- 8 tubes with red dot - exposure tube
- 2 tubes with green dot - control tube
- 10 tubes with green dot-holding tubes

(ii) - 10 slide-units with a screw cap on either side and provided with a 20 mm filling hole

(iii) - Insecticide impregnated papers

(iv) - Sheet of plain paper for lining of holding tubes

(v) - 20 spring wire clips (8 copper clips for exposure tubes and 12 silver clips for holding and control tubes)

(vi) - Glass aspirator tube

(vii) - Adhesive tape, log probit paper

(viii - Impregnated papers Organochlorine /organphosphate / carbamates /synthetic pyrethroid

Methodology

- Insert a piece of white paper in each holding tube and put a silver spring wire clip to keep the paper in position.

- Now put 15-25 mosquitoes per tube in each holding tube with the help of sucking tube through filling hole in sliding unit.

- Keep the holding tubes in upright position for 1 hour and damaged mosquitoes should be removed

- In exposure tubes put insecticide impregnated papers of different concentrations and one control paper impregnated with oil (solvent). To keep the paper in position use copper spring wire clip.

- Now transfer mosquitoes in exposure tubes/control tube from holding tubes with the help of sliding unit.

- Leave the exposure tubes standing upright with screen end up for 1 hour

- At the end of exposure period, transfer the mosquitoes to holding tubes. A small cotton pad soaked in glucose should be kept at the top of screen.

- Keep the holding tubes for 24 hours in a place with diffuse light, temperature , 25 + 5^{0}C and R.H. 70-80%.

- Mortality counts are made after 24 hours. For each concentration at least four replicates should be used

- If control mortality is between 5-20 per cent it can be corrected by Abott's formula. The tests with control mortality more than 20 per cent are unsatisfactory and should be repeated.

$$\textbf{Abotts's formula} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

General Remarks

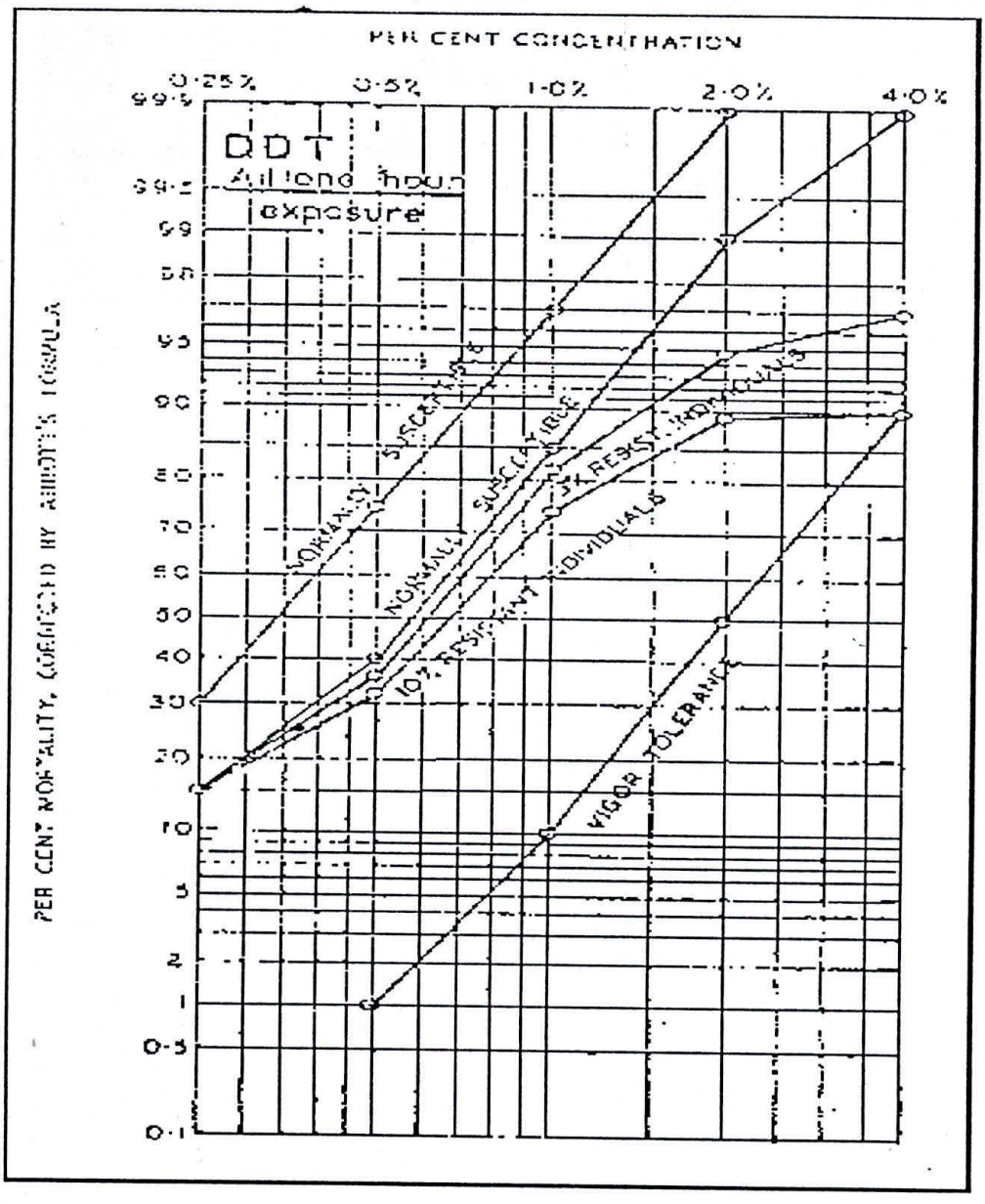
1. Each impregnated paper may be used upto 20 times and upto 3 weeks after removal from the packet

2. After removal of impregnated paper, the packet should be resealed carefully with plastic tape

Result/interpretation

1. Percentage mortality obtained for each concentration can be put in log-probit graph paper.

2. Regression line may be fitted by eyes and LC 50 and LC 95 values can be read from graph.


PER CENT CONCENTRATION
0·25%
0·5%
1·0%
2·0%
4·0%
DDT
Auilong hour
exposure
PER CENT MORTALITY, CORRECTED BY ABBOTT'S FORMULA
NORMALLY SUSCEPTIBLE
NORMALLY SUSCEPTIBLE
10% RESISTANT INDIVIDUALS
5% RESISTANT INDIVIDUALS
VIGOR TOLERANCE

(Fig. 8)

LARVAE

Susceptibility status of larval population of mosquitoes can be determined by exposing late 3rd or early 4th instar larvae to various insecticide concentration in 500 ml glass beaker for a period of 24 hrs. and thereafter larval mortality is recorded as per the method recommended by WHO using WHO test kit. Based upon the larval mortality, the susceptibility status of larvae against particular insecticide is ascertained.

As per the guide lines the following criteria is used for determining the susceptible or resistant , tolerent status of adults and larvae.

	Range of Mortality	Status
1.	Between 98% - 100%	Susceptible
2.	Between 80% - 98%	Tolerant (Verification required)
3.	Below 80%	Resistance

Proforma of mosquito larval survey

Locality - _____ Date of collection - _____

PER DIP DENSITY IN + VE BREEDING PLACES

	Sullage water drains	Septic Tank	Cesspits	OHT	Cistern/ Barrel	Ornamental Tank	Wells	Irrgn. Canal	Seepage Water	Rice Field	Lake	Rain Water Collen.	Rejected Tyre Utensil	Others
ANOPHELINES														
L-I-II														
L-II														
PUPA														
CULICINES														
L-I-II														
L-III-IV														
PUPA														
AEDES														
L-I-II														
L-III-IV														
PUPA														

Proforma for Indoor Adult Mosquito Collection Record (MHD)

Distt.

P.H.C.

Village.

Date

Time

Method: Aspirator.

LastSpray on:

House/ Cattle shed

Weather: Clear/Cloudy/Rainy

Time Spent

S.No.	Mosquito Species	Houses Abdominal Conditions				Total		Density P.M.H. (houses)	Cattle Shed Abdominal conditions				Total		Density P.M.H. (Cattle Shed)
		UF	F	SG	G	F	M		UF	F	SG	G	F	M	
1.	Anophelines														
2.	Culicines														
3.	Aedes														

UF = Unfed F = Fed SG = Semigavid G = Gravid

2.Vector Control

The major thrust for the control of vector-borne diseases has to be on vector control, as the elimination of pathogens/parasites in human or zoonotic reservoir of infection is not in the realm of practicability.

The main objectives of vector control is to keep the vector density at low level to minimise vector-reservoir contact and to curtail the longevity of vector species to interrupt disease transmission. Vector control measures are undertaken where population aggregate for the sake of feeding, resting, breeding etc. particularly during the high density period.

2.1 PERSONAL PROTECTION MEASURES

2.1.1 Anti- adult measures

Several personal protection measures are available for providing protection against the mosquito bite. They can be used as supplementary measures in remote and inaccessible areas or against exophilic and endophagic vector species depending upon their feasibility, cost effectiveness and sustainability.

i) **Physical methods**

This include protective clothings, use of bednets, screening of windows/doors etc.

ii) **Repellents** - These are substances applied to the skin, clothings or mosquito net to repel the mosquitoes and prevent them from biting. The most commonly used repellents are DMP (Dimethyl phthalate) and DEET (Diethyl toluamide). They provide protection for 3-4 hours.

iii) **Impregnated bed nets**

Pyrethroids are fast acting, broad spectrum insecticides with low mammalian toxicity. Impregnation of bednet with synthetic pyrethroid enhances its potential for reduction or interruption of disease transmission against endophagic or exophagic species of mosquitoes. Impregnated bed nets produce deterrent, repellent and killing action and help in reducing man mosquito contact.

iv) **Coils**- Mosquito coils containing natural pyrethrum and herbal products are used in many countries for protection from mosquito bites. Use of Tortoise, Rooster brand coils available in the market last 6-7 hours.

v) **Mats** - The mat is impregnated with synthetic pyrethroids viz. Allethrin/bioallethrin and heated on a plate fitted in a small electric device. Mosquitoes are either repelled or knocked down by the vapor action of the pyrethroid. The mats provide protection from mosquito bite for 10-12 hours.

vi) **Indoor residual insecticidal spray**

Selective spraying is recommended against vector mosquito species which predominently rest and feed in indoor situations. Depending upon the susceptibility status of the vector mosquito species to various organochloroine , organophosphate and synthethic pyrethroids. The insecticide is to be chosen to which the local vector is amenable to control. The details about the insecticide, dosage, the formulation and application etc. are given in the Table- 1.

vii) **Space spraying by Mist, Thermal fogging or ULV spray**

Space spraying has been successfully used to control outbreaks of vector-borne diseases such as malaria, dengue, Japanese encephalitis, Western equine encephalitis etc. The space spray is usually undertaken to control the resting population of mosquitoes either by using the natural pyrethrum extract diluted in kerosene oil or malathion during outbreak situations to interrupt the disease transmission by crisis. This is done in the form of mist, thermal fogging or ULV spray . Insecticides formulation and their dosages for space spray are given in Table- 2.

2. **Antilarval measures**

Anti- larval measures are used as an adjunct to other methods of control and are rarely used as main method of control except against container breeding species or against those mosquito species which breed in confined or specific small water bodies such as *Aedes aegypti*, *An. stephensi* and *An. sundaicus*. Antilarval measures can also be tried in an area where vector species are resistant to commonly used insecticide or exhibit exophily and exophagy or under those situations where adulticide measures are not cost effective or tend to endanger the environment.

Antilarval measures in tropical countries are mainly used in urban or peri urban areas. These measures can be used in certain specialised situations like minning, irrigation wells, tanks etc. if they are operationally feasible and cost effective. The basic idea of all antilarval measures is to prevent, reduce or eliminate the breeding places. Certain commonly used antilarval measures are briefly discussed below:

i) **M.L.O. (Mosquito larvicidal oil)** - Oiling is done in situations where breeding is temporary and permanent measures may not be cost effective. Oiling of a breeding sites kill the larvae by choking their spiracles with oil film & cutting the oxygen supply. It also deter the adult mosquitoes from egg laying.

ii) **Paris green (Copper aceto-arsenite)** - It has been successfully used in malaria control programme for the control of anopheline and culicine breeding. It is applied as dust or granular formulation.

iii) **Abate (Temephos) and Baytex (Fenthion)** - Widely used under urban Malaria Scheme for the control of breeding of anopeheline and culicines mosquitoes . Larvicide formulations and their dosages are given in Table - 3.

2.3 Environmental management for vector control

WHO expert committee on Vector Biology and Control in 1979 defined environmental management as follows:

" The planning, organisation, carrying out and monitoring of activities for modification and/or manipulation of environmental factors of their interaction with man with a view to preventing or minimising vector population and reducing man-vector pathogen control."

This approach which should be carried out prudently and skillfully is naturalistic and involves an attempt to extend and intensify natural factors which limit vector breeding, survival and contact with man. But these measures have many constraints and limitations viz.

i) Selective application,
ii) Require high degree of inter-sectoral coordination,
iii) Capital investment of some of the methods is high,
iv) Maintenance is very essential and
v) Active and sustained community involvement.

This is most simple and dependable method of elimination or prevention of mosquito breeding by identifying the active and potential breeding sites of mosquitoes. Environmental management of vectors are much suited for the vectors of urban malaria and Dengue/DHF as their vectors mainly breed in overhead tanks, coolers and other man made containers in domestic and peridomestic situations.

i) **Drainage**

Different species of mosquitoes are known to be associated with varied type of water bodies. It may be impounded rain water, seepage water, natural water courses or man made water courses etc. These can be eliminated by formulating an effective drainage system which will not permit water stagnation and mosquito breeding.

ii) **Mosquito breeding associated with the construction of Roads/Railways**

High mosquitogenic potential is generated during the construction of roads and railways and most of these breeding places can be eliminated if proper engineering methods are followed. The breeding places includes burrow pits, culverts and quarry pits etc. Such breeding places need special attention by Railway Health Authorities who may use a combination of various vector control measures in an integrated way.

iii) **Irrigation and mosquito breeding**

Engineers should incorporate various engineering devices in consultation with public health experts to include an inbuilt system to drain off the seepage water for its better utilization in agriculture.

2.4 **Biological control** -

The predators, pathogens or parasites may be used for the control of larval breeding. Some of the important biocontrol agents are given below:

i) **Larvivorous fishes**

Of several biological agents, larvivorous fishes are still considered to be the most potential and effective predators for the control of mosquito breeding in unused wells, pools, ponds, lakes, fountains, paddy fields etc. The *Poecilia reticulata* and *Gambusia affinis* are the two most important larvivorous fishes used world over for the control of mosquito breeding. They were introduced in India in 1910 and 1928 respectively and have since been acclimatized in various types of ecological conditions. They are considered to be most important larvivorous fishes for field operation because of their small size, voracious feeder on mosquito larvae, hardiness, agility and adaptability to variety of habitats. The *Poecilia reticulata* commonly known as guppy is a bottom feeder , can tolerate very high degree of pollution. It may be used effectivity for the control of *Culex* breeding whereas *G. affinis* is a surface feeder and prefers to breed in clean oxygenated water and is suitable for the control of anopheline breeding. Fishes can be easily cultured on large scale and require minimum efforts for transportation to any distant place.

ii) **Biocides-**The toxins of certain spore-forming bacteria viz. *Bacillus thuringiensis* variety *israelensis* H14 strain and *Bacillus sphaericus* have recently been shown to hold great promise as a microbial control agent for mosquitoes. These bio-larvicides are host specific, safe to predators and friendly to the environment. Of several indigenous and imported formulations of bio-larvicides, wettable powder formulations of *B. sphaericus* (Spherix) and BTI-H-14 Bactoculicide formulations were found to be highly effective in controlling the larval population of mosquitoes

2.5 Integrated vector control

The integrated vector control may be defined as the application of one or more than one vector control method simultaneously or consequently in a given area to control vector borne diseases. When available options are selected on the basis of epidemiological paradigms, vector behaviour, human behaviour and environmental aspects, it becomes selective vector control. This approach is quite appropriate but requires effective planning, and judicious use of national resources. This requires technical competence, managerial skills and sound understanding of vector and its environment.

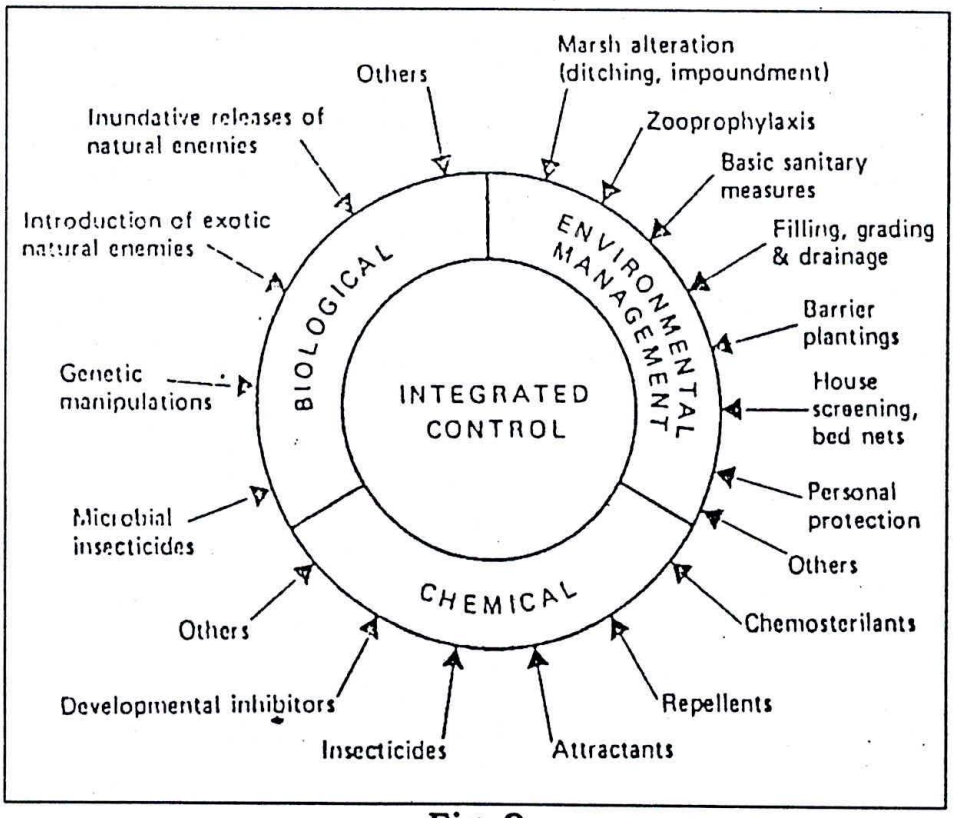


Fig. 9

2.6 Public Health Education and Community participation in the control of vector borne diseases

The role of public health education is vital for the effective imlementation of vector control measures, in respect of vector-borne diseases, as the problem mainly revolves around man and his environment. The aim of the health education for the control of vector-borne diseases like malaria, filariasis, Japanese encephalitis, Dengue/DHF and Kala-azar should be to familiarise and motivate the people by highlighting the following aspects of the disease :

- Causation and mode of disease transmission
- Awareness about the early signs and symptoms of the disease
- Educating the masses to co-operate with the public health workers for the early diagnosis of disease by clinical symptoms and laboratory diagnostic tests
- Usefulness of taking proper and adequate treatment of disease
- Knowledge about the breeding, feeding and resting behaviour of vector species
- Usefulness of insecticidal spray for the control of mosquito/sandfly borne diseases to bring down vector density and to curtail their longevity to interrupt disease transmission
- To restrain local inhabitants from mud plastering of walls of houses, cattlesheds etc. for a minimum period of two months after the spray to retain the residual effect of insecticide for the effective control of mosquitoes and sandflies
- Personal prophylactic measures by using ordinary impregnated bednets or repellents to prevent mosquito/sandfly bite
- To educate masses to sleep on the cots/benches instead of on floor to prevent sandfly or flea bite
- To undertake bio-environment measures to reduce the breeding, resting and feeding places in and around human habitations, cattlesheds etc.
- Use of mass media like radio,television,cinema slides, newspapers, posters and film showsetc. Involvement of Social workers, teachers, school children, public health workers Voluntary Health Organisations/ Resident Welfare Associations should be involved for

disseminating the above given information by holding group meetings or by inter-personal, communication, Health exhibitions etc. to get rid off/ reduce mosquito breeding.

COMMUNITY PARTICIPATION

WHO Alma Ata Declaration (1973) envisaged that community participation should be considered as a crucial component of Primary Health Care (PHC). The idea of community participation was developed with the fond hope that it will make disease control programme more effective. The integration of activities for the control of communicable diseases was considered advantageous to improve the quality of preventive care, reduce morbidity and mortality due to communicable diseases and encourage the participation of people. Involvement of community for the success of any vector control programme assumed still greater significance as the problem revolves mainly around man and his environment. The community will perceive the impact of control measures which will stimulate their active involvement in PHC socially, culturally and technically.

Consequent upon the resurgence of Kala-azar in Bihar in 1977 Plague in Maharashtra and Gujarat in 1994 and Dengue/DHF in Delhi, Haryana and Punjab during 1996, it was observed that the public health education about vector-borne diseases is poor and community participation was practically nil. It is felt that for the control of vector-borne diseases , community may be motivated to co-operate and partcipate for the effective implementation of vector control strategy. After motivation, the community would be able to extend their full co-operation in getting their dwelling units, cattlesheds etc., sprayed with insecticide and should restrain from mud plastering the insecticide treated surface for a minimum period of two months for the retention of residual effect of insecticide. Besides, the community may be motivated to undertake bio-environmental measures like removal of garbage from in and around the houses, pigsties, cattlesheds, filling of all cracks and crevices. The shelters should be made more ventilated and lighted to prevent the breeding, resting and feeding of vector species.

For the success of vector control programme, there has to be a frequent interactions between the health workers and the people so that they may accept the control programme as the " People's Programme" and only this approach will be fruitful for the effective implementation of vector control strategy vis a vis control of vector-borne diseases in various parts of the country.

Activities to be undertaken by various agencies for the control of vector-borne diseases are summarised below:

3. Sandflies - vectors of Visceral and Cutaneous leishmaniasis

Sandflies are mainly involved in the transmission of visceral and cutaneous leishmaniais in various parts of the country. Whereas, visceral leishmaniasis or Kala-azar is transmitted by *Phlebotomus argentipes*, the cutaneous leishmaniasis which is not a major problem in the country is transmitted by *Phlebotomus papatasi, P. salehi and P. sergenti.*

P. argentipes is widely distributed species in India and found in abundance where climate is warm and moist. *P. argentipes* can be identified on the basis of silvery white legs.

P. papatasi is found mainly in plains where climate is hot and dry. It is yellowish brown in colour.

3.1 Morphology of sandfly

Adult sandfly is a small, delicate insect of about 2-3 mm. in length. It is light yellowish to greyish brown in colour with large conspicous dark eyes. The males and females can easily pass through ordinary mosquito net. The body of sandfly is completly covered with long hairs, head bears piercing and sucking type of mouth parts and only female sandfly can sucks the blood whereas males feed on plant saps. They can be easily recognised in nature by the presence of a pair of long and elongated wings which remain erreet upward on the body and makes 'V' shaped appearance while resting. The males are identified on the basis of male genitalia at the terminal end of abdomen and females by the presence of spermatheca.

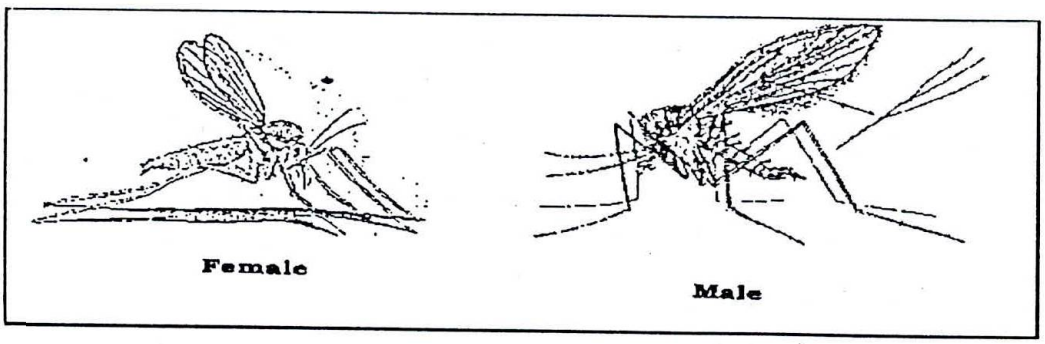


Fig. 10

Life cycle

The life cycle of sandflies is comprised of four stages viz. egg, larva, pupa and adult fig.11.

i) **Egg** - The eggs are laid in moist cracks and crevices containing decaying organic matters. The female *P. argentipes* lay eggs ranging from 5-68 whereas *P. papatasi*, lays eggs ranging from 7-69.

The freshly laid eggs are creamy white in colour, however, their colour changes from dark brown to black after laying. The eggs hatch in about 3-4 days under laboratory conditions at 28 + 2 C and 95% R.H.

ii) **Larva** -The larva is creamy white in colour and possesses a number of hairs on its body. The body is divided into head, thorax and abdomen. After emergence, larvae feed on decaying organic matters. Sandflies have four larval stages. The total larval period of *P. argentipes* and *P. papatasi* was recorded to be 11-29 days and 12-26 days respectively.

iii) **Pupa** - Pupa is elognated and coma- shaped in appearance and is a non-feeding stage. The pupal period varies from 6-10 days for *P. argentipes* and 6-13 days for *P. papatasi* under optimum laboratory conditions.

iv) **Adult** - The adult emerges through a longitudinal slit on the mid-dorsal part of cephalothorax of pupa. Mating in sandflies usually occur on host while feeding but may also takes place after feeding.

Life cycle from egg to emergence of adult was found to be 20-36 days for *P. argentipes* and 22-45 days for *P. papatasi* (Fig.11).

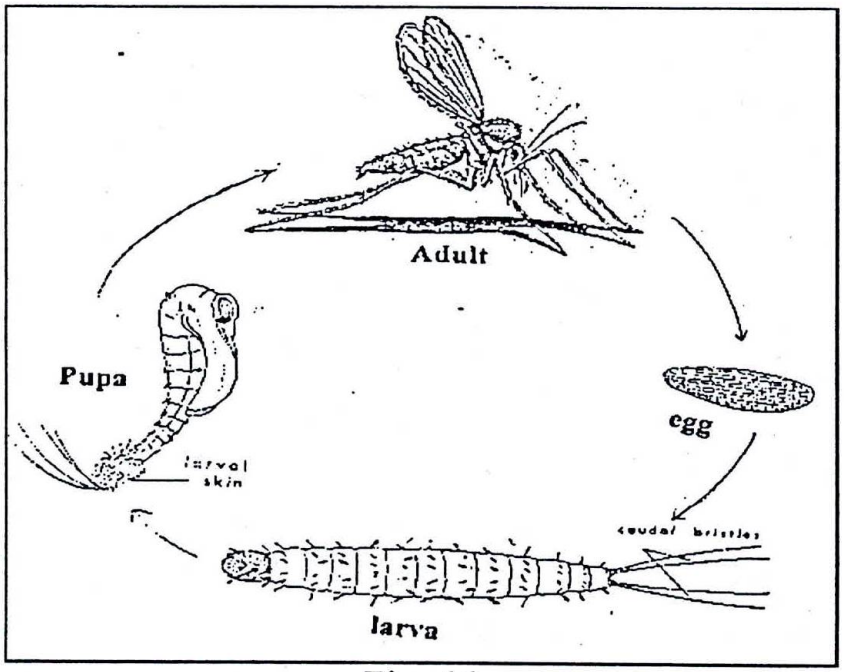


Fig. 11

3.3 Habits and habitats

i) Habits - Sandflies are active during night hours and during day time they remain hidden in cracks, crevices in dark corners of houses and cattlesheds. *P. argentipes* and *P. papatasi* are generally found in mud plastered houses and adjoining cattlesheds.

ii) Feeding habits - Female *Phlebotomus* feed on a varity of mammals at night . *P.argentipes* mainly feed on bovine, whereas *P. papatasi* prefers to feed on human beings.

iii) Resting habits - *P. argentipes* and *P. papatasi* mainly rest in indoor situations in houses, cattlesheds and mixed dwellings etc.

iv) Flight range and movement - Sandflies moves by hopping movements and can not fly long distances. The flight range of *P. argentipes* has been recorded to be 207-505 meters from their breeding places.

v) Longevity - The maximum longevity of *P. argentipes* has been reported to be about 24 days.

vi) Breeding habit - *P. argentipes* lay eggs in the soil having lot of moisture and organic debris in and around cattlesheds and human dwellings.

3.4 Sampling Techniques

Sandflies can be collected by torch light and suction tube method similar to that of mosquitoes. The density of sandflies is expressed in term of Per Man Hour Density (PMHD) (Proforma enclosed)

Other methods of sandfly collections are:

i) Sticky trap method

ii) CDC light traps

iii) Funnel traps

iv) Bait collection

3.5 Susceptibility status to insecticides

P. argentipes, vector of Kala-azar has been reported to be highly susceptible to DDT. However, *P. papatasi* vector of cutaneous leishmaniasis

is highly resistant to DDT and Dieldrin but susceptible to Malathion and other insecticides.

3.6 Control of sandflies -

i) **Prophylatic measures** - use of bed nets and repellents to prevent man/sandfly contact.

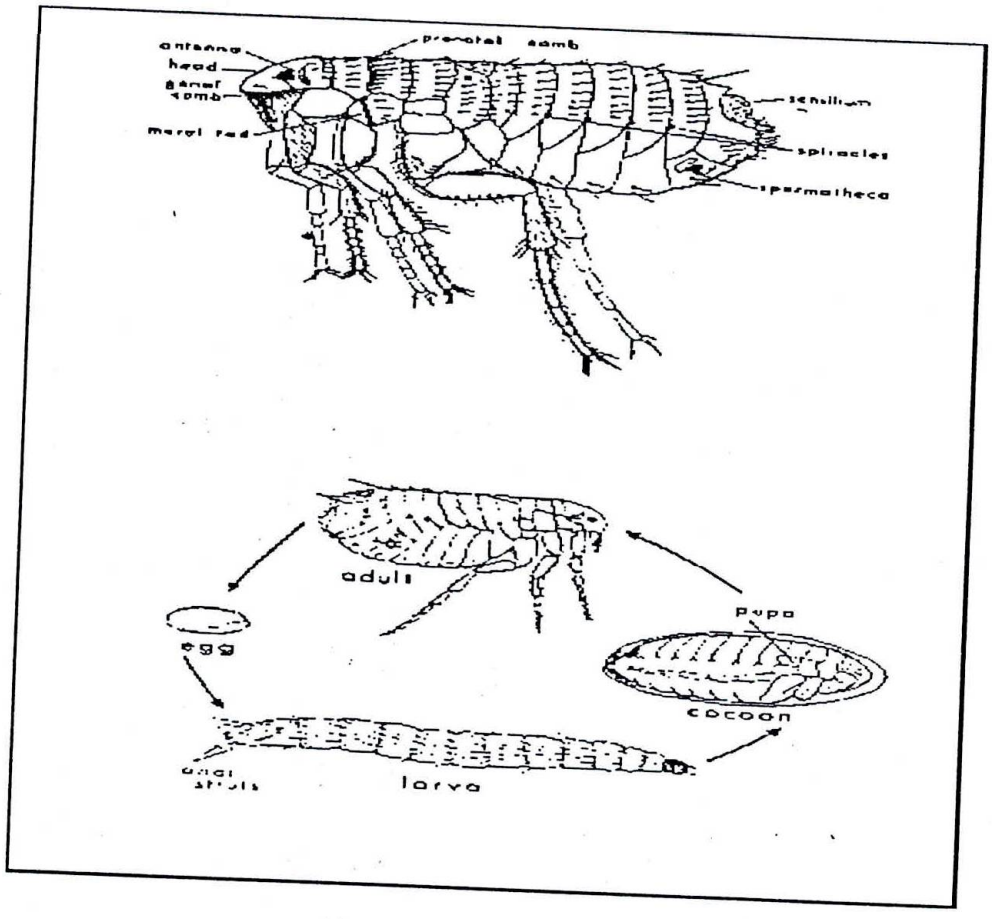
ii) **Environmental measures** - These measures are used to control/eliminate breeding sites of sandflies in and around houses by filling the cracks and crevices.

iii) **Chemical Control** - *P. argentips* can easily be controlled by undertaking residual spray of DDT. Two rounds of indoor residual spraying is undertaken in endemic areas @ 1 gm per sq. meter upto 6 feet height.

4. Rat fleas, vector of plague

Plague which ceased to be the major public health problem in the country during 1966 has resurged in the form of Bubonic plague in Beed Distt. of Maharashtra and pneumonic plague in Surat Distt. of Gujarat during 1994. This has necessiated to intensify disease/vector surveillance to detect plague activity and to monitor flea index in various parts of the country to undertake timely intervention measures.

The Bubonic plague in India is transmitted by three species of fleas namely *Xenopsylla cheopis, X. astia* and *X. brasilensis*. Fleas are laterally compressed insects and their body size varies from 1-4 mm. The body is divided in head, thorax and abdomen and is covered with backwardly directed spines. It has three pair of legs and hind pairs of legs are very well developed and modified for jumping. The mouth parts are of piercing and sucking type and both the sexes suck blood.



4.1 Life cycle - There are four stages in the life cycle of flea viz. egg, larva, pupa and adult. (fig.13).

i) **Eggs** - The eggs are laid in clusters in and around the nest of host animals. It is small in size measuring 0.4 to 0.5 mm. in length, oval in shape and glistering white in appearence when laid but changes to dull yellow colour after few hours of laying. A single *X. cheopis* female may lay

300-400 eggs during its life time. The incubation period of egg is about 2-4 days

ii) Larva - The 1st stage larva hatches out from the egg in 2-4 days. Larvae are small, 13 segmented worm like creatures without leg and its body is covered with hairs. It has chewing types of mouth parts and feed on all types of organic debris. There are 3 larval stages which last for 14-16 days. The 3rd stage larva when fully grown empties it's alimentry canal and pupates. The larva spins a loose meshed cocoon of thread around it to which particles of debris adheres.

iii) Pupa - Pupa is usually enclosed in a cocoon and emerges into adult after 8-10 days under favourable climatic conditions (Temp. 27C and Relative humidity 80-90%).

iv) Adult - After emergence adults usually takes blood meal after 24 hours. Mating usually occurs on host.

4.2 Biology and ecology -

(i) **Habit and habitat** - Fleas are ectoparasites of warm blooded animals specially rodent species. They are generally found on host's body while taking blood or as free living and their immature stages are found inside the rodent burrows.

(ii) **Feeding habit** - Both the sexes feeds on a variety of animals. The female feed for the development of eggs and its survival. Flea species have definite host preference but they can also feed on other hosts also. *X. cheopis* and *X. astia* generally found on rodent species.

(iii) **Resting habit** - After taking blood meal fleas rest in rodent burrows, cracks and crevices having microclimatic condition favourable for the maturation of their eggs.

(iv) **Dispersal/movement** - Fleas move from one place to another by jumping movement. Flea can jump 7 to 8 inches vertically and 14-16 inches horizontally. They can also disperse from one place to another with their animal hosts.

4.3 Sampling Techniques -

Fleas can be collected from the rodents after trapping the rodents using live traps (wire cages) in domestic, peridomestic and sylvatic situations. Traps are laid in evening hours and retrieved in the morning. The positive traps with rodents are to be transported in cloth bags to laboratory and fleas can be collected by combing the rodents. Various indices used for monitoring rodent and flea are given below:

Indices used to determine rodent/flea density

a) Trap positivity rate = $\frac{\text{No. of Traps found +ve for rodents}}{\text{No. of traps laid}} \times 100$

b) Total flea index = $\frac{\text{Total No. of fleas collected}}{\text{Total No. of rodents examined}}$

c) Specific flea index = $\frac{\text{Total No. of fleas collected of a species}}{\text{No. of rodents examined}}$

d) *cheopis* index = $\frac{\text{Total No. of } X.cheopis \text{ collected}}{\text{No. of rodents examined}}$

The *cheopis* index must be kept below 1.0 in plague endemic areas to prevent occurrence of the disease.

Proforma for monitoring rodent/flea population is enclosed.

4.4 Preservation and mounting of fleas - The fleas retrieved from the captured rodents should be preserved in 70% alcohol containing a drop of glycerine. The tubes should be labelled properly giving the following details.

Name of the area -
Date of collection -
Name of host -

i) Mounting procedure

a) Transfer the flea to water for one hour to get rid off 70% alcohol

b) Keep flea material in 10% KOH solution at room temperature for one to two days till the specimen become yellowish transparent

c) Keep material in water for few minutes

d) Transfer to 5% aqueous solution of glacial acitic acid for 30 minutes

c) Transfer to water for an hour with several changes

a) Permanent mounting

(i) After step (e) pass the fleas through 50%, 70% and 90% alcohol for dehydration, thereafter, treat with absolute alcohol for one hour

(ii) Clear the specimens in clove oil for few hours, preferably for a day

(ii) Keep the flea material in xylol for 10 minutes

(iv) Mount flea specimen in Canada balsam

(b) Temporary mounting

After step (e) fleas can be directly mounted in a drop of Hoyer's medium and can be dried over hot plate

Hoyer's medium

Distilled water - 50 ml
Gum arabic - 30 gms
Chloral hydrate - 200 gms
Glycerine - 20 ml

4.5 Susceptibility Tests of fleas to insecticides -

Determination of susceptibility tests of fleas to various insecticides is a pre-requisite for undertaking effective vector control measures. Susceptibility test could be under taken as per standard WHO method (WHO Tech. Report Series No. 443). In this method fleas are exposed to the insecticides impregnated filter paper strips in test tubes for a period of one hour and percentage mortality are observed after 24 hours of exposure.

4.6 Current insecticide susceptibility status of flea

Recent studies carried out on the susceptibility status of *Xenopsylla cheopis* from Distt. Beed(Maharashtra), Varanasi (Uttar Pradesh) and Delhi shows that the species is highly resistant to DDT & HCH but susceptible to Malathion and Deltamethrin.

4.7 Control of fleas

The flea population can be controlled by the application of appropriate insecticide as dusting powder. The main sites of treatment are rodent burrows and run ways. The houses and other structures, the bottom of all walls and the floor for an area of about 15-30 cms from wall should be treated with the dust formulation. Dust should also be applied on the top of the wall and along the rafters where rat runways are present. Other rodent habitats such as piles of wood, debris, wooden structures etc. should also be treated with the insecticide dust.

Keeping in view that the vector flea species are resistant to DDT and BHC the other insecticides like Malathion (5.0 %), Deltamethrin (0.005%) and Permethrin (0.5 %) dust can be used for the control of rat fleas.

Application

For the application of dust the hand operated dusters are quite useful. The rodent frequented sites should be thoroughly covered with dust. To control fleas on wild rodents about 30 gms of the dust formulation is sufficient for each burrow.

Annexure -

Proforma for surveillance of rodent/flea population

Area visited -

Date of visit -

No. of Traps laid -

No. of traps +ve -

Traps positivity -

Total No. of rats trapped/Rodent species -

Total No. of Flea retrieved/ Flea index -

No. of sera samples collected -

No. of tissue smears of heart, lung, liver and spleen collected -

No. of tissues taken for culture -

Proforma for Indoor Sand fly Collection

Name of Distt. _____ PHC_____

Village _____ Date of Collection _____

S.No			Sandfly Species Collected													
	Time Spent	Habitat	*P. argentipes*				*P.papatasi*				*Sergentomyia sp.*				Insecticide Spray Status	Remarks
			M	F	T	DPMH	M	F	T	DPMH	M	F	T	DPMH		
1.																
2.																
3.																
4.																
5.																
6.																
7.																
8.																
9.																
10.																
11.																
12.																
13.																

Mosquito Control measures which may be undertaken by Individuals and Community

Type of activity	Individual action	Community action
Reduction of Man: Mosquito contact	i) Screening of houses/bedrooms ii) Use of protective clothings to prevent mosquito bites iii) Use of repellents to prevent mosquito bites iv) Use of ordinary or insecticide impregnated mosquito nets	i) Site selection of houses in the villages ii) Deforestation and clearance of vegetations near the dwellings (specific to *An.. dirus* vector of malaria in North East India. iii) Vector deviation by shifting of cattlesheds/ piggeries away from human dwellings (specific to zoophilic anophelines/ culicines) iv) Wire meshing of vent pipes of septic tanks to prevent emergence of and oviposition by adults (specific to *Cx. quinquefasciatus*)
Destruction of adult mosquitoes	i) Use of adulticides or aerosols available in the market	i) Indoor residual spraying of chemical insecticides ii) Thermal or cold fogging (ULV)
Destruction of larvae	i) Emptying water containers periodically, scrubbing and cleaning (specific to *Ae. aegypti*). ii) Use of temephos sand granules for water containers (specific to *Ae. aegypti*) & *A. stephensi* iii) Refrain from throwing garbage into the drains and drain cleaning (specific to *Cx. quinquefasciatus*)	i) Larviciding ii) Rearing and release of larvivorous fish viz. *G.affinis* & *P.reticulata* iii) Flushing of drains and their periodic cleaning iv) Removal of algae and other water plants from the ponds to prevent breeding of vectors of malaria and Japanese encephalitis
Source reduction and Source alteration	i) Proper disposal of unused containers, tyres etc. ii) Covering water containers iii) Filling up of breeding sites	i) Community cleaning-up campaign to remove trash and water-retaining debris for *Aedes* control ii) Installation of proper latrines, drainage and water supply to prevent the breeding of *C. quinquefasciatus* iii) Intermittent irrigation of rice-fields for J.E. vector control iv) Filling up of low lying areas or pumping out of water for malaria/filaria vector control v) Reclamation of land

Plan of action for control of DF/DHF vectors in urban and Rural areas

Control Methods	Expected Agencies	Activities Expected	Ways and Means
Source reduction methods for larval control	Community	I) Remove/reduce non-essential water containers conducive for mosquito breeding	i) Health education
			ii) Mass media (Radio, TV, Film shows, News papers etc.)
		ii) Protect water containers from larval breeding by providing lids or cover	iii) School children/housewives health education
			iv) Volunteers
			v) PHC workers
			vi) Community leaders
	Government	1.Solid waste management to prevent mosquito breeding	I) To set up a core working committee for inter-and intera-sectoral coordination
		2. Provision for reliable piped water	
		3. Legislative measures	
		4. Monitoring and assessment	

Draft

DIS 14.2

PREVENTION AND CONTROL OF OUTBREAKS OF MENINGOCOCCAL MENINGITIS

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
(DIRECTORATE GENERAL OF HEALTH SERVICES)
GOVERNMENT OF INDIA
22 SHAMNATH MARG, DELHI 110054

AUGUST 1997

CONTENTS

ABOUT THE DOCUMENT

Target audience:

This document has been developed primarily for the District level officers who will be involved in National Disease Surveillance Programme. However, it can be of good use to State level officers and PHC medical officers.

Objectives:

At the end of the session, the participants will,

- have better understanding of the disease and its public health importance.
- refresh knowledge about clinical presentations and treatment modalities.
- get a clear view about the techniques and methods for diagnosis.
- learn about the components involved in the surveillance.
- appreciate the actions to be taken for prevention of impending outbreak.
- acquire the knowledge of outbreak control methods.

Method of interaction:

Presentation followed by guided discussions with the help of facilitators.

Note for facilitators:

The document is designed for a two (120 minutes) hours continuous session. First 30 minutes can be utilised to make a brief presentation of the contents. Next 75 minutes can be devoted to guided discussion giving particular emphasis on surveillance, prevention and control aspects (including formats). The last 15 minutes can be used for summing-up the document. All the participants should be provided with a copy of the document preferably one day before the session and instructed to take notes, if any, on it only.

1. Introduction:

Meningitis means inflammation of meninges. It is a very serious disease and if not properly treated in time, the patient may die. The disease is caused by a variety of organisms including bacteria, viruses and fungi. Pyogenic meningitis is an acute infection of meninges with bacteria. The common bacteria responsible for pyogenic meningitis are *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumonae*. Among all these, meningococcal meningitis caused *by N. meningitidis* (meningococci) is common in India and has great epidemic potential.

2. Indian situation:

2.1. The disease is reported round the year from different parts of the country, with peak incidence between January to March. Several outbreaks of meningococcal meningitis have been reported from different parts of India in recent past and the disease has continued to become endemic in the country. Names of the states and districts which reported outbreaks are shown below.

States (and districts) of India, which have reported outbreaks of meningococcal meningitis in recent past.

Name of state	District reported outbreak
Delhi	Delhi
Gujarat	Ahmedabad, Mehsana
Madhya Pradesh	Sagar, Damoh, Jabalpur, Rewa, Bhopal, Gwalior, Indore, Ujjain and Vidisha
Orissa	Kalahandi, Phulbani and Koraput
Bihar	Singhbhum, Gumla and Sahebganj
Andhra Pradesh	Vizag, Vizianagram and Srika-kulam
Rajasthan	Jhalawar
Maharashtra	All the districts of the state

2.2. The epidemic of meningococcal meningitis usually comes after a gap of 10-15 years, when the pool of susceptible population becomes large enough to trigger this process. The epidemic takes about 4-5 years to subside.

3. Causative agent:

3.1. The disease is caused by bacteria, *N. meningitidis*. It is a Gram-negative diplococcus, non-motile, usually encapsulated and may be intra or extra cellular. It is a fragile organism, susceptible to cold and drying.

3.2. The capsular polysaccharides of the organism differentiate thirteen serogroups. Among these serogroups of *N. meningitidis*, only serogroup "A" is reported from India.

4. Source of infection and its transmission:

4.1. Primarily the "carriers" (humans, who carry the organism in nasopharynx without getting the disease) are the source of infection. Sometimes, the patients of meningococcal meningitis can also be the sourse of infection.

4.2. The infection is transmitted through droplets during sneezing, coughing and direct contact with nasopharyngeal secretions. There is no extra-human reservoir of the organism.

5. Incubation period:

The incubation period of the disease is between 2-10 days, **commonly 3-4 days**.

6. Pathogenesis:

As mentioned above, the organism is present in the nasopharynx of carriers. Some of these carriers may develop mild upper respiratory infection only. After entry into the nasopharynx of a host, the organism can adhere to and enter the nonciliated cells of the nasopharyngeal mucosa. This colonisation is not sufficient for causing meningitis. In some instances, the organisms transmigrate through these cells to the submucosal space, where they have access to enter capillaries and arterioles and lead to a systemic infection. In this whole process the antiphagocytic capsular and surface antigens play a major role. This acute systemic infection can be manifested in three ways: meningitis (most of the cases), meningococcemia without meningitis and meningitis with meningococcemia.

7. Risk factors:

- In endemic situation the attack rate is highest in children of 6 months to 1 year age group.
- About 50 per cent of cases occur in children below 5 years and 80-85 per cent of total cases occur in less than 25 years age group.
- Attending physicians, health personnel and household members of patients are at more risk of getting the disease.
- Overcrowding, enclosed population (hostel, jail, remand home etc.) and low socio-economic status can also increase the risk of disease spread.

8. Clinical manifestations:

8.1. Meningococcal meningitis is commonly presented with sudden onset fever, headache, vomiting, photophobia, altered consciousness and stiff neck. There may be petechial rash, diastolic hypotension, focal neurological signs and convulsions. In severe case, patient is usually comatose. In patient under one year, the disease is usually presented with fever, irritability, lethargy, convulsion, bulging fontanelle, petechial rash, hypotonia, vomiting and neck rigidity.

The clinical presentations of meningococcal meningitis are indistinguishable from other acute bacterial meningitis.

8.2. When the disease is manifested as Meningococcemia, bacteremia and sepsis occur without meningitis. Three-fourth of such cases may develop characteristic petechial rash in axillae, flanks, wrists and ankles. About 10-20 per cent of all meningococcemia may lead to fulminating stage with vaso-motor collapse, shock and high fatality rate. **Both the types of the disease may co-exist in endemic and epidemic situations.**

9. Case fatality:

Injection with penicillin has been found to be most effective for successful treatment of patients. However, case fatality rate (CFR) can be, as high as, 30 per cent or more during epidemic mainly due to low public awareness, delay in hospitalisation and improper management. With early diagnosis, specific antibiotic therapy and other supportive measures, CFR can be brought down to 5-10 per cent.

10. Clinical laboratory findings:

Apart from Gram staining of CSF, clinical laboratory studies are of little value in establishing the diagnosis. In acute bacterial meningitis, which includes meningococcal meningitis, the characteristic CSF findings are:

• Colour:	Turbid/Purulent
• Pressure:	Increased
• Mononuclear cells:	<50/cu.m.m.
• Polymorph cells:	200-3000/cu.m.m.
• Protein:	> 45 mg/dl.
• Sugar:	< 40 mg/dl.

11. Differential diagnosis:

The common diseases, which have clinical features almost similar to meningococcal meningitis are: other acute bacterial meningitis, cerebral malaria, encephalitis, aseptic meningitis and brain abscess. A thorough history taking, clinical examination and laboratory investigations can help to establish the diagnosis.

12. Laboratory confirmation of diagnosis:

12.1. Culture of meningococci from CSF is "the confirmatory test". But it is not a very easy method. Moreover, the culture becomes difficult after the patients have taken antibiotics. Other methods (serologic) include Counter immuno-electrophoresis (CIE) and Latex agglutination tests. Like Gram staining, these tests are also performed on CSF. The diagnostic rate is highest (70-80 per cent) with Gram staining and Latex agglutination. This can further be increased through combination of tests.

Techniques for Gram staining of CSF:

Atleast 20 drops (1 ml) of CSF should be collected in a sterile tube. Do **not refrigerate** but hold at room temperature before staining. Processing should start immediately after collection.

- Centrifuge CSF at 2000 rpm for 10 minutes
- Draw off the supernatant and reserve for Latex agglutination test.
- Use a drop of sediment to make a smear on a glass slide. Air dry and fix gently by passing through flame.
- Flood the slide with ammonium oxalate-crystal violet solution and let stand for 1 minute.
- Rinse gently with tap water and drain off excess water.
- Flood smear with Gram's iodine solution and let stand for 1 minute.
- Rinse with tap water as above.
- Decolourise with 95 % ethanol for 5-10 seconds.
- Counterstain with safranin for 20-30 seconds or carbol-fuchsin for 10-20 seconds.
- Rinse the slide with tap water and blot dry.

Results: Examine the smear under oil-immersion lens with bright field condenser. Meningococci appear intra- or extra-cellularly as Gram-negative coffee-bean shaped diplococci.

General method for performing Latex agglutination tests of CSF:

- Take about 0.5 ml of supernatant of centrifuged CSF.
- Shake the Latex suspension gently until homogenous.
- Place one drop of specific latex suspension on a ringed glass slide or disposable card.
- Add 30-50 micro-litre of CSF to suspension.
- Rotate by hand or by a rotator at 100 rpm for 2-10 minutes.

Results: Read under bright light without magnification.
Negative reaction: The suspension remains homogenous and slightly milky in appearance.
Positive reaction: Within 10 minutes, agglutination (visible clumping) of the latex particles occurs.

Demonstration of bacteria in the Gram stained smear made from the centrifuged deposit of cerebro-spinal fluid (CSF) is an easy and cost-effective method that can be used at Primary Health Centre level. In field situation, Latex agglutination test can be performed easily and satisfactorily.

12.2. When CSF samples are to be sent to laboratory, **refrigeration should not be done** and the samples are to be sent at room temperature with in 2 hours of collection. From each patient, about 3 ml of CSF should be drawn and collected in 4 small sterile tubes in divided quantity for biochemical, culture, microscopy and serological tests. **If the quantity of CSF drawn/collected is less, then the person sending the sample should decide upon which test(s) is to be done at laboratory.** In such situation, depending upon the facilities available, serology and microscopy are the best options in order of preference. Each sample should accompany detailed information as indicated below,

- Sample identification No.:
- Name of patient:
- Age:
- Sex:
- Complete address:
- Presenting features with duration:
- Provisional diagnosis:
- Treatment given:
- Date of collection:
- Test (s) to be done:

13. Clinical management:

13.1. Patients with meningococcal meningitis require supportive treatment, as well as, antimicrobial therapy. The primary areas of supportive treatment are:

- Bed rest
- Antipyretic
- Sedative
- Good nursing care
- Maintenance of fluid and electrolytes balance
- Prevention of respiratory complications in comatose patients
- Use of anticonvulsants in patients with convulsions

13.2. For antimicrobial therapy, **Crystalline benzyl penicillin is the drug of choice.**

- 300,000 to 400,000 units of penicillin/Kg body weight/day should be given by I.V. drip or in divided doses 2-4 hours.

Alternatively, Chloramphenicol can be given.

- The dose is 100 mg/Kg body weight/day intravenously in 6 hourly divided doses.

This treatment can be given for a total duration of 7 days. Patients become non-infectious after 24 hours of starting specific antimicrobial therapy. Therefore, **they should be kept separately for 24 hours after the starting of antibiotic.**

A four days course with penicillin has been found to be as effective as any longer course of antimicrobials. This fact has special relevance during outbreak situation. In large outbreak, even the great majority of patients can be successfully treated with a single dose of long acting oily preparation of injectable chloramphenicol (100 mg/Kg; maximum 3 gm. I.M) or long acting penicillin.

14. SURVEILLANCE:

14.1. Early warning signal: Like other epidemic prone diseases, surveillance is the most effective tool for prevention and control of outbreaks of meningococcal meningitis. If properly implemented, surveillance can generate early warning signal of an impending outbreak by detecting sudden increase in number of cases/deaths or its clustering in time and space. This early warning should enable the health authorities in confirming the diagnosis and controlling the outbreak at the earliest.

14.2. Case definitions: The prerequisite of a surveillance is identification of patients (cases). The following case definitions are to be used in the surveillance of meningococcal meningitis.

Case definitions of Meningococcal meningitis

Suspect case: Sudden onset fever, severe headache and stiff neck with or without skin rash.
In patient under one year of age, a suspect case occurs when fever is accompanied by a bulging fontanelle.

Probable case: Suspect case with vomiting and positive neck rigidity with or without positive Kernig's and Brudzinski's signs OR suspect case with either cloudy/purulent CSF or petechial skin rash.

Confirmed case: Suspect or Probable case AND any one of the followings:
positive CSF for Gram-negative diplococci in direct examination, detection of meningococcal antigen in CSF or positive cultivation of the organism from CSF/blood/skin rashs.

Kernig's sign is tested by passively extending the patient's knee when his hip is fully flexed. This movement causes pain and spasm of the hamstring group of muscles.

Brudzinski's signs is tested by passively flexing the patient's neck. This movement causes an involuntary flexion of hip, knee and arm joints.

Both these signs become positive when meningeal irritation affects the lower part of the spinal subarachnoid space.

Use of case definitions at different levels

The peripheral health workers (MPWs) will use the "suspect" case definition, while the Medical officers of PHC, CHC etc. will use the "probable" one. The "confirmed" definition will only be used by the hospitals where facilities for laboratory confirmation are available.

14.3. Type of surveillance: Two types of surveillance are necessary in context of meningococcal meningitis. Since clinical presentations of acute meningitis due to all causative bacteria and some other diseases (see differential diagnosis) are indistinguishable, PHCs (including sub-centres), CHCs, Taluk and Sub-Divisional hospitals should use "suspect" and "probable" case definitions for **passive surveillance** of acute meningitis. Whereas, all district hospitals and medical colleges should use "confirmed" case definition for the **sentinel surveillance** of meningococcal meningitis.

- PHC, CHC, Taluk & Sub-Div. hospital: Passive surveillance of acute Meningitis.
- District hospital & Medical college: Sentinel surveillance of meningococcal meningitis.

14.4. Identification of sentinel centres: The sentinel centres should include all medical colleges and district hospitals of the state. In medical college, Head of PSM Department can be the In-charge of the Centre. He has to act in close collaboration with Medicine, Pediatric and Pathology / Microbiology Departments. For District hospital, the Superintendent or his designated subordinate officer may be the In-charge of the Centre.

14.5. Collection of Information: For passive surveillance of acute meningitis, minimum information is to be collected. Information on **name, age, sex, address and date of onset** will be sufficient to continue vigil on the disease situation. This information should be compiled in a linelist manner. However, for sentinel centres, more epidemiological and laboratory information is to be collected on each case as per format in Annex-I.

14.6. Role of Medical college: Being sentinel centre having better facilities, the medical colleges in addition to the above will also

- Conduct antibiotic sensitivity/resistance and serogroup typing tests for the Centre, as well as, of the samples sent from the districts hospitals.
- Perform cross-checking of CSF samples (both +ve and -ve) sent from the district hospitals.
- Extend diagnostic support during epidemic situation.

14.7. Frequency of reporting: All the reporting centres should send monthly report. Formats for passive and sentinel surveillance centres are in Annex-II & Annex-III. The **regularity of complete reporting, including "Nil" report has to be ensured.**

In the event of impending/continuing outbreak, information should flow daily at all levels.

14.8. Flow of information: All the passive and sentinel centres should send monthly report to the District Health Officer through FAX or by special messenger within 3rd day of next month. However, the sentinel centres should also send a copy of the report to the state nodal officer through FAX/speedpost. The district health officer will send consolidated monthly report of his district, as per format in Annex-IV, to the state nodal officer within 7th day of next month by FAX/speedpost. The state nodal officer will send the monthly report of his state to NICD within 15th day of next month by FAX with a copy to CBHI. Format to be used at state level is in Annex-V . With gradual development of HMIS services, NICNET will be used in near future. Flow chart of information is in Annex-VI. **However, in case of emergency, FAX, telephone, telegram should be used for sending daily reports at all levels.**

14.9. Data utilisation & monitoring: The data generated / received at different levels are to be scrutinised and interpreted monthly for local utilisation. Comparison of data should be made with that of previous month of the same year and same month of the preceding years. Properly drawn charts and graphs can help in better understanding of the situation. **Special attention should be given to identify geographical clustering of cases at the earliest.**

14.10. Feedback: Regular feedback on the reports in form of acknowledgement, discussion during monthly meeting, clarification, appreciation, advice etc. should flow from higher to next lower level.

15. Notification of the disease:

15.1. Meningococcal meningitis is not a notifiable disease in India. Presently an institution based passive monthly reporting of cases and deaths exists in the country. These monthly reports from the States are compiled annually at Central level by CBHI (Central Bureau of Health Intelligence). Besides being passive, the reporting is sometimes irregular and incomplete. Thus, this system is ineffective to address the needs of health administrators to look for the trend and foresee any impending outbreak.

15.2. If there is a sudden increase or clustering of cases or deaths due to acute meningitis/meningococcal meningitis, information should be notified immediately (by telephone or FAX) to the next higher level. The district/state health authorities will be

responsible to initiate investigation. If the outbreak is confirmed, National Institute of Communicable Diseases (NICD), Delhi, should be notified forthwith.

16. Actions to be taken in impending outbreak:

- Immediate reporting of suspicion to the next higher health authority.
- Immediate arrangements for laboratory confirmation of diagnosis.
- Continued analysis and monitoring of information on cases and deaths on a spot map.
- Early institution of the specific treatment to patients.
- Monitoring of number of cases and deaths graphically in time frame.
- IEC regarding chemoprophylaxis of household contacts of patients.

17. Control of outbreak:

When an outbreak is reported, the Rapid Response Team should be activated and mobilised by the district/state health authorities for taking up and helping in early implementation of the following control measures.

17.1. Outbreak investigation:

With the first indication of an outbreak, a thorough investigation should be carried out immediately to

- confirm the outbreak
- confirm the laboratory diagnosis
- define the areas affected
- assess the magnitude of problem (morbidity and mortality) in terms of "Time", "Place" and "Person".

Appropriate and early recommendations to control the outbreak is the most important objective of this investigation. Format for writing the outbreak investigation report is in Annex-VII

In outbreak situation, laboratory confirmation of each case is neither required nor possible.

17.2. Strengthening of surveillance:

- Active surveillance of the cases and deaths should be started in the area by health staff. **For this "suspect" case definition can be used** in community.

- Daily reporting of cases and deaths should be started at all levels from periphery to State Health Directorate.

17.3. Patient care:

Provision should be made to treat and follow-up all cases at hospital/CHC/PHC. If the situation demands, "Camp hospitals" should be established in school buildings or similar structures. Earliest institution of specific antibiotic can cut down mortality drastically. Information already available on microbial sensitivity/resistance can give right direction in this matter.

17.4. Health Education:

- Vigorous IEC activity should be started to diffuse the fear and confusion, if any, in the community.
- Recognition of early features of the disease by the community members and importance of earliest hospitalisation are two most important areas of IEC.
- Household contacts, particularly those sleeping in the same room of patient, should be warned about the need to obtain immediate medical attention at the first sign of fever and/or headache.

17.5. Chemoprophylaxis:

Since all the contacts of patients are at a very high risk of getting the disease, they should receive chemoprophylaxis for 2-4 days with **Sulfadiazine tablet** as per following schedule:

- Adults: 1 gm 12 hourly
- School children: 500 mg 12 hourly
- Pre-school children: 250 mg 12 hourly

If the organism is resistant to Sulfa, **Rifampicin** can be given orally. The duration is 2 days. For adults 600 mg and for children 10 mg/Kg body weight to be given 12 hourly.

17.6. Immunisation:

The primary means of controlling epidemic of meningococcal meningitis is vaccination. **In India, bivalent vaccine (against serogroups "A" & "C") is presently imported from out side by the Central Govt., primarily for immunising the Haj pilgrims.**

A & C

Ideally, in an outbreak of meningococcal meningitis, whole of the community should be vaccinated to cut down the transmission of the disease. **One dose is sufficient as it is considered a booster following wide spread mild and subclinical upper respiratory infection due to *N. meningitidid* in the community.**

About 7-10 days are required for the development of immunity after vaccination, which is longer than the average incubation period of the disease. Thus, vaccination can not prevent the secondary cases. It also has no effect on established carriers.

In children below 2 years, the vaccine has poor immunogenic response. But, as the outbreak takes years to subside, **a second dose after 3 months** can be given to these children.

In a country like ours, it may not be feasible and economical to immunise the whole population. But, immunisation if and when decided, should be targeted to the high risk groups, who are to be identified at first. They include: clinicians, laboratory officials, health staff and people staying in segregated places (jail, hostel, residential school, barrack, camp, remand house etc.), as well as, in difficult terrain and remote tribal areas. Once these groups are identified, their vaccination needs to be started as rapidly as possible to achieve maximum benefit in terms of cases prevented. The period of immunity varies from 1-3 years.

Dose & route of immunisation

The vaccine after reconstitution with the diluent (supplied along with), should be used within 24 hours. The dose is 0.5 ml subcutaneously irrespective of age.

17.7. Other measures:

- Closure of schools and banning of large gatherings etc. have not been shown to be effective in curtailing the spread of epidemics.
- **The information of the outbreak should be provided to the geographically contiguous districts/states.**

ANNEX-I

NATIONAL DISEASE SURVEILLANCE PROGRAMME

Meningococcal meningitis

(Format for collecting information on individual case in sentinel centres)

Name of the Centre: District:

Patient's name: IPD Reg.No.:

Age: Sex:

Complete residential address: Address during last 10 days:

Chief complaints with duration: Fever:
Headache:
Nausea/vomiting:
Other (specify):

Date of onset:

Date of hospitalisation:

Clinical diagnosis:

Laboratory investigations & results:

Final diagnosis#:

Specific treatments given:

Out come: Cure & discharged/Died@/Absconded/LAMA*:

Date of outcome:

Additional information in case of Medical college:

- Antibiogram:
- Serogroup:

Level of information to count a "case".
@ Level of information to count a "death".
* LAMA: Left against medical advice

ANNEX-II

NATIONAL DISEASE SURVEILLANCE PROGRAMME
Meningococcal meningitis

(Format for sending report on Acute meningitis by PHC, CHC, and other Passive reporting centres)

Name of Centre: District:

Reporting month: Year:

Date of reporting:

Number of cases*	Number of deaths

---

* Cases include deaths also

Signature of in-charge
Date:

ANNEX-III.

NATIONAL DISEASE SURVEILLANCE PROGRAMME
Meningococcal meningitis

(Format for sending report on meningococcal meningitis by sentinel centres)

Name of the centre: District:

Reporting month: Year:

Date of reporting:

Age group (in years)	No. cases* Male	Female	Total	No. deaths Male	Female	Total
0 - < 1						
1 - < 5						
5 - 9						
10 - 14						
15 - 24						
25 & above						

* Cases include the deaths also.

Additional information in case of Medical college:

- Antibiogram:
- Serogroup:

Signature of In-charge
Date:

ANNEX-IV

NATIONAL DISEASE SURVEILLANCE PROGRAMME
Meningococcal meningitis

(Format for sending report by district health office)

Name of district:

Reporting month:

Date of reporting:

	Number of cases	Number of deaths
Acute meningitis		
Meningococcal meningitis		

---

* Cases include deaths also

Signature of DHO
Date:

ANNEX-V

NATIONAL DISEASE SURVEILLANCE PROGRAMME
Meningococcal meningitis

(Format for sending report by state nodal office)

Name of state:

Reporting month:

Date of reporting:

	Number of cases*	Number of deaths
Acute meningitis		
Meningococcal meningitis		

---

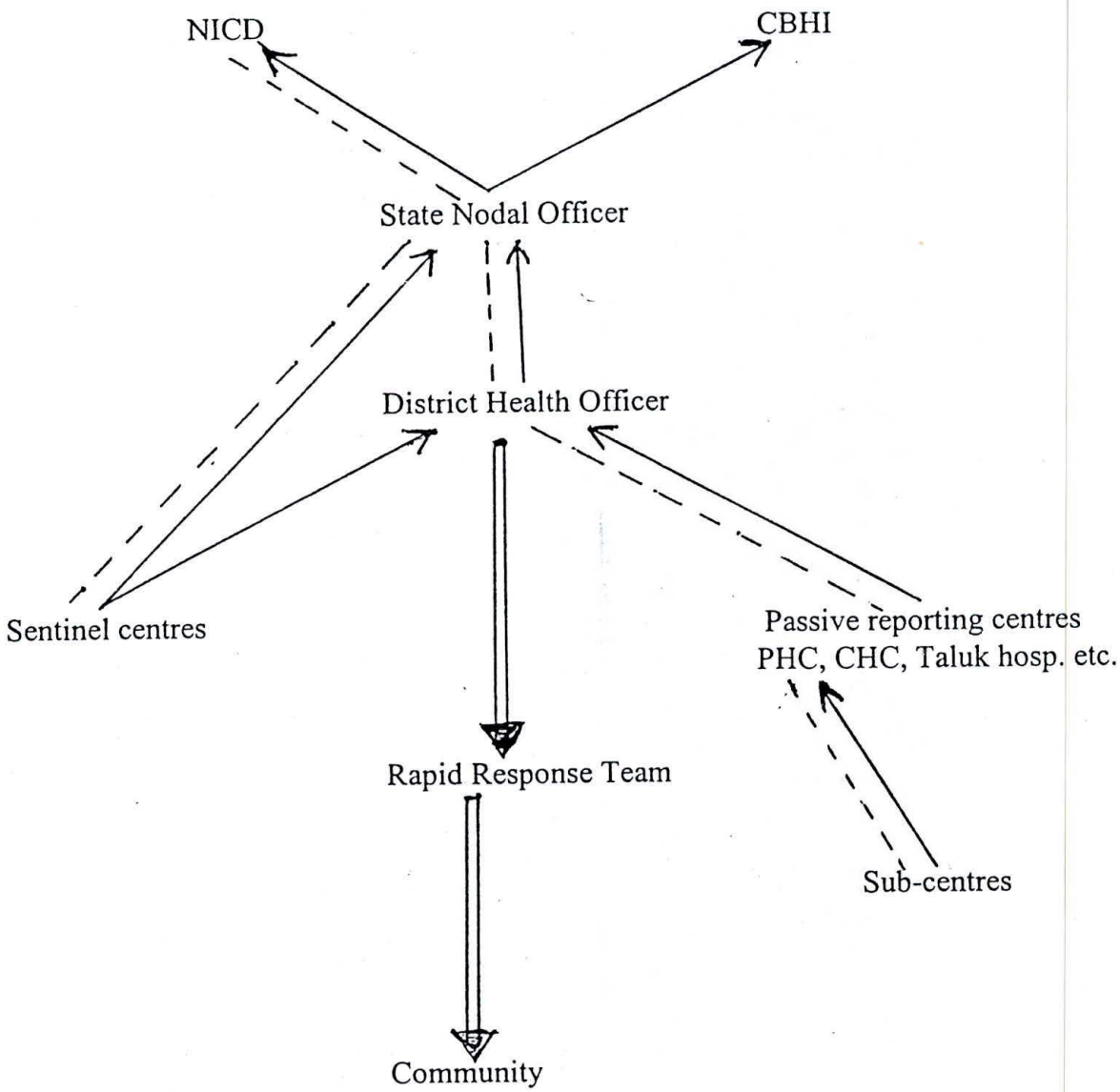
* Cases include deaths also

Signature of state nodal officer
Date:

ANNEX-VI

NATIONAL DISEASE SURVEILLANCE PROGRAMME
Meningococcal meningitis

Chart showing flow of information:



Direction of flow of report ——→
Direction of flow of feedback - - - - - →
Direction of action at community level ⟹

ANNEX-VII

NATIONAL DISEASE SURVEILLANCE PROGRAMME

(OUTBREAK INVESTIGATION REPORT)

General information:

State :.....

District :.....

PHC/Town :.....

Village/Ward :.....

Population :.....

Background information:

Person reporting the outbreak :.....

Date of report :.....

Date investigation started :.....

Person(s) investigating the outbreak :.....

Details of investigation:

Describe how cases were found (may include (a) house to house search in the affected area; (b) visiting blocks adjacent to the affected area; (c) conducting records review at local hospitals; (d) requesting health workers to report similar cases in their areas etc.).

Descriptive epidemiology:

- Cases by time, place and person (attach summary tables and relevant graphs and maps).
- Age-specific attack rate and mortality rates.
- High risk age group and geographical areas.

Description of control measures:

Description of measures for follow-up visits:

Brief description of problems encountered:

Factors which, in your opinion, contributed to the outbreak:

Conclusions and recommendations:

INVESTIGATION & CONTROL OF OUTBREAKS

CHOLERA

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
(DIRECTORATE GENERAL OF HEALTH SERVICES)
22-SHAM NATH MARG, DELHI - 110 054

JULY 1997

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1. Notification of cholera cases

1.1. Cholera is endemic in India and several outbreaks of the disease have been reported. Because cholera has the potential of rapid spread leading to an acute public health problem, special attention is required to be given to the surveillance and prompt follow up action on reported cases of cholera.

1.2. A suspect case of cholera must be notified immediately by messenger, telephone or fax to the local health office. Weekly notification of confirmed cholera cases is required to be made by the state health authorities to the Directorate General of Health Services (Director, Central Bureau of Health Intelligence, Pushp Bhavan, Madangir Road, New Delhi - 110062) and endorsed to Director, National Institute of Communicable Diseases, 22 Shamnath Marg, Delhi -110054, (Phone:- 2521272, 2521060, 2913148; FAX: 2922677; Telegram: COMDIS, DELHI). The age, sex and address of the patient should be included.

1.3 If appropriate measures are taken, cholera remains a focal disease restricted to a limited habitation. Therefore, reporting of taluka and district help in identifying the affected area.

1.4. The first suspect case of cholera in the area must be notified immediately to the local health officer. Laboratory confirmation should be obtained at the earliest opportunity and the results intimated to local health office as soon as these become available.

2. Clinical manifestation and case definition

2.1. In a majority of the cases, the infection is mild with no overt symptoms or only mild diarrhoea. However, in a few cases the onset is rapid with severe watery diarrhoea and vomiting, resulting in loss of large amounts of fluids and electrolytes.

CASE DEFINITION

Presumptive

- Severe dehydration or death from acute watery diarrhoea in a patient 5 years of age or older
- Acute watery diarrhoea in a patient 5 years of age or older in an area where an epidemic is occurring

Confirmed

- *Vibrio cholerae* O1 or O139 is isolated from the stool samples of any patient with diarrhoea

2.2. The clinical features of moderate to severe cases of cholera are the following:

- acute onset
- severe watery diarrhoea
- vomiting
- signs of dehydration and electrolyte imbalance

DEHYDRATION	SEVERE DEHYDRATION
• increased thirst • restlessness irritability • decreased skin turgor • dry mouth	• lethargic • floppy (hypotonia) • unable to drink • decreased urine • fall in BP • tachycardia • unconscious

HYPOKALEMIA
• hypotonia • cardiac arrhythmia • paralytic ileus • cardio-respiratory failure

Note:

Any two or more signs of dehydration in a patient with history of acute watery diarrhoea and vomiting

Any one or more signs of severe dehydration or hypokalaemia in a patient with history of acute watery diarrhoea and vomiting

2.3. The symptoms of mild cases of cholera are clinically indistinguishable from non-specific acute diarrhoea. In more than 90% of the cases, cholera is mild.

2.4 It would also be useful to know if:

- there are any other laboratory confirmed cases in area

OR

- there is a clustering of cases clinically compatible with cholera

Any one of the two above will support the presumptive diagnosis of cholera. It would also facilitate better understanding of the epidemiology of disease and institution of appropriate control measures.

3. Causative agent

3.1. There are more than 60 serogroups of *Vibrio cholerae*, but only serogroup O1 and 0139 cause cholera.

3.2. *V.cholerae* O1 occurs as two biotypes - classical and El Tor. Each biotype also occurs as two serotypes - Ogawa and Inaba.

3.3. Almost all the recent cholera outbreaks have been caused by the El Tor biotype. Cases caused by the classical biotype have not been reported in India since 1980. The El Tor biotype also causes a higher proportion of asymptomatic infections than the classical biotype and survives longer in the environment.

3.4. In late 1992, large scale epidemics occurred in India and Bangladesh caused by a new serogroup - *V.cholerae* O139.

4. Reservoir

4.1. Man is the only host. Patients remain infectious usually for a few days after recovery from clinical symptoms. Occasionally the carrier stage may persist for several months. Antibiotics, to which the strain is susceptible, shorten the period of communicability.

4.2. *V.cholerae* can survive for long periods in the environment and can live in association with certain aquatic plants and animals, making water an important reservoir for infection.

5. Mode of transmission

5.1. Infection usually spreads through contaminated water and food.

5.2. The dose of *V.cholerae* required to produce illness depends on the susceptibility of the individual. It can be affected by the level of acidity in the stomach (the vibrio is destroyed at pH 4.5 or lower) and by immunity produced by prior infection. In endemic areas, breast-feeding protects infants and young children.

COMMON SOURCES OF INFECTION	
Drinking water • contaminated at its source • contaminated during storage • ice made from contaminated water	**Food** • contaminated during or after preparation • fruits and vegetables, 'freshened' with contaminated water and eaten raw • fruits and vegetables, grown at or near ground level and fertilised with night soil or irrigated with water contaminated with human waste, and eaten raw

6. Incubation period

6.1. Incubation period varies from a few hours to 5 days, usually 2-3 days.

7. Clinical management

7.1. Early treatment, in most cases by oral rehydration therapy, can reduce the case fatality of cholera to less than 1%. If treatment is delayed or inadequate, death from dehydration and circulatory collapse may follow rapidly.

7.2. The clinical condition of the patient should be monitored during and after rehydration until diarrhoea stops. Rapid loss of fluids and salts can result in dehydration, acidosis and potassium depletion if symptoms of diarrhoea and vomiting persist. Rehydration therapy should continue to replace ongoing loss of fluids and salts. Young and malnourished children need special attention.

ORAL REHYDRATION THERAPY

- Recommended ORS solution
  - * WHO formula (see annex 5)
- Unsuitable ORS
  - * ORS with high glucose content
  - * ORS with saccharine and colouring agents

7.3. Nearly 80 to 90% of the patients can usually be adequately treated with oral rehydration salt (ORS) solution alone, without intravenous therapy and antibiotics.

ORAL REHYDRATION THERAPY

- give 100 ml/kg body weight of ORS solution in the first 3-6 hours to correct dehydration
- if the patient is thirsty and wants to drink more, allow to drink
- after rehydration has been achieved, continue giving ORS solution for replacement of ongoing losses. Plain water and home available fluids can be taken

7.4. In severely dehydrated patients ORS solution should be started as soon as the patients are able to drink, even before the initial intravenous therapy has been completed. Patients can also be permitted to drink plain water. For preparation of ORS at home, see annex 6.

Quantity	*Infants*	*Older children/ Adults*
30 ml / kg body wt 70 ml / kg body wt	First 1 hour Next 5 hours	First 30 min Next 2½ hours
100 ml / kg body wt	6 hours	3 hours
• reassess clinical condition every 1-2 hours; if hydration is not improving give IV infusion more rapidly	• assess for signs of overload as patient recovers - evidence of swelling, shortness of breath or puffiness	• if patient can drink, start ORS solution along with IV infusion. When signs of severe dehydration disappear, continue with ORS

7.5. Intravenous therapy may be required for those with clinically severe form of cholera. IV fluids should be used only for the initial rehydration of patients with *severe dehydration*, including those who are in shock. Ringer's lactate solution is the preferred fluid for intravenous rehydration. Normal saline solution is less effective for intravenous rehydration, but can be used if Ringer's lactate solution is unavailable. ***Plain glucose solutions are ineffective and should not be used.***

IV FLUID THERAPY

- Preferred
  - * Ringer's lactate solution
- Suitable
  - * Normal saline
    (*does not correct base acidosis and potassium losses*)
- Unsuitable
  - * Plain glucose (dextrose) solution
    (*is not effective for rehydration, correction of base acidosis or replacement of potassium losses*)

7.6. Patients on intravenous fluid therapy should be monitored at regular intervals to check for signs of improvement in the clinical condition of the patient. Care must be taken to ensure that the patient is not overloaded.

7.7. In severe cases, antibiotics can reduce the volume and duration of diarrhoea and can shorten the period during which the cholera vibrios are excreted. Antibiotics can be given orally as soon as vomiting stops, usually within 3-4 hours of starting rehydration. ***There is no advantage in giving injectable antibiotics which are expensive.***

COMPLICATIONS OF IV FLUID THERAPY

Pulmonary oedema is caused when too much IV fluid is given, and especially when metabolic acidosis has not been corrected. The latter is most likely to occur when normal saline is used for IV rehydration and ORS solution is not given at the same time. When the guidelines for IV rehydration are followed, pulmonary oedema should not occur. ORS solution never causes pulmonary oedema.

Renal failure may occur when too little IV fluid is given, when shock is not rapidly corrected, or when shock is allowed to recur, especially in persons above the age of 60. Renal failure is rare when severe dehydration is rapidly corrected and normal hydration is maintained according to the guidelines.

- Guidelines for Cholera Control, WHO 1993

7.8. ***Use of antibiotics for mild cases is not recommended.*** This will hasten the development of antibiotic resistant strains as well as exhaust supplies which may be needed for severe cases. Patients who benefit most from antibiotic treatment are those with severe dehydration.

7.9. The choice of antibiotics should take into account local patterns of resistance to antibiotics. Knowledge of antibiotic sensitivity patterns in the immediate or adjacent areas is important. Studies conducted at NICD, Delhi; National Institute of Cholera and other Enteric Diseases (NICED), Calcutta and King Institute of Preventive Medicine, Chennai have shown that *Vibrio cholerae* that are currently prevalent are resistant to furazolidone, cotrimoxazole, ampicillin, nalidixic acid and streptomycin. These continue to be sensitive to tetracycline and norfloxacin.

No anti diarrhoeal, anti-emetic, antispasmodic, cardiotonic or corticosteroid drugs should be used to treat cholera. Blood transfusion and volume expanders are not necessary.

- Guidelines for Cholera Control, WHO 1993

7.10. Patients should be encouraged to take food, after severe vomiting has stopped, usually within 3 to 4 hours after starting rehydration. Breast-feeding of infants and young children should be continued.

8. Health education

8.1. Health education and public awareness and co-operation are important to control an outbreak. If the community knows how the outbreak spreads and what measures they can take in their own families, the risks can be considerably reduced. It is also important

that the public should know that treatment is simple and effective and there should be no cause for panic. ORS packets should be widely accessible. While the key messages will essentially remain the same for all areas, the language and style may be adapted to local needs.

The Guidelines for Cholera Control, WHO 1993 have suggested several messages. Some of these are given below:

KEY POINTS FOR PUBLIC EDUCATION

To prevent cholera

- drink water from a safe source or water that has been disinfected (boiled or chlorinated)
- cook food or reheat it thoroughly and eat it while it is still hot
- avoid uncooked food unless it is peeled or shelled
- wash hands before preparing or eating food
- wash hands after using toilet or any contact with excreta
- dispose off human excreta promptly and safely

Remember

- with proper treatment, cholera is not fatal
- take patients immediately to a health facility
- give increased fluids. If ORS packets are available, give ORS solution as soon as diarrhoea starts
- cholera vaccination is *not* recommended

THREE SIMPLE RULES FOR CHOLERA PREVENTION

- Cook your food
- Drink safe water (chlorinated or boiled)
- Wash your hands

IS YOUR WATER STORED PROPERLY?

- Clean water can become contaminated again if it is not stored safely
- Water should be stored in a clean container with a small opening and a cover. It should be used within 24 hours
- Pour the water from the container or use a tap or ladle to draw water from the container. Do not dip a cup or glass in a container to draw water

8.2. It is particularly important to inform the public that most cases of cholera can be treated with simple measures and that vaccination is not effective.

9. Laboratory support

9.1. Treatment of cholera does not depend on the results of laboratory examination. However, laboratory examination of specimens from the first few suspected cases is important to confirm diagnosis and to determine the characteristics of the organism.

9.2. A sufficient number of stool specimens should be examined to identify the causative organism and test its sensitivity to antibiotics. Once the presence of cholera is confirmed, ***it is not necessary to examine specimens from all cases or contacts***. In fact, this should be discouraged since it places an unnecessary burden on laboratory facilities and is not required for effective treatment.

9.3. Stool specimens or rectal swabs should be sent to the laboratory in a transport medium (e.g. Cary-Blair medium, VR medium, Alkaline Peptone Water). If a transport medium is not available, cotton tipped rectal swab soaked in the liquid stool should be placed in a sterile plastic bag and tightly sealed. Specimens should be collected before the patient has received any antibiotics.

9.4. Full particulars of the patient(s) from whom samples have been collected must be sent along with the samples as many factors can influence the results of the laboratory tests. The information that should accompany each stool sample is given below:

- name
- name of mother or father
- sex
- date of onset of symptoms
- provisional diagnosis
- clinical outcome (recovered, under treatment, dead, not known)
- antibiotic received prior to collection of sample - Y/N/not known
- date sample collected
- full address

9.5. Apart from prior treatment with antimicrobials, the conditions of collection and transportation of samples can influence laboratory tests. The recommended practices and precautions to be taken to minimise deterioration in the quality of the sample are given in the box.

9.6. Keep an inventory of all laboratories in the district which can undertake culture and identification of *V.cholerae* 01. The names of these laboratories should be known to the medical officers of the peripheral health facilities. Each laboratory must be well stocked with

the media and other reagents. The available stocks should be verified well before the expected seasonal increase of cases of diarrhoeal diseases.

9.7. In districts considered at high risk where outbreaks have been reported in the past, orientation session of the paramedical personnel, laboratory technicians and medical officers is recommended for updating their knowledge and skills in the correct procedures for collection, storage and transportation of laboratory samples.

9.8. The states could also identify a reference laboratory to perform antibiotic sensitivity tests.

COLLECTION AND TRANSPORTATION OF STOOL SAMPLES

- collect the stool sample before the patient receives an antibiotic
- use a clean cotton tipped swab and introduce well into the rectum. When this is done well, the swab will become moist and may be faecally stained.
- alternatively, collect freshly passed liquid stool in a bottle or a cotton tipped swab.
- send the sample to the laboratory in a tightly sealed screw capped sterile bottle *if the sample can reach the laboratory within two hours.*
- send the sample to the laboratory in a tightly sealed screw capped sterile bottle with Cary-Blair transport medium (or VR medium or Alkaline Peptone Water) *if it will take more than two hours to reach the laboratory.*
- if transport medium is not available, soak strips of blotting paper with liquid stool. Send these to the laboratory in carefully sealed plastic bags to prevent drying.
- send the samples using a cold chain. If this is not possible send at ambient temperatures.
- bottles or plastic bags should be placed in separate plastic bags each to prevent leakage of the potentially contaminated material.
- each sample should be labelled. Detailed information as indicated at 9.4 should be sent for each sample.

9.9. More complicated procedures such as phage typing and toxin testing are undertaken by the national reference laboratory at the National Institute of Cholera and other Enteric Diseases (NICED), P-33, CIT Road, Scheme XM, Beliaghata, Calcutta - 700 010, Phone:-3504598, 3505533, 3504478, 3500448 FAX:-3505066.

10. Prevention and control of an outbreak

10.1. The risk of an outbreak of cholera can be minimised and an outbreak can be prevented from spreading further by taking measures given below. There are no other alternatives for the control of an outbreak of cholera.

- provision of safe water
- adopting safe practices in food handling
- sanitary disposal of human waste
- personal and domestic hygienic practices

10.2. The above steps are required both as long-term measures to prevent cholera as well as measures to be taken in a focal area where an outbreak is anticipated. Community participation is essential to preempt an outbreak so that safe practices are followed for storing water and for food handling.

10.3. When an outbreak occurs or when the risk of such outbreaks is high, the co-operation of other government departments, non-governmental agencies and the community often becomes necessary. Such help will be more forthcoming if mechanisms for interaction have been developed before the onset of an outbreak. It might be useful to convene a meeting of the concerned departments, community representatives and the NGOs before the expected seasonal increase of cases of diarrhoeal diseases. Some mechanism for briefing the press should also be established. Some suggested areas in which the government departments and NGOs can assist may be seen at Annex 7.

10.4. According to WHO guidelines, chemoprophylaxis, vaccination and travel & trade restrictions have been found to be ineffective and are not recommended for the control of an outbreak of cholera or for the prevention of its spread to other areas.

INEFFECTIVE MEASURES

- chemoprophylaxis
- vaccination
- travel and trade restrictions (*cordon sanitaire*)

10.5. Mass chemotherapy is not only ineffective in preventing the spread of cholera, but it also diverts manpower and resources from effective measures. In several countries, it has contributed to the emergence of antibiotic resistance in the vibrio, depriving severely ill patients from valuable treatment. The value of selective chemotherapy of household contacts is also doubtful. It is not recommended as a routine measure.

10.6. Vaccines that are currently available do not have high vaccine efficacy rates. 2 doses are required for primary immunization. In those who are immunised, protection lasts for 3-6 months only. Vaccination does not reduce the incidence of asymptomatic infections or prevent the spread of infection. Vaccination campaigns divert resources and manpower from more useful control activities. Inadequately sterilised needles and syringes may transmit the parenterally transmitted infections such as HIV and Hepatitis B. ***No country requires travellers to have a cholera vaccination certificate.***

RISK OF CHOLERA TRANSMISSION THROUGH FOOD TRADE

Although there is a theoretical risk of cholera transmission associated with international food trade, the weight of evidence suggests that this risk is small and can normally be dealt with by means other than embargo on importation.

A large number of tests carried out on commercially imported foods from affected countries (most recently from South America) have not detected *Vibrio cholerae* 01. Indeed, although individual cases and clusters of cases have been reported, WHO has not documented a significant outbreak of cholera resulting from commercially imported food.

- Guidelines for Cholera Control, WHO 1993 page 29

10.7. Travel and trade restrictions between countries or different areas within a country do not prevent the spread of cholera. Majority of the infected individuals have no symptoms. Setting up check-posts requires massive inputs and diverts attention from other more useful control measures.

11. Preparatory action in anticipation of an outbreak

11.1. Alert health personnel and hospitals to report increase or clustering of cases of diarrhoea. If a case of dehydration following diarrhoea is seen in children 5 years or older, cholera should be suspected and notified to the local health office immediately. All health facilities including those having only OPD should maintain records of patients seen. Address of the patients should be recorded and maintained locally for use in case an outbreak occurs. If there is a sudden increase in cases or clustering of cases in an area, field investigations should be carried out and necessary corrective action taken. An effective surveillance system can provide an early warning signal and help in initiating appropriate actions to quickly contain outbreaks of all waterborne diseases, including cholera.

Particular attention may be given in the pre-monsoon period before the expected seasonal increase of water-borne diseases; however, these measures are expected to be in place round the year.

11.2. Ensure that the health personnel are adequately trained in oral rehydration therapy and that the recommended guidelines are followed in the hospitals. If necessary, orientation sessions or retraining may be organised. Early treatment can save many lives.

11.3. Arrange random checks for water quality for coliform organisms (faecal contamination). Special attention may be given to high risk pockets. In places where water is found to be of unsatisfactory quality follow-up action may be taken with the concerned authorities for water supply. If feasible, chlorination should be carried out to render water safe for drinking (Annex 3).

11.4. Health educational activities should be carried out in the community to promote safe practices especially before the monsoons when the seasonal increase of cases of diarrhoeal diseases can be expected.

11.5. Check that adequate stocks of essential supplies are available and have been distributed to the peripheral health institutions well in advance of the expected seasonal increase of cases of diarrhoeal diseases. ORS packets should be available in all the health facilities. It is recommended that adequate stocks of bleaching powder, chlorine tablets, IV fluids and appropriate antibiotics are in stock in case of an emergency.

11.6. **A nodal officer should be identified at the state and district levels** with the responsibility of collecting and analysing relevant surveillance reports and for ensuring that appropriate follow-up action as required is taken up promptly. The name of the nodal officer should be made widely known so that she/he could be contacted in case of an emergency or if clarifications or additional information is required by the medical and health personnel in the periphery.

12. Investigation of an outbreak

12.1. Recognition of early warning signals, timely investigations and the application of specific control measures can limit the spread of an outbreak and prevent deaths. Control measures are most effective when selective interventions are applied early.

12.2. When an increase in the number of cases of diarrhoeal diseases is reported, the areas from where the cases are reported should be checked. Diagnosis should be confirmed of as many cases as possible to check that it is clinically compatible with cholera.

12.3. Stool samples from a few typical cases may be collected and sent for laboratory examination (see 9)

12.4. Line listing of cases, including age, sex and address should be made. Active search should be made for more cases.

12.5. Identify sources of drinking water. Check water quality for bacteriological contamination. If piped water supply, check for possible leakage. If a common source of water supply has been identified, inform public not to use the water for drinking purposes or for washing utensils etc.

12.6. Arrange alternate source for water, including tankers if possible. If possible chlorinate water source. Distribute tablets for domestic chlorination of water along with instructions for its proper use, if necessary (Annexe 4). Contact concerned authorities for water supply.

12.7. Alert health facilities and hospitals in the area. Make sure that adequate supplies of ORS, IV fluids, appropriate antibiotics and other essential supplies are available.

PRECAUTIONS AT TREATMENT CENTRES

- quarantine not necessary, although restricted contact between patients and community may be encouraged
- strict infection control measures such as face mask, gloves or special clothing for hospital staff and visiting family members is not required
- frequent hand-washing is recommended
- safe disposal of excreta and vomit is necessary

12.8. Arrange health educational activities in the community regarding personal and domestic hygiene, recommended sources of safe water supply, oral rehydration therapy and health facilities where patients can be taken for treatment (see 8).

12.9. Active case reporting should be continued for at least one week after the last case.

OTHER PRECAUTIONS AT HOME AND IN THE COMMUNITY

- bedding, clothing, mattresses can be disinfected by thorough drying in the sun
- the simplest method for a family or a small rural health centre to dispose of cholera stools is by putting them in a pit latrine or burying them.
- In large hospitals, liquid stools and vomit can be disinfected before disposing these in the toilet by 4% hypochlorite. Semi-solid and other waste can be incinerated.

12.10. On confirmation of a focal outbreak of cholera, take precautionary measures as indicated in para 11 in other potentially high risk pockets in the district.

12.11. Notify immediately to the concerned state officer as soon as a clinically compatible case of cholera is reported.

12.12. The detailed procedures for conducting outbreak investigation, data analysis and report writing are given in a separate document (Outbreak Investigations - A Field Guide). A suggested format for report writing may be seen at Annex 8.

ACKNOWLEDGEMENT

The manual has been edited by Dr Jotna Sokhey, Director NICD; Dr Rajesh Bhatia, Consultant (Microbiology); Dr D.C.Jain, Joint Director (Epidemiology) and Dr Jagvir Singh, Deputy Director.

SAFE WATER

- boiling for 1 minute will kill or inactivate *V.cholerae* and other common organisms that cause diarrhoea. Boiling is, however, expensive and not practical especially in areas where outbreaks of cholera and other diarrhoeal diseases are most likely to occur because of fuel shortages

- when surface water/ handpump water is contaminated, this source should be closed for drinking water purposes. This information should be prominently displayed indicating that the source of water is not fit for use. In Delhi, shallow handpumps are painted red. Alternate water source should be provided, including water tankers during the course of an outbreak

- where it is feasible chlorination of the water source, such as a draw-well should be immediately organised

- in urban areas, immediate co-ordination with the agency responsible for water supply should be organised to ensure chlorination of water source and repair of water pipes, if indicated

- chlorine releasing tablets may be used for domestic purposes in the area of an outbreak

- community should be encouraged to use narrow mouthed containers for water storage to reduce secondary transmission in the family

RECOMMENDED MINIMUM CHLORINE LEVELS IN WATER DISTRIBUTING SYSTEMS

- 0.5 mg/litre - at all sampling points in a piped water system
- 1.0 mg/litre - at standpost
- 2.0 mg/litre - in tanker trucks at filling

Annex 3

CHLORINATION OF DRINKING WATER

PREPARATION OF STOCK SOLUTION
(1% solution in 1 litre of water)

Add to one litre of water any of the following:

- calcium hypochlorite (70%) — 15 gram

OR

- bleaching powder or chlorinated lime (30%) — 33 gram

OR

- sodium hypochlorite (5%) — 250 ml

OR

- sodium hypochlorite (10%) — 110 ml

The stock solution should be used within one month. It should be kept in a closed container in a cool place away from light

CHLORINATION OF WATER

(Add stock solution to water)

- 0.6 ml or 3 drops — 1 litre of water
- 6 ml — 10 litres of water
- 60 ml — 100 litres of water

Allow water to stand for 30 minutes before using. The residual chlorine level should be 0.2 to 0.5 mg/litre

Annex 4

DOMESTIC CHLORINATION OF DRINKING WATER

- crush commercially available chlorine-releasing tablet
- put in the water container with 20 litres of water
- allow to stand for 30 minutes
- use water within 24 hours

Containers with a narrow mouth are recommended for the storage of drinking water.

Annex 5

Composition of ORS (net weight = 27.9 gm)	
Ingredient	**Weight (gm)**
Sodium Chloride IP	3.5
Potassium chloride IP	1.5
Sodium citrate IP	2.9
Glucose anhydrous IP	20.0

Note:-To be used in one litre of potable water.

Annex 6

HOW TO PREPARE ORS SOLUTION FROM A 1 LITRE PACKET

- Mothers must be taught on how to measure one litre of water. It is important that a measure which is commonly available in the homes is identified and mothers told the exact number of such measures that will make 1 litre.
- There is no need to boil water for preparing the ORS solution. Clean water which the household normally uses for drinking purposes can be used.
- Hands must be washed before making the ORS solution.
- Full packet of ORS must be used. Generally the mother will tend to save a part of the packet in order to use it later. It is important to emphasise that the whole packet is to be mixed in one litre of water.
- The container should be kept covered. The solution can be used for 24 hours and should be discarded if not consumed within this period. Fresh solution should be prepared, if required.

Annex 7

INTER DEPATMENTAL COMMITTEE
SUGGESTED AREAS OF RESPONSIBILITY AND ACTION

District administration

- mobilize resources by organizing meetings with
  - concerned government departments
  - non-governmental agencies
  - community leaders
- ensure supplies of ORS packets and other essential items
- ensure adequate quality monitoring of water samples
- arrange safe water supply
- ensure adequate facilities for transportation of serious patients to district hospital, if necessary
- provide relevant information to the press
- monitor status of control activities
- repair leakage in pipe water supply

District Health Office / Municipal Health Office

- alert health personnel to report cases and to monitor trends
- arrange active surveillance in affected area
- ensure that treatment guidelines are followed in hospitals and other health facilities
- ensure availability of ORS packets and other essential items
- arrange health educational camps and distribution of health educational material
- arrange chlorination of water sources if possible
- arrange water quality monitoring
- convene meeting under district administrator to seek co-operation of other government departments and NGOs

Concerned Department (s) responsible for water supply

- repair leakage in pipe water supply
- arrange potable water supply, including water tankers if necessary
- arrange chlorination of water
- ensure water quality monitoring

Other government departments such as social welfare, education, tribal welfare and NGOs

- dissemination of relevant information
- promotion of oral rehydration therapy
- reporting clustering of acute diarrhoea, jaundice (*Pilia*) cases

Panchayat members, village pradhans, community leaders

- dissemination of relevant information
- promotion of oral rehydration therapy
- reporting cases of acute diarrhoea and jaundice (*pilia*)
- monitoring chlorination of water sources such as wells
- arranging transportation of serious cases to hospital

OUTBREAK INVESTIGATION REPORT

General Information

State :_____

District :_____

Town/PHC :_____

Ward/Village :_____

Population :_____

Background Information

Person reporting the outbreak :_____

Date of report :_____

Date investigations started :_____

Person(s) investigating the outbreak _____

Details of Investigation

Describe how the cases were found (may include: (a) house-to-house searches in the affected area; (b) visiting blocks adjacent to the affected households; (c) conducting record reviews at local hospitals; (d) requesting health workers to report similar cases in their areas, etc.):

_____

_____

_____

_____

_____

Descriptive Epidemiology

- Cases by time, place and person (attach summary tables and relevant graphs and maps).
- Age-specific attack rates and mortality rates
- High-risk age-groups and geographical areas.

Description of Control Measures Taken

_____

Description of Measures for Follow-up Visits:

Brief Description of Problems Encountered

Factors Which, in Your Opinion, Contributed to the Outbreak

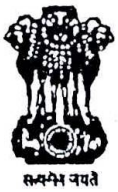
Conclusions and Recommendations

Date

(Name and Designation)

INVESTIGATION & CONTROL OF OUTBREAKS

JAPANESE ENCEPHALITIS



NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
(DIRECTORATE GENERAL OF HEALTH SERVICES)
22-SHAM NATH MARG, DELHI - 110 054

AUGUST 1997

Contents

1. Introduction

1.1. Japanese Encephalitis (JE) is a disease of public health importance because of its epidemic potential and high case fatality rate. In patients who survive, complications may lead to life long sequelae.

1.2. The first major outbreak of JE occurred in Bankura and Burdwan districts, West Bengal, in 1973 and since then has spread to many states/UTs of country.

1.3. JE is a mosquito-borne zoonotic disease. The virus infects mainly animals and birds. Man is an incidental host. The risks increase in areas where ecological conditions facilitate transmission to man.

1.4. Though JE is primarily a disease of rural agricultural areas, where vector mosquitoes proliferate in close association with pigs and other animal reservoirs, its epidemics have also been reported in peri-urban areas where similar conditions may exist.

2. Notification of cases

2.1. If an outbreak of JE is suspected it must be reported immediately to the district health office. The state health authorities must be informed through the quickest mode of communication, preferably through telephone, fax or e-mail of the details of the outbreak including investigation and control measures initiated. The National Institute of Communicable Diseases, 22-Sham Nath Marg, Delhi - 110 054 (Phone:- 2521272, 2521060, 2913148; FAX:- 2922677; Telegaram:- COMDIS, DELHI) is expected to be kept informed of the action taken.

2.2. The total number of overt cases per village will be few and the local health personnel must be alert about any case(s) of encephalopathy in their areas. Active surveillance through key individuals in the community should be encouraged in the high risk pockets, for example, places where piggeries are commercially established.

3. Causative agent

3.1. JE is caused by a group B arbovirus (flavivirus). The virus is antigenically related to other flaviviruses including dengue and yellow fever viruses.

4. Mode of transmission

4.1. The infection is transmitted through the bite of an infected culicine mosquito. In human beings, viraemia is mild and lasts for a short duration. Infection in man is the dead end of transmission. Man to man transmission has not been documented.

4.2. The transmission cycle is maintained in animals and birds.

5. Reservoir of infection

5.1. JE virus has its natural cycle in wild or domestic vertebrates and mosquitoes. The animal hosts include pigs, cattle and horses. Water birds such as pond herons, cattle egrets, poultry birds and ducks play a significant role in the natural history of JE virus.

5.2. Pigs are the major vertebrate hosts. Although, infected pigs do not manifest any overt symptoms of disease they develop tremendous viraemia and can infect the mosquitoes **The pigs are considered as amplifying hosts.**

5.3. The currently available evidence does not indicate major role of cattle in transmission of disease.

5.4. Horses develop active disease but viraemia is rarely present in high titre or for long periods. Horses are unlikely source of mosquito infection.

5.5 Infection in man appears to be correlated with living in close proximity with animal reservoirs, especially pigs

6. Vector

6.1. Mosquitoes belonging to the *Culex vishnui* group (*Culex vishnui, Culex pseudovishnui, Culex tritaeneorhynchus*) are the most important vector species in India. 11 more species of mosquito have been incriminated as vectors of JE.

6.2. Culex mosquitoes generally breed in water bodies with luxuriant vegetation. Irrigated rice fields, shallow ditches and pools are common breeding places.

6.3. Culex mosquitoes are zoophilic, feeding primarily on animals and wild birds. They rest outdoors in vegetation and other shaded places but in summer may also rest indoors. The mosquitoes are outdoor as well as indoor feeders.

6.4. Epidemics usually coincide with the monsoons and post-monsoon period when the vector density is high. However, in endemic areas, sporadic cases may occur throughout the year.

6.5. Female mosquitoes get infected after feeding on a viraemic host. They can transmit the virus to other hosts after an extrinsic incubation period of 9 to 12 days. The mosquitoes remain infected for life. The average life period of a mosquito is about 21 days. Culex mosquitoes can fly for long distances (4-5 km).

7. Incubation period

7.1. The incubation period in man, following mosquito bite probably varies from 5 to 15 days.

8. Clinical manifestations

8.1. The clinical features of JE are those of encephalopathy. The patient will give a history of acute onset with fever and change in behaviour or sensorium lasting for more than 24 hours. Focal neurological deficits may or may not be present.

8.2. Disturbances of sensorium are reflected as lethargy, somnolence, irritability, apathy or loss of consciousness. The patient may develop difficulty of speech and other neurological deficits like ocular palsies, hemiplegia, tremor and ataxia. There may also be loss of bladder and bowel control. The focal neurological signs may be stationary or progressive.

CLINICAL LABORATORY FINDINGS IN ACUTE ENCEPHALITIC STAGE
Blood: moderate to high polymorph leucocytosis (total count ranging between 10,000 and 35,000 per cu.mm with neutrophils ranging between 50 and 90%).
Cerebrospinal fluid (CSF): the cell count generally ranges between 10 to 1000 per cu.mm. with a predominance of lymphocytes. The protein may be raised slightly to 60-100 mg percent and sugar may also be slightly elevated.

8.3. In majority of the cases, however, the infection is mild with no overt clinical symptoms or mild fever with headache. Individuals develop immunity after infection. In endemic areas cases are, therefore, seen more often in children under 15 years of age as the adult population is already immune through natural infection. In virgin areas, cases may be seen in all age groups.

8.4. Meningitis should be excluded as a cause of the encephalitic syndrome, so that specific treatment is started in time. Treatment of JE, which is a viral infection, is symptomatic. Other causes of encephalopathy include other viral infections, drugs and toxins. Some of the causes are listed in the box.

8.5. One of the complications of malaria caused by *P.falciparum* is encephalopathy. Since malaria is relatively common, the diagnosis of malaria should also be considered. The diagnosis is confirmed if field investigations indicate malariogenic conditions, illness is clinically compatible and anti-malaria treatment is effective. The facilities for making blood slides and reading these slides should be available at each PHC.

8.6. Typhoid fever is another important endemic disease which can give rise to symptoms mimicking Japanese Encephalitis. It should be excluded by careful history and physical examination and blood culture for *S.typhi*, if necessary.

COMMON CAUSES OF ENCEPHALOPATHY	
Infectious	
• Vaccine Preventable Diseases	Pertussis, measles
• Viral	Japanese encephalitis, mumps, rubella, herpes simplex, enteroviruses, cytomegalovirus, Epstein Barr, rabies
• Other	Enteric fever, malaria, tuberculosis, toxoplasmosis, cryptococcal infection, leptospirosis
Non-infectious	
• Fluid & electrolyte imbalance	Hyper/hyponatremia Alkalosis/acidosis
• Metabolic	Thiamine deficiency, diabetic acidosis, hypoxia, hypoglycaemia, uraemia, hyperbilirubinemia
• Toxic	Heavy metals (lead, mercury, arsenic) Insecticides, Carbon - Monoxide
• Physical	Heat hyperpyrexia
• Tumours/Abscess	

8.7. Epidemiological and entomological investigations are useful in leading to a presumptive diagnosis of JE. The presence of risk factors such as the vector and amplifying hosts supports the diagnosis of JE.

8.8. The diagnosis of JE is confirmed by laboratory tests.

8.9. Case definition may be seen at Annex 1.

9. Case fatality rate and sequelae

9.1. Case fatality rate is high in severe cases. Case fatality rates of 20 to 40% have been recorded.

9.2. Patients who recover from the acute episode may have neurological sequelae. These occur with variable frequency and depend on age and severity of the illness. The commonly observed sequelae are:

- mental impairment
- severe emotional instability
- personality changes
- paralysis

10. Laboratory confirmation of diagnosis

10.1. The diagnosis of JE can be confirmed by serological tests. The tests include detection of IgM antibodies which appear after the first week of onset of symptoms and are detectable for one to three months after the acute episode.

A rising antibody titre of IgG antibody in paired sera taken at an interval of 10 days or more is confirmatory.

10.2. IgG antibodies indicate previous infection and are useful for conducting sero-epidemiological studies to determine the extent of silent infection and immunity levels in the local population.

10.3. Antigen for conducting the tests is not yet commercially available. Antigen for detection of IgG antibody is produced in limited quantities for operational research and outbreak investigations at the National Institute of Virology (20-A, Dr. Ambedkar Road, Pune - 411 001; Phone:-627301, 627302; FAX: 622669). With the antigen received from NIV, the National Institute of Communicable Diseases (NICD) supports outbreak investigations on the request from the state health authorities.

10.4. Isolation of virus from blood or CSF is not routinely done for diagnostic purposes as viraemia is of short duration.

10.5. Blood for serological studies should be carefully collected taking due universal precautions. Specimen containers should be clearly labelled. Each specimen should be accompanied with the detailed information about the case as given in the box so that the results could be scientifically interpreted.

INFORMATION WHICH SHOULD ACCOMPANY EACH SAMPLE

- name of the patient
- name of the mother/father
- age
- sex
- complete residential address
- name of the hospital/PHC sending the sample
- registration number of the patient
- date of onset of illness
- date of hospitalisation
- date of collection of sample
- history of immunisation against JE
- provisional diagnosis
- brief clinical findings
- results of clinical laboratory investigations

11. Clinical management

11.1. There is no specific treatment of JE. However, supportive treatment and good nursing care can significantly reduce case fatality rate. It is, therefore, important that cases are referred to a hospital as early as possible if encephalitis is suspected and treatment commenced without waiting for serological laboratory results.

11.2. In the acute phase, clinical management is directed at maintaining fluid and electrolyte balance. Keeping the airway open in a comatose patient is important. Patients with hypoxia may require oxygen.

11.3. If the patient has convulsions, appropriate drugs are prescribed.

11.4. There is no evidence that gammaglobulins or corticosteroids have a beneficial effect and these are not recommended.

11.5. Physio-therapy may be necessary in the convalescent stage.

11.6. Long-term neurological sequelae may require specialised care.

12. Prevention and control of an outbreak

12.1. A surveillance system should be established so that any case of encephalopathy is immediately reported to the local health authorities. Necessary field investigations must be carried out in the area of residence of the patient to check for amplifying host and vector.

12.2. The preventive measures are directed at reducing the vector density and in taking personal protection to prevent the bite of mosquitoes. The isolation or destruction of the amplifying hosts (usually pigs), which are the main source of infection, is not practical as these animals do not show any overt signs of illness and it is not possible to identify infected animals.

12.3. Immediate measures are called for to reduce the density of mosquitoes by spray of insecticides. The public should also be informed to take necessary precautions against mosquito bite such as use of full sleeved clothes, mosquito nets at night and mosquito repellent creams.

12.4. Long-term measures include the recommended steps for vector control under the National Malaria Eradication Programme (NMEP).

12.5. Patients should be referred to the district hospital as the peripheral health facilities may not have adequate resources to manage serious cases.

12.6. Pockets of high risk should be identified so that these areas could be given more attention with regard to control measures, health educational activities and field supervision. Such areas would include villages and peri-urban areas where pigs are reared, especially if these are close to paddy fields which are rich breeding places for Culex mosquitoes.

12.7. Isolation of patients and disinfection of secretions and excretions are not required as JE virus is not transmitted person to person.

RECOMMENDED MEASURES FOR CONTROL OF JE OUTBREAK

- Vector control
- Prevention of man-mosquito contact
- Prevention of animal reservoir-mosquito contact ? if feasible
- Health education

12.8. A killed JE vaccine is produced at the Central Research Institute (CRI), Kasauli from the brain of suckling mice inoculated with the Nakayama JE strain. The vaccine is not recommended for use for the control of an outbreak. Two doses of 1 ml each (0.5 ml for children under the age of 3 years) should be administered subcutaneously at an interval of 7-14 days. A booster injection of 1 ml should be given after a few months (before 1 year) in order to develop full protection. Re-vaccination may be given after 3 years. Since the risk of JE is not universal and is limited to focal areas, JE vaccination is not included in the national immunization programme in India.

12.9. Arrange health educational activities in the community regarding prevention of mosquito bites by use of mosquito nets and mosquito repellent creams, avoiding sleeping in or near places where pigs are housed, taking measures like mosquito proofing, residual insecticide spray in piggeries and reporting suspected cases at the health facilities early.

13. Community Participation

13.1. Community participation is essential for the prevention and control of an outbreak of JE. The community must be encouraged to take steps to protect themselves from mosquitoes by using bed-nets. The co-operation of the community is also important during the periodic insecticide spray.

13.2. In pockets of high risk, active surveillance of JE should be encouraged so that first case(s) are immediately reported to the local health authorities.

13.3. Co-ordinated efforts by government departments such as agriculture, animal husbandry, flood control, irrigation and industrial development are essential so that risk factors for mosquito breeding could be reduced. In places where piggeries are being established with government support, the concerned departments must be aware about the potential risk of JE and must take necessary precautionary measures for vector control in collaboration with the local health authorities.

13.4. In an event of an outbreak, the co-operation of other government departments will help to bring it more effectively under control. An inter-departmental committee for outbreak prevention and control should be constituted which should meet periodically. Panchayat members, key community representatives and NGOs should be included as members of the committee. A meeting of the committee should be convened before the expected seasonal increase of water and vector borne diseases. In districts where risk factors of JE exist, status of control measures for JE should also be assessed. The suggested areas of responsibilities of the various departments is given at Annex 5.

13.5. Press release and other mechanisms for briefing the press should be considered to that the press has access to reliable information.

RISK FACTORS FOR JE OUTBREAK
• high density of Culex mosquitoes • presence of amplifying hosts (pigs) • paddy cultivation

14. Investigation of an outbreak

14.1. The investigation of an outbreak of JE is similar to the investigation of other epidemic prone diseases. The first principle is to receive early warning signals, confirm diagnosis and to take prompt measures for control of the outbreak. Control measures are most effective when selective measures are applied early.

14.2. Line list of cases, including age, sex and address should be maintained. Active search should be made for more cases. Serum samples should be collected for laboratory confirmation of diagnosis.

14.3. Vector surveillance should be immediately initiated and should include collection of adult mosquitoes, identification of mosquito species and density and assessment of susceptibility of vectors to available insecticides.

14.4. On confirmation of an outbreak of JE, take precautionary measures in other potentially high risk pockets in the district.

14.5. After the outbreak is over, a detailed report of the outbreak must be written. Format for outbreak investigation report given at Annex 2.

CASE DEFINITION

SUSPECT CASE

- High grade fever of acute onset with at least two of the following:
- Decrease in level of consciousness independent of convulsions
- Significant change in mental status either in behaviour or personality
- Convulsions

PROBABLE CASE

- Suspected case of Japanese Encephalitis, and
- Usually not more than a few cases (1-2) in one village.
- With or without signs of meningeal irritation and varying degree of neurological deficits
- Pleocytosis in CSF of more than 10 cells/mm^3 in clinically compatible illness.

CONFIRMED CASE

- Serology for JE antibodies.
- Demonstration/isolation of virus/antigen from CSF, brain tissue or rarely blood.

Annex 2

OUTBREAK INVESTIGATION REPORT

General information

State :----------

District : ----------

PHC/Town :----------

Village/Ward :----------

Population :----------

Background Information

Person reporting the outbreak :----------

Date of report :----------

Date investigations started :----------

Person(s) investigating the outbreak :----------

Details of investigation

Describe how cases were found (may include (a) house to house search in the affected area; (b) visiting blocks adjacent to the affected area; © conducting record reviews at local hospitals; (d) requesting health workers to report similar cases in their areas etc.):

_____
_____
_____
_____
_____

Descriptive epidemiology

Cases by time, place and person (attach summary tables and relevant graphs and maps)

Age specific attack rates and mortality rates

High risk age groups and geographical areas

Vaccination status of cases, unaffected population

Prevalence and density of JE vectors

Prevalence of reservoirs specially pigs

Description of control measures

_____
_____
_____
_____

Description of measures for follow-up visits:

_____
_____
_____
_____

Brief description of problems encountered:

_____
_____
_____
_____

Factors which ,in your opinion , contributed to the outbreak

_____
_____
_____
_____

Conclusions and recommendations

_____
_____
_____
_____

Date (Name and designation)

Annex 3

Format for MPWs for reporting suspected JE case

Village_____

District_____

Name_____

Age_____Sex_____

Address_____

Fever____Yes/No__
Date of onset_____
Headache__Yes/No__
Stiffness in Neck____Yes/No__
Fits __Yes/No__
Weakness of extremities ____Yes/No__
Vomiting___Yes/No_
Unconsciousness ____Yes/No__

Recovered/still suffering/death with date_____

House : Kacha/Pacca

Animals - Pigs/Cattle/Buffalo/Goat/Hens/Ducks etc.

Sleeping habit - Outside/same room with animals/separate room

Format for medical officers for reporting JE case

District_____ Village_____
House No._____ Head of Family_____
Date of Survey_____

Particulars of Patient:

Name_____Age(Years)_____Sex: M/F
Father's Name_____ Date of onset of illness _____
Recovered / Still suffering / Died on_____
Date of investigation_____
Informant_____

Symptoms

Fever (more than 100 ° F):	Yes / No
Headache :	Yes / No
Neck rigidity :	Yes / No
Convulsions:	Yes / No
Unconsciousness :	Yes / No
Sequelae:	
Some form of paralysis :	Yes / No
Involuntary movements:	Yes / No

Similar illness in family/neighbourhood:
Name_____Age_____Sex_____Date of onset_____

Clinical laboratory investigations

Samples sent for confirmation of diagnosis (and results, if available)

Diagnosis

(Signature & Designation)

Annex 5

INTER-DEPARTMENTAL COMMITTEE
SUGGESTED AREAS OF RESPONSIBILITY AND ACTION

District administration

- mobilize resources by organizing meetings with
  - concerned government departments
  - non-governmental agencies
  - community leaders
- ensure vector control measures
- ensure adequate facilities for transportation of serious patients to district hospital, if necessary
- provide relevant information to the press
- monitor status of control activities

District Health Office / Municipal Health Office

- alert health personnel to report cases and to monitor trends arrange active surveillance in affected area
- arrange health educational camps and distribution of health educational material
- arrange vector control measures
- arrange drugs and fluids in nearest hospital in adequate quantity
- convene meeting under district administrator to seek co-operation of other government departments and NGOs

Concerned Department (s) responsible for agriculture and animal husbandry

- ensure measures for reducing factors favouring the breeding of mosquitoes
- support measures for vector control
- take precautionary measures where piggery development projects are initiated
- report suspected cases of JE

Other government departments such as social welfare, education, tribal welfare and NGOs, and Panchayat members, village pradhans and community leaders

- ensure measures for reducing factors favouring the breeding of mosquitoes
- support measures for vector control
- take precautionary measures where piggery development projects are initiated
- report suspected cases of JE
- arrange transportation of serious cases to hospital

OUTBREAK

INVESTIGATION & CONTROL

A FIELD GUIDE

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
(DIRECTORATE GENERAL OF HEALTH SERVICES)
22-SHAM NATH MARG, DELHI - 110 054

AUGUST 1997

CONTENTS

1. ROLE OF OUTBREAK INVESTIGATIONS

1.1. Many communicable diseases are endemic in India. An effective surveillance system is essential for planning, implementation and monitoring the disease control programmes. Many of these diseases have seasonal patterns and cyclic trends which can be discerned through the surveillance system. These diseases can also cause outbreaks with the potential to spread rapidly and cause many deaths. Outbreaks of new and reemerging infections may also occur.

1.2. Precautionary measures taken in anticipation of an outbreak can prevent an acute public health emergency and save lives. While outbreaks cannot always be predicted or prevented, recognition of early warning signals, timely investigations and application of specific control measures can limit the spread of the outbreak and prevent deaths. Control measures are most effective when selective interventions are applied early.

1.3. The primary purpose of an outbreak investigation is to control the outbreak, limit its spread to other areas and assess how prevention strategies can be further strengthened to reduce or eliminate the risk of such outbreaks in the future.

2. PREPAREDNESS FOR OUTBREAK INVESTIGATIONS

2.1. The field guide has been designed to help in making decisions regarding investigations, specific interventions and follow-up measures. While adaptations may be necessary at local levels depending on the field situations and available resources, it is important that preparatory action is taken so that the district is able to meet the eventualities if an outbreak occurs. Some of the recommended measures are given in the Box.

RECOMMENDED PREPARATORY ACTION

- Identify a nodal officer at the state and district levels.
- Establish a routine surveillance system.
- Constitute an inter-disciplinary team at state/district levels (rapid response team).
- Train medical and other health personnel.
- List the laboratories at regional /state/district level.
- List 'high-risk' pockets in the rural / urban area.
- Establish a rapid communication network.
- Undertake IEC activities for community participation.
- Ensure that essential supplies are available at the peripheral health facilities and buffer stocks are maintained at the district level.
- Set-up an inter-departmental committee, including NGOs.

2.2. The identification of a nodal officer at the state and district levels is important for receiving information about unusual events and for ensuring that necessary follow-up action is taken in a timely and effective manner.

2.3. An inter-disciplinary expert team at state and district levels (rapid response team) comprising of epidemiologist or public health specialist, microbiologist, clinician(s), entomologist and concerned programme officer should be constituted and necessary administrative orders issued authorising the team to move quickly to the site of the outbreak if such a request is made by the nodal officer. This is particularly important in the event of an unusual outbreak for which the services of the expert team may be required at short notice.

2.4. Concerned medical and health personnel should be trained in the principles of outbreak investigations including recognition of early warning signals, epidemiological and entomological parameters, differential diagnosis, laboratory support and specific control interventions.

2.5. Some laboratory tests are expected to be done at the PHC level; others may be available only at the district level. For some laboratory tests, samples may need to be sent to the state, regional or national levels. A list of the laboratories with full address, telephone and fax numbers along with the type of tests conducted is recommended to be maintained. The nodal officer should identify gaps in laboratory services at each level that can be filled within the given resources. Special emphasis must be placed on proper correction of clinical samples, their storage and transportation. A suggested checklist of services at district level is given at Annex 1.

2.6. An effective routine surveillance system must be established in each district. Data should be regularly analysed as suggested in a seperate module **"Suveillance of Epidemic Prone Diseases"** available from National Institute of Communicable Diseases (NICD), 22-Shamnath Marg, Delhi-110 054; Phone: 2521272, 2521060; FAX: 2922677; (E-mail: jotna@del2.vsnl.net.in). Early warning signals will be missed in the absence of a reliable surveillance system.

2.7. It is important that adequate facilities are established at the district level for rapid and efficient analysis of the surveillance data. The nodal officer and other key personnel should receive training in the use of computers. Software such as EPI INFO are particularly useful in maintaining and analysing line lists of cases of various diseases. Such analysis provides valuable epidemiological information regarding high risk groups, areas and factors.

2.8. In each district there are likely to be some areas which will be at a higher risk of outbreaks because of inadequate facilities such as water

supply, poor sanitation, or some areas may have poor transportation and communication facilities which may impact negatively on early notification of an outbreak and health seeking behaviour of the community. Spot maps of these areas may be prepared so that special attention could be given to the surveillance reports from these areas.

2.9. In the event of an outbreak, the state officer is required to be notified immediately. The district officers may also need technical and other support in the event of an unusual outbreak or if the diagnosis is not confirmed. Since the National Institute of Communicable Diseases (NICD) is the nodal office at the national level, it is expected that notification of the outbreak would be made immediately to NICD, also indicating if any technical support is required or not. It is obvious that the communication network must be rapid in order to optimize its effectiveness. Under the national disease surveillance programme, it is expected that the district and state levels will be linked to NICD through e-mail and fax. Telephone facilities are expected at the PHC level.

2.10. The prevention and control of outbreaks require the close and active cooperation of the community. Community level IEC activities should be supported so that key messages regarding the control of the diseases and prevention of outbreaks are known. Health education material which has been prepared in advance and field tested will be useful if there is an outbreak in the area because such material may be required at short notice. The medical and health personnel should establish contact with community leaders and other key personnel in their areas which would be useful in receiving early warning signals and in soliciting community support during an outbreak. Local private practitioners could provide valuable support.

2.11. The nodal officer at the district level, in consultation with the concerned programme officers, must ensure that essential supplies are in place in the peripheral health facilities and that adequate buffer stocks are maintained at the district level. Inventories should be checked before the expected seasonal increase of cases. Some life saving medicines such as ORS packets may also be kept at the village level especially in the high risk pockets and areas which might become inaccessible during the monsoons.

2.12. When an outbreak occurs or when the risk of such outbreaks is high, the cooperation of other government departments, non-governmental agencies and the community often becomes necessary. Such help will be more forthcoming if mechanisms for interaction have been developed before the onset of an outbreak. It might be useful to convene a meeting of the concerned departments, community representatives and the NGOs before the expected seasonal increase in cases of diseases. Some mechanism for briefing the press should also be established. Some suggested areas in which the government departments and NGOs can assist may be seen at Annex 2.

To include Panchayat Presidents in list, list ZP

2.13 Detailed demographic, environmental and cultural profile of district (including maps) should be available.

3. DISEASES COVERED IN THE FIELD GUIDE

3.1. The diseases included in the field guide can broadly be classified into the four following groups:

- endemic diseases with the potential of causing focal or large outbreaks, e.g. malaria, cholera, measles, viral hepatitis, meningococcal meningitis etc.
- diseases for which eradication or elimination goals have been set. A single case of such diseases should be treated as an outbreak, e.g. poliomyelitis, guineaworm and yaws
- rare but internationally important diseases with high case fatality rates with the potential of importation due to conducive epidemiological conditions, e.g. yellow fever
- outbreaks of unknown aetiology

3.2. An attempt has been made to prepare a common methodology and format for investigations to simplify its use.

3.3. First reports about an outbreak will be based on clinical syndrome and diagnosis will be presumptive. Suggestions have been made for clinical syndromes, outbreaks of which are more commonly reported. Investigations must be made, including epidemiological, entomological and laboratory, to rule out the more common causes first. A thorough knowledge about clinical symptoms and epidemiological parameters is important for outbreak investigations. In case of doubt, second opinion should be taken. It may also be necessary to seek the help of the rapid response team.

3.4. If the outbreak is unusual, case fatality rates are very high, aetiology cannot be determined or if the clinical syndrome had not been reported in the area before, the state officer and NICD would be expectecd to assist the district authorities in investigations.

3.5. During the course of investigations, universal infection control precautions are expected to be implemented. While investigating outbreaks of haemorrhagic fever or if parenteral route of transmission is suspected, protective gear such as gloves, masks etc. are expected to be worn by health personnel treating or investigating patients. Procedures that require the collection of blood and other clinical samples should be undertaken carefully.

4. DEFINITION OF AN OUTBREAK

4.1. **An outbreak or epidemic is defined as the occurrence in a community of cases of an illness clearly in excess of expected numbers.** While an outbreak is usually limited to a small focal area, an epidemic covers larger geographic areas and has more than one focal point.

4.2. The number of cases which are needed to be called an outbreak varies according to several factors. It depends on past historical patterns of the disease, case fatality and complication rates, potential of spread to other areas. For some diseases even a single case (acute poliomyelitis, guinea worm, unusual acute severe episode of an illness of unknown aetiology) constitutes an outbreak.

4.3. In places with established epidemic cycles, it may be worthwhile to initiate necessary preventive measures and alert treatment centres in the area in anticipation of a cyclic increase in cases. Similar precautionary measures can be taken to minimize seasonal increase in cases.

4.4. States and districts should establish criteria on the number of cases that constitute an epidemic based on their local situations.

4.5. Increase in the total number of cases does not, however, necessarily indicate increase in the incidence of the disease. Variations in the number of reporting sites, completeness of reporting, geographical size of the catchment area and size of the population are factors that must be taken into consideration while analysing reports.

5. TRIGGER EVENTS FOR OUTBREAK INVESTIGATIONS

5.1. It is useful to have a short list of 'warning signals' which should trigger an investigation. If personnel at the local levels are alert about these signals and respond rapidly, it may be possible to arrest the outbreak in the early stages when control measures are most effective and can usually be undertaken within local resources.

5.2. It is important, however, that the list of such 'trigger events' is kept as short as possible so as not to divert resources from routine health care services and control programmes. At the same time, an important purpose of a surveillance system is to prevent outbreaks or detect them in the early stage, and therefore the health personnel must be responsive to the warning signals. Some suggested trigger events are listed below in the Box.

SUGGESTED TRIGGER EVENTS FOR OUTBREAK INVESTIGATIONS
• clustering of cases/ deaths in time and space • unusual increase in cases or deaths • unusual increase in bacteriologically proved cases, even if total cases are not increased • patients older than 5 years of age with severe dehydration following diarrhoea (usually accompanied with vomiting) • acute febrile severe illness of unknown etiology • acute haemorrhagic fever • acute fever with altered sensorium • acute flaccid paralysis in a child • even one case of suspected plague • even a single case of measles or any other epidemic prone disease from a tribal or other poorly accessible area • even one case of a disease which is not known to be present in an area • shifting in age distribution of cases • occurrence of two or more epidemiologically linked cases of meningitis • unusual isolates • high vector density • disasters

5.3. Intensified surveillance activities are recommended and health personnel alerted in the event of the following:

- disasters
- high vector density detected by vector surveillance
- unsatisfactory quality of water detected by water monitoring
- unusual isolate detected by a laboratory from clinical/ environmental sample

6. FIELD INVESTIGATIONS AND ANALYSIS OF REPORTS

6.1 Verification of the Diagnosis

6.1.1. **The first principle of outbreak investigation is to confirm the diagnosis of as many reported cases as possible**. Much time and effort may be wasted due to misdiagnosis. The reported cases should be investigated by a medical officer to confirm the diagnosis. The majority of the cases are expected to fall within the standard case definitions. In situations of doubt, whether an illness meets the case definition, a second opinion may be sought.

6.1.2. Laboratory confirmation of clinically-diagnosed cases or identification of the etiological agent may sometimes be necessary. Under such a situation, samples should be collected carefully from a few selected cases. **It is not necessary to collect specimen from all cases** as it is not essential for the outcome of outbreak investigations and control measures. Collection of unnecessary samples is discouraged as it

places a heavy load on the laboratory and some tests are very expensive. The types of tests, sample collection and transportation procedures have been detailed in a separate module.

6.2 Epidemiological Features and Standard Case Definitions

6.2.1. It is necessary to know the clinical and epidemiological features of the diseases to effectively investigate and control outbreaks. For easy reference, the epidemiological parameters should be available in tabular form.

6.2.2. Differential diagnosis of some of the important and common clinical syndromes have been given in this document. Medical officers are encouraged to expand the list and include other conditions prevalent in their areas.

6.2.3. Standard case definitions are summarised in a separate module **"Case Definitions of Epidemic Prone Diseases"** available from NICD.

6.3 Criteria for Investigating an Outbreak

6.3.1. After an outbreak has been confirmed, a decision must be made and resources committed to investigate it, and to initiate follow-up activities to limit its further spread. Specific interventions will depend on the cause of the outbreak and mode of transmission. For example, if dengue fever/ dengue haemorrhagic fever is suspected or confirmed, vector surveillance and vector control activities should be taken up with high priority. Vector surveillance should be immediately undertaken in some of the other high risk pockets even if no case has been reported from the area. Similarly, if cholera is suspected, water quality monitoring should be stepped up and the health facilities alerted. Specific suggestions have been discussed later in this module.

6.3.2. It is the responsibility of the local medical officer to arrange for the treatment and follow-up of cases and contacts. It should be noted that during an outbreak a higher percentage of more severe forms may occur. Case fatality rate may increase due to mitigating factors such as high malnutrition rates in young children or limited knowledge in the community about the danger signs of severe illness. Precautionary measures need to be taken and decide where and when to seek medical help in a timely manner. For example, post measles pneumonia kills many childrens. Mother must consult the health facilities whenever signs and symptoms of pneumonia appear for proper treatment of cases. It will only be possible if mothers know about signs and symptoms of pneumonia i.e. increased respiratory rate and indrawing of ribs.

6.4 Case-Finding Through Active Surveillance

6.4.1. After establishing the existence of an outbreak and verifying the diagnosis, it becomes important to accurately define and count the cases. During the period of the outbreak, all the cases of the disease under consideration occurring in that area should be identified and listed.

6.4.2. Active surveillance refers to actively seeking out cases. This may include visits or telephone calls to the medical facilities or private practitioners that might expect to admit or attend cases of the disease. Active surveillance through peripheral health personnel, personnel from other government departments, NGOs and key community representatives provides additional information about cases who may not have been seen at government health facilities. Valuable information can be obtained by contacting key community representatives, especially if the outbreak is focal. Such information is useful in defining the extent of the outbreak.

6.4.3. Suspect case definition may be used to identify cases. While it is recognized that there may be some over-reporting of cases, it is nonetheless important that no case is missed, especially if treatment is available. Some of the epidemic prone diseases are amenable to simple and cost effective treatment and case fatality rates are usually less than 1% if such treatment is applied early during the course of the illness (for example, in cholera). In the absence of specific treatment, mortality rates can be very high.

6.4.4. Depending on the disease and the resources available to investigate the outbreak, it may even be desirable to conduct house-to-house visits, especially in the homes of contacts of cases. In some circumstances, community assistance may be enlisted for house-to-house visits.

6.4.5. Completeness of reporting and relevant information on majority of cases are important for determining high risk factors, complications and mortality rates and health seeking behaviour as well as to further understand the clinical pattern and epidemiology of the disease. The information is also necessary to assess the quality of the national programmes (if relevant).

6.4.6. Active surveillance should be maintained until the outbreak is over. Developing a regular schedule of daily or weekly (depending on the urgency of the situation) visits or telephone calls to concerned institutions or individuals will help maintain the flow of surveillance information to those analysing the outbreak and directing control activities.

6.5 *Line-Listing, Defining and Counting of Cases*

6.5.1. It is important that information from surveillance be recorded in a standardized manner. Persons who are ill and meet the case definition for the outbreak disease should be entered onto what is called a "line-listing". This is a list of all reported cases with the relevant data on each case which provides the basis for counting of cases. It is the data-base on which the descriptive epidemiology of the outbreak can be made and it can serve as the basis for other, more sophisticated, analytical epidemiological studies, such as risk-factor analysis, vaccine efficacy, etc.

6.5.2. The line list suggested for field use is shown in Annex-3.

6.6 *Descriptive Epidemiology*

In investigating an outbreak, it is necessary to provide a detailed description in terms of time, place and person.

6.6.1. Cases by time

The onset of illness of the cases should be graphed by hours (for example, in food poisoning), days, weeks or months, as appropriate. This type of graph is commonly referred to as an epidemic curve. The epidemic curve can be helpful in identifying the index (first) case or cases, and may even suggest patterns or modes of transmission. During outbreaks, analysis of cases by time will help to document the trend of the epidemic and to monitor the effectiveness of the containment measures.

In case of endemic diseases, it is also useful to present previous data on a line graph. Such graphs help to demonstrate the magnitude of the outbreak compared to the previous reported incidence, rapidity of spread of the disease and evaluation of control efforts.

6.6.2. Cases by place

A map of the area or even a rough sketch can be drawn showing where each reported case resides to indicate geographical distribution of cases and to identify high risk pockets. In some situations, serial spot maps, by week or by month (or by disease generation) may provide insight into the pattern of the spread of the disease over time.

Cases tend to cluster, and it may also be useful to mark affected schools or other institutions on the map in addition to residential locations. Such mappings may assist in identifying the sources of infection.

Status of water supply, environmental and sanitary conditions, and population density are important factors in the outcome of outbreak investigations and control. These should be as descriptive as possible.

6.6.3. Cases by person and population characteristics

Cases should be described in terms of age, sex, occupation, socio-economic parameters, migration, vaccination history and other relevant characteristics.

It is usually sufficient to group cases by age-groups by 0-11 months, 1-4 years, 5-14 years, 15-45 years and ≥ 45 years. However, in the case of vaccine preventable disease outbreaks which usually affect young children, grouping with smaller intervals will be needed such as <1 year, 12-23 months, 24-59 months, 5-9 years, 10-14 years and ≥ 15 years.

While preparing tables, the population characteristics should be grouped accordingly. While calculating the age-specific attack, complication or mortality rates, the proportion of the age group under study to total population should be taken into account while calculating rates.

6.7. Description of environmental conditions

The study of environmental conditions and the dynamics of its interaction with the population and causative agents will help in the formulation of hypothesis on genesis of the epidemic, which will make the basis for the control measures to be taken. With respect to physical environment, the data on rainfall, humidity and temperature are available in the district meteorological centres. Information on natural disasters like floods, cyclone, drought, earth quake etc., as available may be utilised for the description of the epidemic. Man made situation like developmental projects on irrigation and industries may create environmental conditions conducive for disease transmission. Similarly information on drinking water supply and environmental sanitation is crucial for the investigation.

6.8. Additional steps during investigation of outbreaks of vaccine preventable diseases

6.8.1. The determination of immunization coverage levels is crucial if the outbreak is due to a vaccine preventable disease. The immunization status in the community can be assessed through immunization performance records available at the PHC. Percentage immunization coverage is estimated by dividing the total number of doses (measles and BCG) or third doses (OPV and DPT) of the vaccine administered to a specific age-group by the total population of that age-group in the affected village(s) or urban area. The data may be summarised for example for OPV as shown below.

Reported vaccination coverage for OPV

Year	Population	Estimated No. of infants	Vaccination performance					Percent coverage*
			0 dose	1 dose	2 doses	3 doses	Booster	

* Percent coverage= No. given 3 doses x 100 / Estimated number of infants

6.8.2. The age-groups vaccinated should be fully assessed as inclusion of older children may give a false sense of higher coverage. The immunization activities, including periodicity of the immunization sessions, quantities of vaccines received and cold chain system, should be reviewed.

6.8.3. Information on immunization coverage may also be available through past vaccination coverage evaluation surveys. If no such coverage evaluation surveys have been performed recently, or if estimates of immunization coverage by reports of doses administered are unavailable or suspect, (a large number of vaccinations may be given by the private sector and are not reported) then it may be useful to conduct a coverage evaluation survey as a part of the outbreak investigation which may also be used to estimate vaccine effectiveness.

6.8.4. Immunization status is an important descriptive as well as analytical parameter. The immunization status of each case must be carefully investigated to ascertain the number of doses of the vaccine received by the patient. Immunization cards or immunization registers should be checked to verify the immunization status. Verbal history should be used only if such records cannot be obtained. Ideally, the place of immunization should also be examined to assess quality of the cold chain.

6.9. Laboratory investigations

6.9.1. The results of laboratory investigations should be listed in the outbreak investigation report. Results of some tests can be obtained locally and quickly. Such tests include water quality monitoring if a water-borne disease is suspected.

6.9.2. Control activities and treatment of patients should not be delayed pending laboratory confirmation of diagnosis. Action should be initiated based on clinical, epidemiological and entomological findings.

6.9.3. Special emphasis must be placed on proper collection, transportation and storage of clinical samples.

6.9.4. It is not necessary while submitting the preliminary report to wait for the results of laboratory tests which have been sent to laboratories outside the district and which may take sometime. The results can be added in the final report.

6.10. Entomological investigations

6.10.1. Entomological investigations are an important aspect of field investigations as a number of outbreaks are vector borne. Entomological investigations are useful to confirm or rule out the probable cause of the outbreak, especially if results of laboratory tests are not available.

6.10.2. High vector density is a warning signal as the risk of vector borne outbreaks increases under such conditions. On the other hand, the risk is low if the density is maintained below critical levels.

6.10.3. Vector control measures are expected to be applied under the National Malaria Eradication Programme (NMEP). High vector density is a reflection of the unsatisfactory field implementation and monitoring of the above programme.

6.11. Determining Who is at Risk of Disease

6.11.1. The descriptive epidemiology will help to define the population groups at high risk of disease in terms of age-groups, geographical location, activity (school, mela, etc.), immunisation status and other characteristics.

6.11.2. It is more appropriate to determine attack rates rather than absolute numbers because rates take into account the variations in the population size of different groups. Such rates are generally computed by dividing the number of cases in a population group by the population size of the same group.

Example: There are 45 cases of measles among 1100 children less than 5 years of age and 52 cases in 3400 children 5-14 years of age in the affected town.

Attack rate = 45/1100 = 0.04 or 40 cases per 1 000 population less than 5 years of age

Attack rate = 52/3400 = 0.015 or 15 cases per 1 000 population 5-14 years of age

6.12. Follow-up Visits

6.12.1. Follow-up visits after the outbreak has subsided are important to make sure that late cases are not missed. Some of the diseases, such

as measles, can lead to secondary infections in children. Post-measles complications, most commonly diarrhoea and pneumonia, can occur at any time up to six weeks after the onset of the illness. Prompt treatment of these secondary infections can save lives.

6.12.2. The frequency and duration of follow-up visits will depend on many factors and decisions will have to be taken at the local level. The factors that will influence decision will be the severity of the disease, its potential for spread to other areas, maximum incubation period and the accessibility of the affected area to routine health services.

6.12.3. It is important to make follow-up visits to evaluate the control strategy and to complete the documentation of the outbreak.

6.13. Documentation

6.13.1. Documentation of the epidemic is the last, but not the least step in outbreak investigation. Important lessons can be learnt if the documentation is complete and data properly analysed. The information will be useful in drawing up long term strategies for reducing the risk of the outbreaks in future and in more effective handling of the outbreak if it were to occur. A suggested format for final report is given in at Annex-4.

6.13.2. It is also important that the report is shared with the concerned officers of other states and districts.

6.13.3. Publication of the report in a technical journal or newsletter will ensure wider accessibility of the information to others who may be interested.

6.13.4. The main findings of the outbreak should also be discussed with the members of the rapid response team and the members of the inter-departmental committee.

7. HEALTH EDUCATION

7.1. Health education and public awareness and co-operation are important to control an outbreak. If the community knows how the outbreak spreads and what measures they can take in their own families, the risks can be considerably reduced. It is also important that the public should know if treatment is available and where to seek medical help. If such information is available there is less likely to be panic and chaos. Community support will also be more forthcoming. While the key messages will essentially remain the same for all areas, the language and style may need to be adapted to local needs.

7.2. It is suggested that action for preparation of health education material and key messages is taken for epidemic prone diseases prior to

an outbreak. This will allow enough time for the preparation and field testing of the material. Some suggestions are given in the documents on specific diseases.

7.3. It is particularly important to inform the public that most cases of epidemic prone diseases can be treated with simple measures if treatment is started in a timely manner.

7.4. It is also important that the public and administrators are aware of inappropriate measures so that much time and resources are not diverted for measures which are ineffective. These include vaccination and chemoprophylaxis for cholera and quarantine measures for most infections.

8. PREVENTION AND CONTROL OF AN OUTBREAK

8.1. Water borne outbreaks

8.1.1. The risk of outbreaks of water-borne diseases such as cholera, acute watery diarrhoea, viral hepatitis, shigellosis and typhoid fever can be minimised and an outbreak can be prevented from spreading further by taking measures for the following:

- provision of safe water
- adopting safe practices in food handling
- frequent handwashing
- sanitary disposal of human waste

8.1.2. The above steps are required both as long-term measures to prevent cholera and other water borne diseases as well as measures to be taken in a focal area where an outbreak is anticipated. Community participation is essential to pre-empt an outbreak so that safe practices are followed for storing water and for food handling.

INEFFECTIVE MEASURES
chemoprophylaxis vaccination against cholera travel and trade restrictions (*cordon sanitaire*)

8.1.3. Mass chemotherapy is not only ineffective in preventing the spread of the disease, but it also diverts manpower and resources from effective measures. In several countries, it has contributed to the emergence of antibiotic resistance, depriving severely ill patients from a valuable treatment. The value of selective chemotherapy of household contacts is also doubtful. It is not recommended as a routine measure.

8.1.4. Vaccines that are currently available against cholera do not have high vaccine efficacy rates. In those who are immunised, protection lasts for 3-6 months only. Vaccination does not reduce the incidence of asymptomatic infections or prevent the spread of infection. Vaccination campaigns divert resources and manpower from more useful control activities. ***No country requires travellers to have a cholera vaccination certificate.***

8.1.5. Travel and trade restrictions between countries or different areas within a country do not prevent the spread of cholera. Majority of the infected individuals have no symptoms. Setting up check-posts requires massive inputs and diverts attention from other more useful control measures.

8.1.6. An important objective of outbreak investigations is to reduce mortality rates by early diagnosis and appropriate treatment. Case fatality rates can be significantly reduced through effective therapy. Specific treatment is not available for some diseases.

8.2 Vector borne outbreaks

8.2.1. Vector control measures should be applied as per the calendar of activities to optimize impact. The control measures should be directed at all stages of the life cycle of the vector.

8.2.2. The risk of vector borne diseases has increased in the urban and peri-urban areas due to the changing life styles and industrial activities which have made the surrounding environment more conducive for the breeding of mosquitoes and other vectors.

8.2.3. The *Aedes* mosquito is a domestic breeder preferring clean water containers. The cooperation of the community is imperative to control mosquito breeding by taking simple precautionary measures. Regular monitoring is essential to ensure that breeding sites are eliminated in a timely manner.

8.2.4. The risk of some vector borne outbreaks increases in the presence of animals or birds. For examples, pigs are considered to be amplifying hosts of Japanese Encephalitis virus. Where piggeries have been established, regular monitoring and periodic anti-vector measures are indicated. The local pradhan and panchayat members should be aware that if there is an acute case of fever with altered sensorium, the local health authorities should be notified immediately.

8.2.5. Bubonic plague and visceral leishmaniasis are other important vector borne diseases. In areas where leishmaniasis is prevalent or there is a potential threat of leishmaniasis or plague, vector monitoring and IEC activities are recommended.

8.3 Food-borne diseases

8.3.1 Food-borne diseases include food poisoning due to toxins produced by micro-organisms (e.g. *Staphylococcus aureus*) and chemicals, as well as food-borne infections (e.g. Salmonella infection). In fact, all the waterborne infections (viral, bacterial and parasitic) can be transmitted through contamination of food. Food-borne outbreaks are very common in our country. Whereas in the past contaminated food processed in the home exposed a few individuals, the food processed and distributed extensively by the industry could result in the exposure of a large number of people.

8.3.2 While investigating an outbreak of food-borne infection, efforts should be made to interview all who are exposed for history of food consumption and illness, if any. Rates of illness in those who did or did not consume a specific food item are compared, and relative risk is calculated for each food item. The implicated food would give the highest attack rates, and/or the highest relative risk. In large outbreaks, a sample of population may be interviewed, or the investigations may be by case control studies. Food handlers and suspected food also need attention during investigations.

8.4 Parenterally transmitted infections

8.4.1 Injections can transmit a variety of infections including HIV and hepatitis B and C. Inadequately sterilized needles and syringes, sharp instruments that penetrate the skin, and unscreened blood are common source of parenterally transmitted infections. Appropriate sterilization of needles (boiling for at least 20 minutes) and screening of all blood donations for HBsAg, HIV, VDRL and malarial parasites will go a long way in preventing these infections.

9. PRECAUTIONARY ACTION IN HIGH RISK POCKETS TO WHICH THE OUTBREAK CAN POTENTIALLY SPREAD

9.1. Alert health personnel and hospitals to report increase or clustering of cases or deaths. All health facilities should maintain records of patients seen, including OPD. Address of the patients should be recorded. If there is a sudden increase in cases or clustering of cases in an area, field investigations should be carried out and necessary corrective action taken. An effective surveillance system can provide an early warning signal and prevent outbreaks.

9.2. Ensure that the health personnel are adequately trained and that the recommended guidelines are followed in the hospitals. If necessary, orientation sessions or retraining may be organised. Early and appropriate treatment can save many lives.

9.3. Arrange random checks for water quality for coliform organisms (faecal contamination). Special attention may be given to high risk pockets. In places where water is found to be of unsatisfactory quality, follow-up action may be taken with the concerned authorities for water supply. If feasible, chlorination should be carried out to render water safe for drinking.

9.4. Health educational activities should be carried out in the community to promote safe practices especially before the monsoons when the seasonal increase of cases of water and vector borne diseases can be expected.

9.5. Check that adequate stocks of essential supplies are available and have been distributed to the peripheral health institutions well in advance of the expected seasonal increase of cases, for example, ORS packets should be available in all the health facilities. It is recommended that adequate stocks of bleaching powder, chlorine tablets, IV fluids, appropriate antibiotics and insecticides are in stock in case of an emergency.

10. SYNDROMIC REPORTING OF OUTBREAKS

10.1. Common syndromes

The monitoring of early warning signals and first reports will be syndromic and diagnosis will be presumptive. These may include acute cases of:

- fever
- haemorrhagic fever
- fever with altered sensorium
- diarrhoeal diseases
- jaundice
- flaccid paralysis
- severe illness of unknown aetiology.

10.2. Acute cases of fever

10.2.1. Clustering and sudden increase of acute cases of fever may be due to malaria, dengue fever or other viral fevers. If the increase meets the criteria of an outbreak, necessary clinical, epidemiological, laboratory and entomological investigations must be conducted to confirm diagnosis.

10.2.2. Fever with rash may be due to measles or chicken pox. Diagnosis can be confirmed by typical clinical presentation, age group affected and vaccination history (measles).

10.2.3. Acute fever are caused by a variety of viruses. The type of virus can be identified only by laboratory tests. However, the type of clinical presentation and other epidemiological and entomological parameters may help in presumptive diagnosis. Some of the diseases such as dengue fever have a relatively small but distinctive form of severe forms such as haemorrhagic fever and shock syndrome. The report of even a single case of haemorrhagic fever from an area which has a reported increase of acute fever cases compatible with dengue fever as well as a high density of *Aedes aegypti* is a strong indication in favour of dengue fever.

10.2.4. Malaria is relatively common in our country. Any increase in cases of acute fever from an area which has other conditions conducive for the spread of malaria, requires that malaria is considered as the cause of the outbreak.

10.2.5. Increase in the acute fever cases may be due to typhoid fever which is also relatively common in many parts of the country. Patients should be examined to see if the clinical presentation is compatible with the case definition of typhoid fever.

10.3. Haemorrhagic fever

10.3.1 Cases of haemorrhagic fever are expected to be relatively rare and few in number. It is therefore very important that notification by the clinicians is made immediately by the fastest means of communications. Investigation of the case of haemorrhagic fever may identify a dengue fever outbreak. If a clustering of acute fever cases have been reported and dengue fever is suspected on clinical grounds and entomological investigations, a reported case of haemorrhagic fever from the area will clinch diagnosis.

10.3.2. Haemorrhagic fever can also be caused by chikungunya virus. The disease is clinically indistinguishable from dengue fever. However, cases of shock syndrome have not been reported and case fatality rates are low.

10.3.3. Severe forms of haemorrhagic fever with high case fatality rates due to yellow fever and ebola fever have been reported from some countries. There has been no report from India. However, epidemiological conditions are conducive for the importation and spread of these infections. Travel history or close contact with those who have recently travelled abroad should be obtained if the above infections are suspected. State and national authorities must be notified immediately by the fastest routes of communication. Infection control precautions must be practiced while investigating or treating patients, and handling infectious biological material.

10.4. Fever with altered sensorium

10.4.1. Fever with altered sensorium (encephalopathy) may occur as a result of complication of some diseases such as malaria, measles and pertussis. Such complications occur in a relatively small proportion of the cases. Even a single case of encephalopathy therefore reflects a relatively large number of cases of the disease in the community. It is probable that clustering of cases of acute fever have already been documented through the routine surveillance system and report of a rare but well recognized and documented complicated case further confirms the cause of the outbreak. It is important that the clinicians are aware of the need to report such cases immediately so that further field investigations could be carried out.

10.4.2. One of the complications of malaria caused by *P.falciparum* is encephalopathy. Since malaria is relatively common, the diagnosis of malaria should be first considered. The diagnosis is confirmed if field investigations indicate malariogenic conditions, illness is clinically compatible and anti-malaria treatment is effective. The facilities for making blood slides and examining them should be available at each PHC.

10.4.3. A case of meningitis usually presents with sudden onset of high grade fever, severe headache, stiff neck with or without altered sensorium. Presence of nuchal rigidity and positive Kerning and Brudzinski signs confirm the clinical diagnosis. Meningitis may occur due to tubercular, viral or pyogenic organisms. A lumber puncture for demonstration and/or isolation of organisms from CSF. A commercially available latex agglutination test may provide the aetiology of some agents of meningitis i.e. *Streptococcus pneumoniae, Haemophilus influenzae B, Meningococci A&C.* It is noteworthy that meningococcal meningitis may occur in epidemic form.

10.4.4. Tuberculous meningitis is relatively common in many parts of our country. Although large number of cases are reported, TB meningitis is not expected to occur in the form of explosive outbreaks. Clinicians must, however, keep in mind TB meningitis as a differential diagnosis, especially if the patient is a young child.

10.4.5. Typhoid fever is usually characterized by gradual onset of malaise, lethargy, headache, myalgia, loss of appetite and fever that increases in stepwise fashion to reach 39-41° C in 5-7 days period. A mental apathy and dullness is common and delirium may develop. At this stage patient may present as fever with altered sensorium. Since typhoid fever is very common in our country, it should always be excluded by careful history, physical examination and blood culture for *S.typhi.*

10.4.6. JE infection is usually mild with no overt clinical symptoms or mild fever with headache. However, the patient may present with signs of encephalopathy. Usually not more than a few such cases (1-2) occur in one village. These patients will give a history of acute onset with fever and change in behaviour or sensorium lasting for more than 24 hours. Focal neurological deficits may or may not be present. Disturbances of sensorium are reflected as lethargy, somnolence, irritability, apathy or loss of consciousness. The patient may develop difficulty of speech and other neurological deficits like ocular palsies, hemiplegia, tremor and ataxia. There may also be loss of bladder and bowel control. The focal neurological signs may be stationary or progressive. In majority of the cases, individuals develop immunity after infection. In endemic areas cases are, therefore, seen more often in children under 15 years of age as the adult population is already immune through natural infection. In virgin areas, cases may be seen in all age groups. Patient should be hospitalised immediately to reduce mortality rates. Cases are confirmed by serology for JE antibodies and demonstration/isolation of virus/antigen from CSF, brain tissue or rarely blood.

10.5. Acute cases of diarrhoeal diseases

10.5.1. Water borne and food borne diseases can present with a variety of clinical symptoms. Acute watery diarrhoea in young children is the most common problem. Cases occur throughout the year, with a seasonal increase in the monsoon and post-monsoon months. Focal outbreaks of acute watery diarrhoea can occur. Such outbreaks have also been reported following outbreaks of measles. Prompt action is important to check the spread of the outbreak and to provide oral rehydration therapy which is life saving. In the absence of ORT, high mortality rates have been recorded. Death may occur within a few hours of severe episodes of acute watery diarrhoea. The younger the age group, the more susceptible they are to dehydration.

10.5.2. While diarrhoeal episodes may also occur in adults, these usually do not result in severe dehydration or death. If cases of severe dehydration or death following acute watery diarrhoea is reported in patients older than 5 years of age, cholera should be suspected and control measures at the field level should be initiated as per guidelines.

10.5.3. Outbreaks of dysentery can occur in children. Such outbreaks have the potential of causing a large number of deaths unless specific treatment is initiated in a timely manner. It is important that the community and the peripheral health personnel are aware of the danger sign of blood in the stools (bloody diarrhoea) so that medical help is sought immediately.

SYNDROMIC REPORTING OF OUTBREAKS

Acute fever

malaria
dengue fever
measles
influenza
other viral infections
plague
typhoid fever

Haemorrhagic Fever

dengue haemorrhagic fever
leptospirosis
chikungunya fever
yellow fever, ebola, hanta, lassa fever (potentially importable infections) **

Acute Fever with Altered Sensorium

falciparum malaria
japanese encephalitis
meningococcal meningitis
other meningitis (pyogenic, viral)
post measles encephalopathy *
pertussis encephalopathy *

Acute Diarrhoeal Diseases

acute watery diarrhoea in young children
acute watery diarrhoea in patients above 5 years of age with severe dehydration (suspected cholera)
dysentery (bloody diarrhoea)

Acute Respiratory Infections (Acute pneumonia)

influenza
leptospirosis
plague
anthrax
melioidosis
hanta virus

Jaundice

hepatitis E
hepatitis B & C (if focal with high case fatality rate) *
leptospirosis
yellow fever (potentially importable) **

Acute Flaccid Paralysis

poliomyelitis

Severe Illness of Unknown Aetiology

* - relatively rare and cases may be few in number

** - not reported in India. If diagnosis is suspected, travel history and close contact with those who have recently travelled abroad should be obtained.

10.6 Acute Respiratory Infections (Acute pneumonias)

10.6.1. Acute respiratory infections leading to pneumonia are major causes of morbidity and mortality in India, especially in early childhood. They can be caused by a variety of microorganisms including bacteria, viruses, fungi and parasites. Most of the pneumonias present as sporadic cases.

10.6.2. Some diseases like plague, anthrax, leptospirosis, influenza, melioidosis and hanta virus infection may result in severe outbreaks with mainly pulmonary involvement. These may affect people from all age groups. Even if a clustering of a few cases in older children and adults is noticed, investigation should be initiated promptly for impending outbreaks of these infections so that appropriate action for treatment of cases and control of outbreak is promptly taken.

10.6.3. Patients with acute pneumonia usually present with acute fever, chills, cough, chest pain, other non specific symptoms, varying degree of respiratory insufficiency, and infiltrate on chest X-ray. In addition, there may be haemoptysis and leukocytosis or leukopenia. Once pneumonia is suspected, the specific etiologic diagnosis is necessary for proper management to prevent mortality and reduce further transmission.

10.7. Jaundice

10.7.1. Although jaundice may occur due to many reasons, viral hepatitis is responsible for the majority of cases with jaundice in our country. At least 6 agents (HAV, HBV, HCV, HDV, HEV and HGV) can cause viral hepatitis.

10.7.2. Feco-orally transmitted hepatitis E virus has been responsible for virtually all the outbreaks of viral hepatitis in India. These outbreaks are invariably linked to contamination of water supply. The expression of icterus appears to increase with increasing age. There is no evidence of a chronic form. A majority of Hepatitis E cases however, occur in young adults. Secondary household cases during the outbreaks are uncommon. The case fatality rate may reach up to 20% among those infected during the 3rd trimester of pregnancy.

10.7.3. Recently, outbreaks of hepatitis B occurred in defined rural communities of Gujarat, Haryana and Rajasthan states which were epidemiologically linked to the use of unsafe injections by unqualified medical practitioners. The outbreaks were marked by high case fatality rates.

10.7.4. Acute viral hepatitis is such a sufficiently distinct clinical syndrome that it usually poses no difficulty in diagnosis. Patient develops nonspecific symptoms including malaise, weakness, anorexia, nausea, vomiting, fever, and mild pain in abdomen. Soon, jaundice and dark

urine follow. The duration of jaundice is variable, but usually lasts 1-3 weeks. There is dramatic elevation of ALT and AST (≥8 times of normal), and mild elevation of alkaline phosphatase (usually only 3 times of normal). However, specific types of viral hepatitis in individual patients can't be distinguished on clinical grounds. The diagnosis is done by serology.

10.7.5. Leptospirosis is emerging as an important cause of jaundice in many parts of country. The disease is usually characterized by abrupt onset of high grade fever, myalgia and conjuctivial suffusion. Rash is occasionally present. If patient present with jaundice, renal involvement and hemorrhage, leptospirosis should be strongly suspected. Meningitis, pulmonary and cardiac involvement may also be present in some cases. Unless treated promptly with antibiotics, it is marked by high case fatality rates. The diagnosis is confirmed by demonstration of rising antibodies, or isolation of leptospires from blood during acute illness and from urine after the first week.

10.8. Acute Flaccid Paralysis

10.8.1. Poliovirus infection is the most important cause of acute flaccid paralysis (AFP) in children. The possibility of polio should be considered for any case of AFP, even in areas with high OPV coverage levels and a very low incidence of poliomyelitis. The diagnosis of paralytic poliomyelitis should be discarded only after another diagnosis has been established.

10.8.2. Acute paralytis poliomyelitis is characterised by fever followed by abrupt onset of weakness or paralysis of limbs which does not progress after first 3 days. Paralysis is not present at birth and is not associated with serious injury or mental retardation. Typical findings on physical examination include: acute flaccid paralysis, muscle tenderness, no sensory loss, absent or depressed deep tendon reflexes, and asymmetrical findings. Wasting of affected muscle is a late finding. Residual paralysis after 60 days of onset of symptoms, or death or unknown follow-up in an AFP case makes the presumptive diagnosis. Isolation of wild poliovirus from AFP cases or contacts confirm the diagnosis of acute poliomyelitis.

10.8.3. Paralytic poliomyelitis is most often confused with Guillain-Barre syndrome (GBS), transverse myelitis, and traumatic paralysis due to sciatic nerve injury. Traumatic paralysis due to sciatic nerve injury following a misplaced gluteal injection can be differentiated by a careful history and physical examination. Fever is usually absent in GBS and the paralysis is symmetrical and distal. There are global hypotonia and global absence of deep tendon reflexes. Cramps, tingling sensation, and hypo-anesthesia of palms and soles are usually present in GBS. Many cases of polio have initially been diagnosed as GBS even by experts. Accordingly,

WHO recommends that stool specimens should be tested for poliovirus on all cases of GBS less than 5 years of age.

Normally it must be displayed Manually
it is important to

Exercise 1

Koraput district was reported to be affected by an "outbreak" of acute gastroenteritis (GE) in July-August, 1991. About 2270 cases and 413 deaths occurred during this period. Most of the cases had only diarrhoea and vomiting.

Q.No.1.1. How will you decide whether or not the district was in the grip of an outbreak?

Yes · How CFR 18.19 %
we need to have more information

Table-1.1 describes the reported data on GE from the Koraput district during 1988-1991.

Table 1.1
Cases and Deaths due to GE in district Koraput, 1988-91

Year	No. of cases	No. of deaths	%
1988	981	225	22.88
1989	375	112	29.8
1990	1387	173	12.47
1991 (upto August)	2380	426	17.99

Mean : Av = 914 → 76.19 per month

Q.No.1.2. Now, will you consider it an outbreak of GE? If yes, why? If not, why?

yes · 3 times the previous years

Analysis of the age distribution of 308 deaths revealed that about 9% of deaths occurred in children below five years of age and about 62% of deaths occurred in adults above 20 years of age.

Q.No.1.3. Does it help you in suspecting cholera as the cause of this outbreak?

Yes

Q.No.1.4. What was the most disturbing features in this outbreak? Was it preventable?

CFR is more than 1 in a short span

ORS to be given at an early stage.

Rectal swabs from 59 cases were examined in the laboratories of NICD, Delhi. 15 samples were positive for *V.cholerae* O1 biotype El Tor. The isolates were sensitive to tetracycline, nalidixic acid, ampilcillin and chloramphenicol, but resistant to furazolidone and streptomycin. The other samples were found negative for any enteropathogens.

Q.No.1.5. What are the possible reasons for 44 of 59 samples being found negative for enteropathogens?

not proper collection —
~~[illegible]~~ given treatment —
late sending. —
defective lab.

Q.No.1.6. Assume yourself as the team leader for the investigation of this outbreak. (i) How will you plan the investigations? (ii) How will you control the outbreak?

(i)

didn't give any chloramphenicol

Exercise 2

During the period from 18 May to 19 June, 1991, 392 cases of clinically suspected typhoid fever were treated at the outpatient department of PHC Galore, district Hamirpur, Himachal Pradesh. Of them, 101 were admitted in the PHC. Analysis of the data on clinical features in the admitted patients revealed that they initially presented with fever (100%), headache (74%), pain abdomen (18%), vomiting (17%), diarrhoea (15%), constipation (7%), palpable spleen (57%) and palpable liver (13%). *Salmonella typhi* was isolated in 10 of 25 blood samples examined in the laboratories of Medical College, Shimla.

Q.No.2.1. What makes this an outbreak of typhoid fever? Comparable data from previous years are not available?

Q.No.2.2. If you want to organise a survey for active search of cases, what case definition will you use?

A line list of admitted cases was available in the primary health centre, Galore. It provided information on name, age, sex, address and date of onset of symptoms for all the 101 cases.

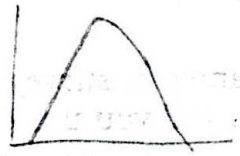
Q.No.2.3. How will you analyse the data? Prepare dummy graphs and tables.

Table 2.1 provides you data on admitted cases by date of onset of symptoms.

Table 2.1
Weekly distribution of admitted cases by week of onset of symptoms

Week ending	No. of Cases
12.05.1991	0
18.05.1991	4
25.05.1991	16
01.06.1991	41
08.06.1991	25
15.06.1991	12
upto 19.06.1991	3
Total	101

Q.No.2.4. Prepare epidemic curve using data given in Table 2.1



Q.No.2.5. What type of epidemic curve have you got? How do you interpret the epidemic curve?

Tables 2.2 and 2.3 describe the cases by age, sex and village.

Table 2.2
Cases of typhoid fever by age and sex, PHC Galore

Age (years)	Male	Female	Total
0-1	0	0	0
2-5	2	8	10
6-15	25	10	35
16-25	18	17	35
26-35	2	5	7
36-50	5	4	9
50 +	0	5	5
All ages	52	49	101

Table 2.3
Cases of typhoid fever by sex and village, PHC Galore

Village	Male	Female	Total
Lanjiana	22	31	53
Daswin	17	1	18
Pahal	1	2	3
Halti	2	3	5
Ghirmani	4	0	4
5 other villages	6	12	18
Total	52	49	101

Q.No.2.6 Using the data available in tables 2.2 and 2.3, can you suggest a hypothesis for transmission of infection?

A marriage function was held in village Lanjiana on 16th May, 1991. The bridegroom belonged to village Daswin. Only males accompanied the marriage party, whereas persons of both sexes participated from bride's side. During this function, water was used from a *Bawri* (a local water body). Analysis of water from Bawri revealed very high contamination; coliform count 1600/100 ml, faecal coli 275/100 ml of water. Those who attended the function started becoming ill after about 2 weeks. Contaminated water from Bawri was suspected as the cause of this outbreak.

Q.No.2.7 Can you provide some alternative explaination for the transmission of infection during the marriage function?

food, Carrier ...

Table 2.4 describes the outbreaks investigated by NICD during 1970-1994. Most of the outbreaks were of GE/cholera and viral hepatitis. Although typhoid fever is a major public health problem in India, only 2 outbreaks were due to typhoid fever.

Table 2.4
Outbreaks investigated by NICD during 1970-94

Disease	No. of Outbreaks
Cholera/GE	41
Viral Hepatiits	27
Japanese Encephalitis	13
PUO	9
Meningococcal meningitis	8
Malaria	6
Polio	4
Dengue fever	4
Smallpox	3
Chickenpox	3
Food poisoning	3
Kala-azar	3
Typhoid fever	2
Epidemic dropsy	2
Vrial encephalitis	2
Salmonellosis	2
Insecticide poisoning	2
Measles	2
Acute haemorrahagic fever	2
Plague	2
Influenza	2
Acute conjuctivitis	1
Herpes Zoster	1
Other/Unknown	7
Total	151

Q.No.2.8 Can you provide some explaination for a small number of outbreaks of typhoid fever investigated by NICD?

Exercise 3

Almost all the outbreaks of viral hepatitis in India are due to faeco-orally transmitted hepatitis E. These outbreaks have been invariably linked to contaminated water supply. An occassional outbreak may be due to hepatitis A. Recently, a few outbeaks of hepatitis B epidemiologically linked to unsafe injections have also been reported. This exercise pertains to an outbreak of hepatitis E.

Residents of a locality in North West Delhi (SD Block, Pitampura : population 1435) felt an unusal increase in the cases of jaundice in April, 1994. The block was inhabited by people belonging to middle or upper socio-economic strata. All the residents had access to and used only sanitary latrine. The Block had piped water supply. There was no other source of water. The water supply was intermittent and did not fulfil the requirements of people. The residents therefore, used on-line-booster pumps to lift the water to their overhead tanks.

Q.No.3.1. How will you plan your investigation to (i) diagnose the disease and (ii) select a suitable case definition to assess the extent of outbreak?

1) Case definition: to collect as many cases as possible, raised as many as not possible. Jaundice only

(Usual attack rate is 1 - 1.5/1000 per year).

Q.No.3.2. A house to house survey revealed 27 cases of jaundice in 1435 population within a reference period of 3 months. The attack rate of jaundice was found to be 1.9% ~~per 1000~~ population. Many outbreaks are on record where a varying number of cases of jaundice (a few cases to many thousands) occurred in a short period. 27 cases in SD Block were also considered an outbreak. How could you have reached to this conclusion?

Yes. Became > > 1 per 1000.

Q.No.3.3. You found that most of the cases were due to hepatitis E (laboratory findings). You suspect contaminated piped water as the major factor in this outbreak. How will you substantiate your suspicion?

attack rate in diff localities then compare during breakage repair

investigate de a Sanitary Survey.

Q.No.3.4. Were you dealing with an unusual clustering of hepatitis B cases, how would you have planned to find out the mode of transmission?

Q.No.3.5. The outbreak in SD Block was due to hepatitis E. Using the data already provided to you, can you speculate (i) why did the outbreak occur at all? (ii) why was the outbreak not explosive?

Exercise 4

A patient from village Banyani (populatin 3403) came to the OPD of PHC on 7.10.1991 with complaints of fever, headache, bodyaches and chills. He also informed the medical officer about a large number of cases having similar illness in the village, of whom many had died.

Q.No.4.1. If you happened to be the incharge of PHC, what steps will you take and why?

A team of doctors from the PHC surveyed the village on 8.10.1991. They examined 756 persons. 696 (92%) of them were presently suffering from fever usually accompanied with rigors and chills. None of them had signs of meningitis. A medical specialist examined 63 patients of fever. 25 of them (40%) had spleen enlargement.

Q.No.4.2. Will you consider this episode as an outbreak of febrile illness? Do you need more information to reach at some conclusion?

Q.No.4.3. What was the most probable cause of this outbreak?

During the survey undertaken on 8.10.1991, 466 blood slides were collected from fever cases. 81 slides were found positive for malarial parasite (Plasmodium vivax 66, Plasmodium falciparum 15). The PHC considered it an outbreak of falciparum malaria. District authorities were

informed about the outbreak and a treatment centre was established at village Banyani to treat the patients.

The state health authorities informed the National Instt. of Communicable Diseases on 1.11.91 about the outbreak. The message stated that a large number of fever cases and 21 deaths had occurred in a village in district Farukhabad.

Q.No.4.4. You do not have access to any other information except this message. Will you include JE and/or dengue fever in differential diagnosis? If yes, Why? If not, why?

JE larvine cans

Dengue in Urban less deaths more wide spread

UP.

NICD team visited the affected area during 1-3 November 1991. The team collected the following data from the PHC.

(i) 2294 blood slides were collected in village Banyani upto 2.11.91. 325 were found positive for malarial parasite (*P.falciparum* 131, *P.vivax* 194). About 40% of the positive slides were due to P.falciparum.

60% 40%

(ii) During the same period, 2184 slides were collected from other villages of PHC within 5 kilometer of village Banyani. 208 were found positive for malarial parasite (*P.falciparum* 36, *P.vivax* 172).

(iii) Malaria situation in PHC in the last five years is shown in Table 4.1.

Table 4.1
Malaria situation in PHC Talgram, 1987-1991

Year	Slides collected	Slides positive for MP		
		P.vivax	P.falciparum	Total
1987	3170	9	0	9
1988	5921	7	0	7
1989	5822	4	0	4
1990	7384	2	0	2
1991 (upto september)	7233*	2	0	2

* 948 slides still to be examined.
Note:- Population of the PHC about 2 lakhs.

(iv) 19 persons died due to suspected malaria; 3 in August, 12 in September and 4 in October.

Q.No.4.5 Why was this outbreak not detected in the early rising phase?

While going through the streets in village Banyani, it was observed that a large number of residents were rolling *bidies* (local cigarette) in front of their houses. On inquiry, it was found that *bidi* rolling and agriculture were the main occupations in village Banyani and a few surrounding villages. It provided the clue to the genesis of the outbreak in the village Banyani.

Q.No.4.6. Can you speculate on the gensis of outbreak of falciparum malaria in village Banyani? It is noteworthy that District Farukhabad did not report any case of falciparum malaria in the last 3 years.

You have been given the responsibility to investigate and control this outbreak.

Q.No.4.7. How will you plan further investigations?

(Note:- See a related exercise in Module on Disease Surveillance)

Annex 1

CHECK LIST FOR MODEL DISTRICT LEVEL LABORATORY FOR DISEASE SURVEILLANCE

1. The laboratory should be able to perform followings:

Microscopy: Rapid presumptive diagnosis of	Cholera Tuberculosis Diphtheria Plague Malaria Filariasis Meningitis
Bacteriological culture of	Stool especially for cholera
Bacteriological examination of	Water for coliform count
Rapid diagnostics for	AIDS Meningitis due to meningococci, pneumococci H.influenzae HBsAg (also for volunteer donors) Syphilis
Non-ELISA based tests	Typhoid fever

2. Laboratory should be equipped to collect, store and transport specimens for following:

Stool, Blood/serum, CSF
Milk/food
vomtius vomitus
for various diseases including arboviral diseases such as JE and Dengue fever, Poliomyelitis, and Measles
Samples from environment such as soil

3. Laboratory should have the facilities to wash and sterilize glassware etc. needed for collection and transportation of clinical and environmental specimens as well as for separating serum from blood samples.

Clinyoscle pleb

4. Laboratory should have adequate inventory/or access to basic material that may be needed for surveillance and investigation of outbreak or to provide support in times of disasters.

5. Standard operating procedural manual should be available with the laboratory.

6. Adequate staff members who are qualified and/or oriented/trained for undertaking disease surveillance work.

7. Sufficient stationery for maintaining the records and dispatching the same to other centers.

8. Laboratory should have access to electronic communication network for rapid transmission of results and/or early warning signals.

Chemical analysis of water have sent to diff place

9. To provide as small laboratory incomplete for

staining to disposable syringes

Daily Cartridge changes

CHECK LIST
FOR DISTRICT LABORATORY FOR DISEASE SURVEILLANCE

S.No.	Activity	Yes/No
1.	**Does the lab undertake microscopic examination for**	
	Stool sample for cholera bacilli	
	Sputum for tuberculosis bacilli	
	Throat aspirate swab for diphtheria bacilli	
	Bubo aspirate/sputum for plague bacilli	
	Peripheral blood smear for malarial parasite	
	Peripheral blood smear for microfilariae	
	CSF for meningitis organisms	
2.	**Does the lab undertake bacteriological examination of**	
	Faeces for cholera bacilli	
	Water samples for coliform count	
3.	**Does the lab have rapid diagnostic kits for**	
	HIV infection	
	Meningitis	
	Syphilis	
	Australia antigen (HBsAg)	
4.	**Are following equipment available in functional condition in lab**	
	Autoclave	
	Microscope	
	Centrifuge	
	Refrigerator	
	Deep freezer (-20c)	
	Balance	
	pH meter	
	Gas meter	
	Incubator	
	Water bath	
	Hot air oven	
5.	**Does lab perform serological tests**	
	VDRL	
	Widal	
	ASO	
	Any other	
6.	**Does lab has full time professionals**	
	Microbiologist/Medical Officer trained in microbiology	
	Technical staff with qualification in lab medicine	
	1.	
	2.	
	3.	
	Supportive staff with experience in lab	
	1.	
	2.	
	3.	

7.	**Does the lab has at any point of time followings**	
	1. 100 sterile glass syringes with needles	
	2. 100 screw capped glass vials for blood collection	
	3. 100 screw capped plastic vials for storage of serum	
	4. 100 plastic/glass petri dishes	
	5. Cary Blair transport medium (50 vials)	
	6. 10 pairs of Gloves	
	7.	
8.	**Are SOPMs available with the lab**	
9.	**Does the lab has an access to NICNET/ERNET**	
10.	**Does the lab has a system of record storing which is:**	
	Manual	
	Computerised	
11.	**Is there any periodic evaluation of working of lab**	
12.	**Is there a stand-by supply of electricity**	
	If yes, what are the equipment which are on it:	
13.	**Does the lab has an inventory of regional and national reference labs where samples can be sent:**	
14.	**Does the lab participate in any external quality assurance scheme in bacteriology/serology?**	

INTER-DEPARTMENTAL COMMITTEE
SUGGESTED AREAS OF RESPONSIBILITY AND ACTION

District administration

- mobilize resources by organizing meetings with
  - concerned government departments
  - non-governmental agencies
  - community leaders
- ensure adequate quality monitoring of water samples
- repair of leakage in water pipe lines
- arrange safe water supply
- ensure supplies of ORS packets and other essential items
- ensure vector control measures
- ensure adequate facilities for transportaion of serious patients to district hospital, if necessary
- strengthening of existing provision under the Drug & Cosmatic Act to curtail over the counter sale of parenteral drugs
- provide relevant information to the press
- monitor status of control activities

District Health Office / Municipal Health Office

- arrange repair of leakage in water pipe lines
- alert health personnel to report cases and to monitor trends
- arrange active surveillance in affected area
- ensure that treatment guidelines are followed in hospitals and other health facilities
- ensure availability of ORS packets and other essential items
- strengthening of existing provision under the Drug & Cosmatic Act to curtail over the counter sale of parenteral drugs
- ensure vector control measures
- arrange health educational camps and distribution of health educational material
- arrange chlorination of water sources if possible
- arrange water quality monitoring
- convene meeting under distrist administrator to seek cooperation of other government departments and NGOs
- check sterilisation practices of medical practitioners for syringes, needles and sharp instruments

Concerned Department (s) responsible for water suppy

- repair of leakage in water pipe lines
- arrange potable water supply, including water tankers if necessary
- arrange chlorination of water
- ensure water quality monitoring

Other government departments such as social welfare, education, tribal welfare and NGOs

- dissemination of relevant information
- promotion of oral rehydration therapy
- ensure vector control measures
- check sterilisation practices of medical practitioners
- reporting of cases

Panchayat members, village pradhans, community leaders

- dissemination of relevant information
- promotion of oral rehydration therapy
- ensure vector control measures
- check sterilisation practices of medical practitioners
- reporting of cases
- monitoring chlorination of water sources such as wells
- arranging transportation of serious cases to hospital

Annex-3

SUGGESTED FORMAT FOR LINE LIST OF CASES

Sl. No.	Name of Patient	Father's/ Husband's Name	Address	Age	Sex	Date of Onset of symptoms	Symptoms	Outcome of illness (still ill/ recovered / died)

Note:- A column on immunisation status should be added for vaccine preventable diseases.

Annex 4

OUTBREAK INVESTIGATION REPORT

General Information

State :_____

District :_____

Town/PHC :_____

Ward/Village :_____

Population :_____

Background Information

Person reporting the outbreak :_____

Date of report :_____

Date investigations started :_____

Person(s) investigating the outbreak _____

Details of Investigation

Describe how the cases were found (may include: (a) house-to-house searches in the affected area; (b) visiting blocks adjacent to the affected households; (c) conducting record reviews at local hospitals; (d) requesting health workers to report similar cases in their areas, etc.):

_____
_____
_____
_____
_____

Descriptive Epidemiology

- Cases by time, place and person (attach summary tables and relevant graphs and maps).
- Age-specific attack rates and mortality rates
- High-risk age-groups and geographical areas.

Descri-ption of Control Measures Taken

_____

Description of Measures for Follow-up Visits:

Brief Description of Problems Encountered

Factors Which, in Your Opinion, Contributed to the Outbreak

Conclusions and Recommendations

Date (Name and Designation)

DISEASE SURVEILLANCE

AT DISTRICT LEVEL

A LABORATORY MANUAL

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
22 SHAM NATH MARG, DELHI - 110 054

Contents

CHAPTER- 1

1. ROLE OF LABORATORY IN DISEASE SURVEILLANCE:

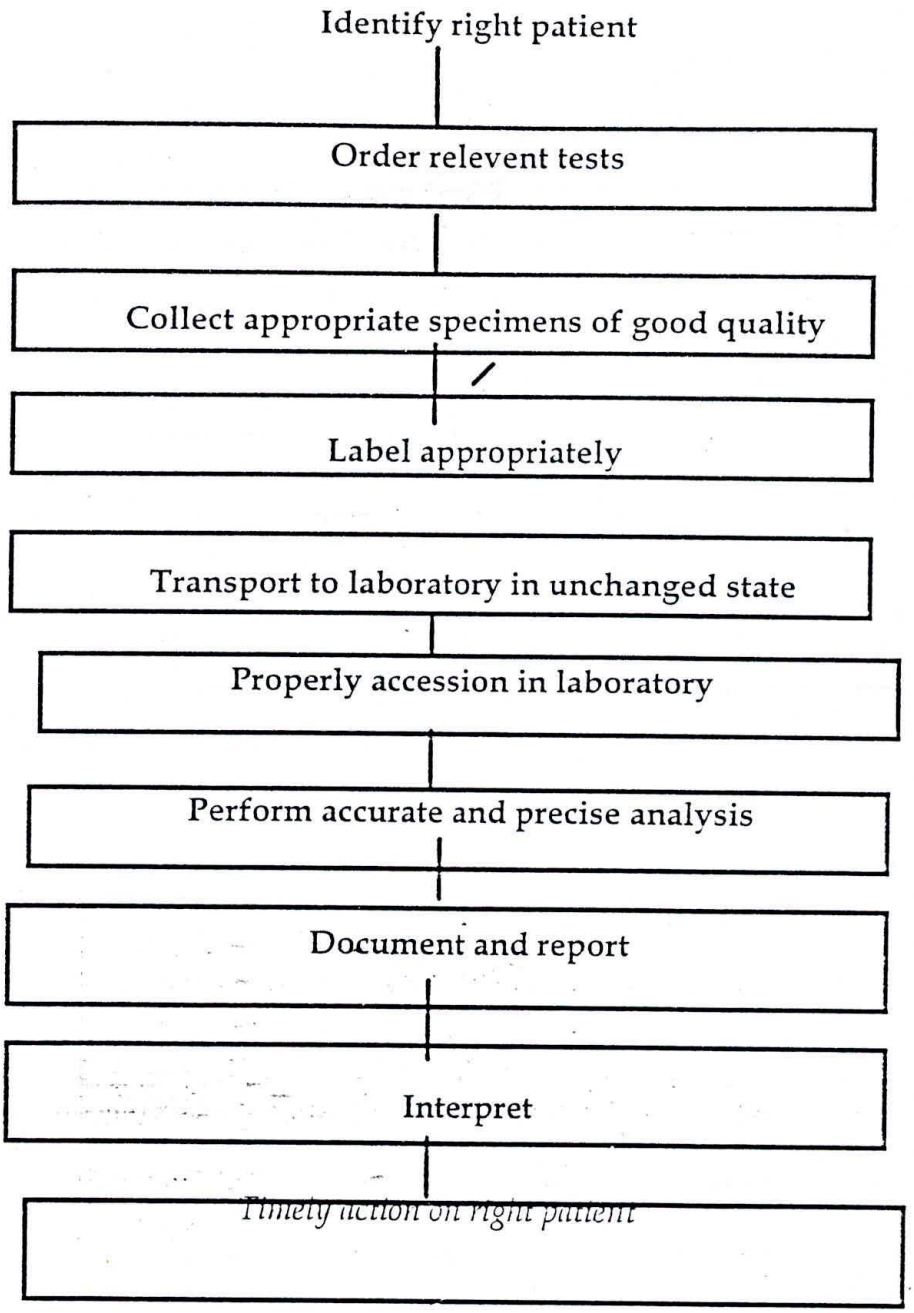
Epidemiological surveillance of a disease is the continuing scrutiny of all aspects of the occurrence and spread of a disease that are pertinent to effective control. It is a dynamic process involving the infectious agent, host, reservoirs, vectors and the environment as well as a complex mechanism concerned with the spread of infection and the extent to which spread has occurred. Surveillance of any particular disease includes systematic collection and evaluation of morbidity and mortality data, reports of investigation of epidemics, laboratory investigations to find out the causative agent, use and untoward effects of biologicals, insecticides and other materials used in control, assessment of immunity status of population and other relevant data for action. The introduction of laboratory techniques in epidemiological services has revolutionised the concept as well as scope of disease surveillance. Now a days laboratory support is considered an integral component of a sensitive system of surveillance.

Role of Laboratory services in surveillance:

1. Diagnosis of a syndrome
   - o Encephalitis
   - o Hepatitis
   - o Meningitis
   - o Pyrexia of unknown origin
2. Tracing the source of infection.
   - o Epidemiological markers
3. Detection of inapparent infections/carriers.
   - o Japanese Encephalitis
   - o Typhoid fever
   - o Meningococcal meningitis
4. Early detection of outbreak:
   - o Meningococcal meningitis
   - o Hospital infections.
5. Retrospective diagnosis.
   - o Rheumatic heart disease
   - o Subacute sclerosing panencephalitis
   - o Nephrotic Syndrome.

6. Detection of New Disease Agents
   - o HIV
   - o Newer Enteropathogens (*V. Cholerae*)
7. Monitoring of treatment
   - o Antibiogram
   - o Sero-Therapy.
8. Quality Control of Biologicals
   - o Vaccine potency testing
   - o Vaccine Safety studies
9. Prevalence studies
   - o Sero-surveys
   - o Immune status
10. Find out natural foci of infection
   - o Plague
   - o Leptospirosis
11. Controlled field trials.
   - o Newer drugs/vaccines
   - o Newer regimens of drugs/vaccines

12. Key to successful laboratory based surveillance lies in :
   1. Right sample collection.
   2. Right time to collect samples for disease surveillance.
   3. Right methodology to be followed for transportation of sample.
   4. Right laboratory to be chosen so as to get prompt and correct results
   5. Right interpretation of lab. Results.



13. Networking of laboratories

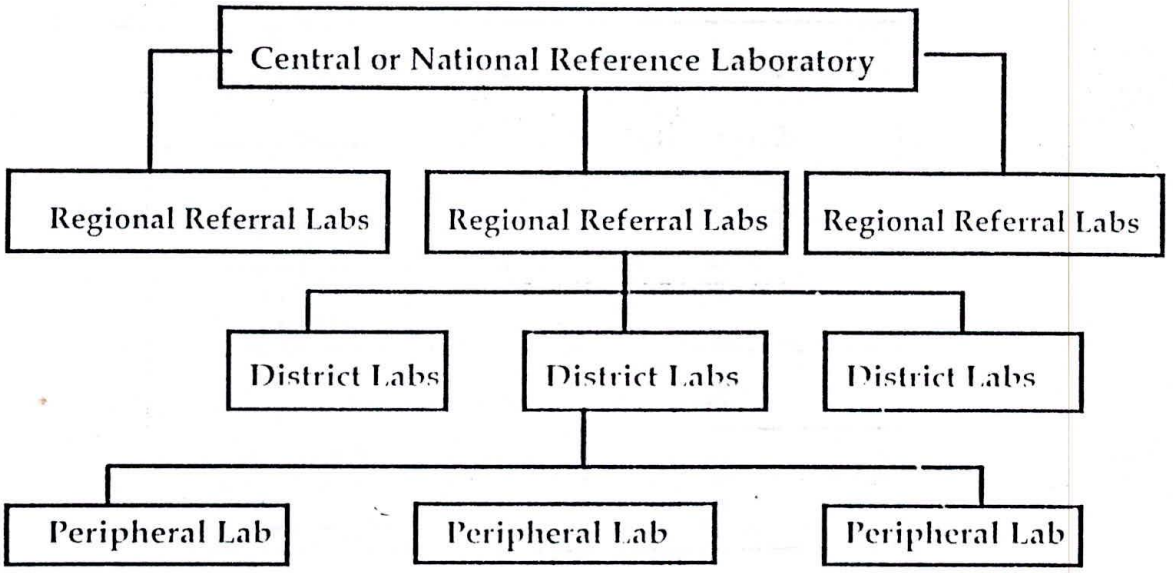


Fig 1: Networking of laboratories

13 Proposed strengthening of district level laboratories under NDSP

PERIPHERAL LABORATORY

These laboratories are located at the point of first contact of patients with the health care services. In most of the developing countries these are available only at primary health centre or community health centre (upgraded primary health centres). These laboratories provide technical support for preventive, curative and promotive services for the individual as well as the community.

Staff

The staff in peripheral laboratories should include one technician and one laboratory assistant/attendant.

Space

Space available in peripheral laboratories should include at least one laboratory-cum-office/record room (1o ft x10 ft) and one store-room combined with other services (16 ft x10 ft).

Other facilities

Other necessary facilities include

- a supply of safe water,
- a reliable source of energy (battery, electricity, solar or kerosene) and
- sterilization facilities and waste deposit

There must also be transport and communication facilities between the peripheral and intermediate laboratories for referral of samples and patients, procurement of supplies and personal discussion.

Equipment and supplies. Necessary equipment and supplies include: good microscopes, centrifuges, transport media, glassware, sterile swabs, reagents for staining (eg. Gram, Albert, Ziehl Neelsen, Romanowsky), kits and reagents for rapid diagnostic tests, sterilized syringes and needles, micropipettes and tips as well as sterile collection bottles for blood/serum and water analysis.

Tests to be performed

These laboratories are expected to undertake tests of public health as well as clinical relevance. Amongst the tests of public health relevance, diseases of greater epidemiological importance should be accorded priority. Testing of environment samples (especially water) also falls into the priorities of public health relevance. Certain serological tests may be of use in studying epidemiological pattern of the important diseases and the same can be performed at peripheral laboratories (Table 1)

Table 1

Suggested microbiological tests at district laboratories

Procedure/Specimen	Disease/Organism
Microscopy for stained smears	
(Gram, Albert,Ziehl Neelsen)	
Nasopharynx and throat	Diphtheria, Vincent's angina
Sputum	Tuberculosis, pneumonia
CSF	Meningitis
	(pyogenic & tuberculosis)
Urethra/vaginal discharge	Gonorrhoea
Stool	Cholera/dysentery
Culture	Cholera
Serological tests	Enteric fever (Widal)
	RPR/VDRL
	Brucella (Tube agglutination)
Dipstick and Particle agglutination test	HBsAg, HIV
Bacteriological analysis of water	

As far as possible, these tests should be reliable, sensitive, specific, rapid, easy to perform and cost effective.

CHAPTER - 2

COLLECTION, STIORAGE AND TRANSPORTATION OF SPECIMENS

CHAPTER- 2

2. COLLECTION, STORAGE AND TRANSPORTATION OF SPECIMENS:

***[Inventory of labs to be added]

2.1 Effective diagnostic microbiology depends upon the correct collection and timing of clinical specimens and their proper transport to the laboratory under optimal conditions. It has been observed that most important and frequent source affecting laboratory analysis is collection and transportation of the specimen. The guidelines for it must be emphasized.

- o Specimen should be in adequate quantity.
- o Specimen must be collected before the administration of antimicrobial agents.
- o Contamination of specimen with externally present organisms or normal flora of body must be prevented.
- o Specimen must be collected at appropriate stage of the disease.
- o Specimen should not get contaminated during storage.
- o Specimen handling should not be risky to individual and/or community.

2.2 Some of the essential precautions which need to be followed are as follows:

- o Apply strict aseptic techniques throughout the procedure.
- o Wash hands before and after the collection.
- o Collect the specimen at the optimum time.
- o Make certain that the specimen is representative of infectious process (e.g. sputum is the specimen for pneumonias and not saliva) and is adequate in quantity for the desired tests to be performed.
- o Collect or place the specimen aseptically in an appropriate sterile container.
- o Ensure that outside of specimen container is clean and uncontaminated.
- o Tightly close the container so that its contents do not leak during transportation.
- o Label and date the container, complete the requisition form.
- o Arrange for immediate transportation of specimen to laboratory.

2.3 Laboratory specimens required for tests for particular causative agents:

Suspected agent/ disease	Specimen	Test
Arbovirus infection	Blood or brain (-70°C)	Isolation
	Blood or serum (+4°C)	Serology
Cholera	Rectal swabs or stool specimens in transport medium, as recommended by the laboratory.	Culture
Gastroenteritis	Stool	Culture, ELISA*
Viral Hepatitis	Serum (+4°C)	ELISA*
Legionellosis	Blood, sputum, in enrichment broth.	Culture/ FA**
Malaria	Blood (thick and thin smears)	Staining
Meningococcal meningitis	Spinal fluid, blood,	Latex agglutination test
Plague	Bubo fluid, blood (in broth or on slants)	Staining, Culture/ FA**
Typhoid fever	Blood in enrichment broth (early in disease); serum	Culture; serological test
Dysentery	Faecal specimens or rectal swabs in enrichment broth.	Culture/ microscopy
Syphilis	Blood/ serum	VDRL/ RPR

* Enzyme linked immunosorbent assay

** Fluorescent antibody test.

Because of alterations in the specimen prior to measurement, the clinical state of the patient will not be necessarily reflected by the result of the laboratory investigation despite the correct laboratory performance. Some of the important specimens and their proper collection and transportation methods are described here so as to ensure quality.

2.4 Blood for serological testing:

Blood is the most important and frequently collected clinical specimen at district level.

Blood should be drawn using sterile (preferably disposable) syringes and needles. Quantity of blood drawn should be minimum 4-5 ml. Vial in which blood is collected should be preferably sterile, dry and properly labeled. The needle and syringe used, as also the vial, should be completely DRY before collecting blood. After drawing blood, the needle should be removed from the syringe before transferring blood from syringe to the vial. Do not shake the blood that is collected in the vial. Let it stand undisturbed at room temperature for 2-4 hours. After the blood has stood at room temperature for 4-6 hours, it should be subjected to the process of serum-separation. If the facilities for separation of serum are not available, then it should be refrigerated at 4°C (NOT FROZEN).

Using a sterile Pasteur pipette, dislodge the retracted clot from the liquid portion of blood, transfer the liquid portion into a clean sterile centrifuge tube having a rubber cork.

Centrifuge at 500g for 5 minutes. Transfer the supernatant(serum) using a sterile Pasteur pipette into sterile clean, dry plastic disposable screw capped vials. Label the vials.

2.4.1 Filter paper method of collection of blood:

Transportation of liquid specimens soaked on filter paper is a simple and safe procedure. All kinds of body fluids can be absorbed onto filter paper strips (Whatman No.1). Cards with incorporated filter paper strips having marked circle (usually 3 cm diameter) are commercially available. The details of the patient are written on the card with a ball-point pen. The blood is taken from the finger prick or heal prick of an infant. The site is cleaned with 70% alcohol and wiped dry with sterile gauze. Puncture is made with a sterile disposable lancet and the first drop of blood is wiped away with sterile dry gauze.

Filter paper (marked area) is gently touched onto the second drop of blood and allow the blood to soak till the premarked area is completely filled. Punctured site should not be squeezed to prevent any haemolysis. Blood is applied only on one side of filter paper and once only.

The blood specimen is dried for 3 hours in the air in a horizontal position without letting the specimen come in direct contact with any surface, direct heat or sunlight. **These samples should not be refrigerated.**

The specimen is placed in a plastic bag or an envelope along with few granules of a desiccant and sealed hermetically before transportation or mailing. If stored in a cool and dark place, such specimens give acceptable results even upto three months.

(For collection of **stool, CSF , aspirates** and **water,** see in relevant chapters)

2.5 Labeling of Specimens:

After collection of specimen, it should be immediately labeled and case investigation form should be filled up and should accompany each specimen. The specimen should be kept cool preferably at 2-8°C and sent to laboratory as early as possible. In case of delay the sample should be stored at 2-8°C before transporting to nearest laboratory.

Labels for specimen collection vial

Name_____Age_____

Specimen No._____

Specimen

Date Time

2.6 Storage and Transportation:

In general, infectious materils should be kept at a low temperature during storage and transport, except the CSF samples collected from cases of pyogenic meningitis which should be maintained at room temperature. The types of refrigeration required to achieve various temperatures are as follows:

Temperature (°C)	Type of refrigeration
(+)2-8	Domestic refrigerator
(+)4	Wet ice or frozen ice packs (cold bags)
(-)8	Freezer of domestic refrigerator
(-)20	Freezer cabinet
(-)70	Deep freezer or dry ice.
(-)160	Liquid nitrogen

The quantity of pathogens or antibody in original clinical samples can decline during storage or transportation which seriously affects the diagnostic results. Hence special care should be taken before or during transit of materials to laboratory to protect them from heating or drying.

Precautions

- Repeated thawing and freezing of specimens should be avoided.
- Freeze the specimen only if transport to District/National/Reference Laboratory is assured at -20°C.

Recommendation - Store and transport all specimens at 2-8°C (Lower Compartment of refrigerator) except CSF.

For Transportation of specimen

- wet ice or ice pack should be used.
- Specimen containers relating to single case investigation should be placed in a plastic bag with an absorbent material surrounding the specimen so that even if whole specimen leaks out, it will be absorbed,

- The laboratory report form should be sealed within a separate plastic bag and wrapped round the specimen or attached firmly to the box of specimens.
- The material should be packed in an insulated carton/carrier to transport a specimen to the laboratory.
- All specimens should be considered as potentially pathogenic and accordingly labeled with internationally accepted biohazard label.

Transportation of Virus Isolates/Specimen to Reference Laboratory:

Specimen virus isolates to be sent to other laboratories require special attention for packing of the material and strict guidelines for transportation of samples should be followed.

CHAPTER - 3

DISINFECTION AND STERILIZATION

CHAPTER- 3

3. Disinfection and Sterilisation

Definition:

Sterilisation implies complete destruction of all living micro-organisms including spores. Disinfection means destruction of vegetative forms of organisms which might cause disease or spoilage of food etc. It does not necessarily kill spores. The two terms are not synonymous.

3.1 Disinfection of used laboratory articles

Disinfection of both reusable and disposable glassware and articles contaminated with morbid or culture material is of utmost importance in the laboratory. All the specimens received in the laboratory should be considered as potentially pathogenic. The ideal method of treating such materials is to incinerate all the disposables and decontaminate the reusable articles by autoclaving. These facilities may not be available in every laboratory. For purpose of disinfection, disposal and recycling, all the articles may be divided into three categories.

- Disposables.
- Reusable articles contaminated with morbid material such as pipettes, slides, test tubes etc.
- Material containing or contaminated with bacterial cultures.

3.2 Disposables:

Soak the material overnight in a strong solution of disinfectant before disposing alongwith garbage; 1% sodium hypochlorite, 10% solution of formalin or 3% lysol may be used as disinfectant.

3.3 Reusable articles contaminated with morbid material:

Discard the articles into a jar containing solution. Let them remain in this solution overnight. Drain off the disinfectant. Transfer the material to a metal pot or tray with cover. Pour water and boil for 15 minutes. Cool and drain off the water. Pass on the articles for washing.

3.4 Glassware containing culture material

Discard all the articles containing or contaminated with culture material directly in a metal box or a bucket. Place the box with material in the autoclave and decontaminate by autoclaving (see sterilisation).

Drain off culture medium and pass for washing.

3.5 Disinfection of rooms

Seal all the windows, ventilators and fire places with brown paper and adhesive tape.

Pour 500 ml of formalin and 1000 ml of water in a pan or tray and boil with the help of a spirit lamp or a bunsen burner. Spirit in the lamp should be just sufficient, to boil off the formalin and the lamp extinguishes when there is a small quantity of liquid left in the pan. Seal the door.

Open the door, next morning and spread a piece of lint soaked in ammonia on t table. This will neutralise excess of formalin present in the room.

3.6 Washing of laboratory glasswares:

3.6.1 <u>New glassware</u>

Usually new glassware are slightly alakline. Before washing these have to be neutralized. The method is as follows:

- o Prepare a 2% solution of hydrochloric acid in a big basin.
- o Soak the new glassware in this solution for one day.
- o Rinse twice with clean water and once with demineralized water and dry.

3.6.2 <u>Dirty glassware</u>:

- o Rinse twice in lukewarm or cold water otherwise serum or blood may stick to them and may not be washed.
- o Put the glassware in a bowl containing detergent solution and scrub the inside with a brush. After scrubbing soak the glassware in this solution for 2-3 hours.
- o One by one, take out the articles and rinse under running tap water, then put all the glassware in a container containing tap water (no trace of detergent should be left otherwise this may lead to false results).

- o Drain the water by putting each articles on a wall draining rack.
- o Place the articles in a wire basket and dry in a hot air oven at 60oC.
- o Plug each article with non-absorbent cotton wool or aluminium foil and store in a cupboard to avoid dust.

3.6.3 Pipettes:

- o Immediately rinse in running tap water to remove blood, urine, and serum reagent, etc.
- o If the pipettes were used for infected materials, soak them in cylinder full of disinfectant solution (2% dettol or 2% phenol) for 24 hours otherwise place in a large measuring cylinder full of water.
- o Soak in detergent and rinse as in case of dirty glassware.
- o In case the pipettes are blocked put them in dichromate solution for 24 hours. Next day clean under running tap water, check individually, rinse for a number of times.

3.6.4 Syringes and needles:

- o Immediately after use remove the plunger and rinse the barrel and plunger. Syringe water through the needle forcefully. Finally remove the needle.
- o If the piston is blocked, either soak the syringe for 2 hours in hot water or pipette with the syringe standing on its end, piston down. Alternatively soak the syringe in a container of 10 vol hydrogen peroxide.
- o In case of block needles use the stylet to remove the bock.

3.7 Methods of sterilisation

The common methods of sterilisation used in a microbiology laboratory can be broadly divided into three categories depending upon the materials to be sterilised.

- o Dry heat.
- o Moist heat.

o Filtration.

3.7.1 Dry heat

The two commonly used methods of sterilisation by dry heat are:

(a) Red heat or flaming

(b) Hot air sterilisation.

3.7.1.a Red heat or flaming

Instruments such as inoculating loops and searing irons are sterilised by this technique. For sterilisation of inoculating loop, hold the loop vertically on the blue cone of the flame for few seconds and slowly raise upwards till whole of the wire is red hot. Move the loopholder rapidly downwards through the flame so that several inches of the lo holder is also heated slightly.

3.7.1.B Hot air sterilisation

Items to be sterilised:

This is the best method for sterilisation of dry glasswares such as test tubes, flasks, pipettes, pertidishes, assembled all glass syringes, throat swabs and other sealed materials which can withstand high temperature and where penetration by steamis not possible.

Sterilisation by hot air can be conveniently carried out in an electrically heated oven. A themostat is fitted to control the temperature. Larger units should be fitted with an air circulating fan to ensure uniform temperature in the different parts of the oven.

(Add.: Preferable methods of sterilization)

CHAPTER - 4

MICROSCOPIC EXAMINATION

CHAPTER- 4

4. MICROSCOPIC EXAMINATION:

In a peripheral laboratory, microscopic examination can provide rapid and economical presumptive diagnosis which may have significant bearing upon control and prevention strategies.

4.1 Cleaning and storage of Microscope Slides:

4.1.1 Cleaning of new slides

- Soak the slides in a vessel containing soap water olution for a few hours.
- Place the slides either in running tap water or several changes of clean water for few hours.
- The slides should be wiped dry using a dry, clean, lint-free cloth.
- Always handle the cleaned slides by the edges to avoid finger marks.

4.1.2 Cleaning of used slides:

- Soak the slides for at least 60 minutes in 1-2% hypo-chlorite solution.
- Wash in hot soap water scrubbing both the sides with the brush, taking particular care to wash only a few slides at a time to prevent scratching.
- Clean the slides individually with gauze or cotton wool.
- Transfer the slides to a fresh detergent solution.
- Wash in running tap water or several changes of clean water.
- Wipe dry with a clean lint free cotton cloth.

4.1.3 Storage of Slides

- Initially, after washing and cleaning, the slides should be kept in a dry place or a warm air cabinet.

- Thereafter slides should be stored in packages of 10 which should be wrapped in thick paper and secured with adhesive tape or rubber bands.

4.2 Microscopy for Pyogenic Meningitis:

Pyogenic meningitis is an acute bacterial infection of the meninges, commonly caused in epidemic form by *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*.

For the laboratory confirmation of the diagnosis, the following clinical specimen should be collected.

4.2.1 Cerebrospinal Fluid (CSF)

CSF following lumbar puncture should be collected in 3 separate clean sterile containers (Bijou bottles) for following investigations:

- Biochemical analysis
- Cytological examination
- Microbiological tests

General guidelines for collecting CSF

- CSF should be collected before the start of chemotherapy.
- Never refrigerate or expose it to sunlight, and transport immediately to the laboratory.
- In case of delay in transportation, keep CSF at 37°C.
- Other clinical samples which can be collected are blood and petechial fluids.

4.2.2 Examination of CSF

(a) Macroscopic:

Look for the presence of turbidity, blood or coagulum.

Cytologic examination to be done only when there is no coagulum in the CSF.

Material Required:

- Neubauer's counting chamber, WBC diluting fluid, WBC pipette, compound microscope.

The cell count should be done by the usual procedure of WBC count using a Neubauer's chamber and count the number of leukocytes per cmm of fluid.

The normal CSF should be absolutely clear, free of any coagulum and should not contain more than 0-8 lymphocytic cells/cmm.

In pyogenic meningitis, appearance of CSF is turbid and contains more than 8-10 leukocytes/cmm, the cells being predominantly polymorphs in nature.

(b) Microbiological examination:

- Microscopy

Microscopic examination is required to directly visualise the causative organism in the CSF.

Requirements:

- Clean slides
- Coverslips
- Table top Centrifuge
- Centrifuge tubes
- Pasteur pipettes
- Clean glass vials
- Reagents of Gram's staining.
- Rubber teats
- Discarding jar
- Neubauer Counting chamber
- WBC pipette.

Procedure:

- Transfer about 1-2 ml of CSF in a sterile Centrifuge tube.
- Centrifuge at 3000 rpm for 5 minutes.
- Keep the supernatant fluid for Latex Agglutination test.
- From the deposit, make smears on 3 clean glass slides and air dry.
- In case of a clotted CSF, transfer 3 small pieces of clot on three different glass slides.
- Tease the clots using needles or wooden sticks or the edge of the slide and after spreading make the smears. Air dry.
- In case of scanty CSF, several drops of CSF should be placed at one particular spot on the slide, each being allowed to dry before the next is added. Air dry and heat fix the smear.
- Stain the smears by Gram's staining method as given in Annexure.
- Examine under microscope under oil immersion.

Observations:

- Presence of Gram negative bean shaped diplococci, both intracellularand extracellular suggests the presence of *Neisseria meningitidis* (Meningococcus).
- Other organisms which can be seen are *Streptococcus pneumoniae* (Pneumococcus), which appear as gram-ositive diplococci, *Haemophilus influenzae* which appear as gram negative thin filaments rods.

Gram negative diplococci

4.3 Diagnosis of Pulmonary tuberculosis by sputum examination:

o Tuberculosis is a disease of great public health importance caused by *Mycobacterium tuberculosis* and some other species of Mycobacteria.

- The diagnosis of pulmonary tuberculosis can be established by demonstrating the bacillus in the sputum of the patient by microscopy.

4.3.1 Sputum collection:

- Collect the sample preferably early in the morning.
- For optimum results, 3 consecutive days samples should be tested.
- In case sputum is scanty, a 24 hour collection may be examined.
- A nebulized and heated hypertonic saline may be used to induce sputum production in patients unable to bring out the sputum.
- Sputum should be collected in a sterile wide mouthed container with a tight lid.

The sample should be delivered to the laboratory with minimum delay.

Specimen that cannot be delivered or processed immediately should be refrigerated at 4-8°C for a maximum of 3-4 days.

Materials required for sputum microscopy:

- Properly collected specimen
- Wooden sticks
- Clean glass slides
- Spirit lamp/Bunsen Burner
- Petri dish
- Inoculation hood
- Face masks
- Reagents for Zeihl-Neelsen staining
- Glass Rods, Plastic clay.

Procedure:

Preparation of the smear

- In an inoculation hood or in an isolated room, wearing a face mask, transfer a portion of the sputum to a petri dish.
- Using a wooden stick, tease out a small portion of caseous, purulent or bloody material and transfer it to a clean slide.
- Using the same wooden stick or an inoculting wire loop, spread this material uniformly over a large area, covering at least two thirds of the slide.
- Air dry the slides and flame them immediately and stain according to the Ziehl-Neelsen staining method as given below:

4.3.2 Ziehl Neelsen Staining (Acid fast staining)

Requirements

- Carbol fuchsin solution
- 20% sulfuric Acid
- 25% Alcohol
- Loeffler's Methylene blue
- Distilled water

Staining Procedure:

- Put the heat fixed smears onto a platform made using two parallel glass rods over a wash basin.
- Cover the slide with carbol fuchsin and heat the slide from below until steam uses.
- Allow to stain for 5-8 minutes with intermittent heating, putting fresh carbol fuchsin on the slide time to time.
- Care should be taken not to allow the stain to dry on the slide.
- Wash the slide preferably using distilled water. (Do not use tap water).
- Cover the slide with 20% Sulfuric Acid. Wash the slide with water after one minute. Pour more acid and continue decolorisation till smear is just faint pink.

- Wash the slide again with water.
- Cover the slide with 25% Alcohol for 2 minutes.
- Wash with water.
- Counterstain with Loeffler's methylene blue for 30 seconds.
- Wash the smear with tap water, air dry and examine under oil immersion.

Observations:

Mycobacteria appear as bright pink, slender, slightly curved rods, whereas the background tissue, cells and other organisms are stained blue.

Note:

- Staining jars should never be used as with a positive stained slide. the bacilli may get detached from the slide and float about in staining fluid later on sticking to the negative slides and may give false positive results.
- After examining a positive slide, take care to wipe the lens with a clean tissue paper before examining the next slide.
- Do not record the smear to be negative unless at least 200 microscopic fields have been thoroughly examined under oil immersion objective.

4.4 Diagnosis of Plague

Plague is an ancient scourge of mankind, which is a bacterial disease caused by *Yersinia pestis*. It is endemic in rodents and fleas. In man, plague occurs mainly in three forms, bubonic, pneumonic and septicaemic.

The presumptive diagnosis of Plague can be established by microscopic examination.

Sample collection:

- Bubo aspirate: in bubonic plague should be collected.
- Under all safety precautions collect bubo aspirate by puncturing the bubo with a sterile hypodermic syringe and exudate is withdrawn.
- Sterilize the puncture site with tincture iodine.

- o 10 ml or 20 ml syringe, armed with 18/19 guage needle and a few ml of sterile saline drawn into the syringe, should be used for aspiration of bubo aspirate.
- o Bubo is then punctured and suction applied.
- o If aspiration does not produce fluid, then saline is injected into the bubo again and aspirated again.
- o Transfer the exudate into a sterile container.
- o Label the container.
- o Transport to the laboratory at 2-8°C.

4.4.1 Sputum Collection in Pneumonic Plague:

- o Collect the sputum sample in a sterile wide mouth screw capped container.
- o Label the specimen
- o Transport the specimen to laboratory at 2-8°C.

In the Laboratory:

- o Make three smears out of the same portion of exudate/sputum taking precautions not to form aerosols.
- o Air dry the smear.
- o Stain smears either by Methylene blue/Gram staining/Waysons stain.

4.4.2 Gram Stain:

This is a routine laboratory procedure used for examining specimens suspected to contain bacteriologic agents. Direct microscopic examination of specimens and cultures can provide a rapid presumptive diagnosis. Gram stain results, the shape of cell (cocci, bacilli), the type of cell arrangement (single, chained, clustered) visualized under light microscopy, can provide a quick assessment of what the etiologic agent may be.

Principle:

The Gram stain forms the cornerstone of microscopic bacteriology. It was described by Hans Christian Gram over 100 years ago. Crystal violet (gentian violet) is the primary stain that will bind to the peptidoglycan present in the cell walls of some bacterial cells.

Iodine is added as a mordant to fix the dye. If the cell wall does not contain peptidoglycan then crystal violet is easily washed off with acid or alcohol (decolorizer). A secondary dye, safranin (counterstain), is added after the decolorization step. If the primary did not bind the cells will easily adsorb safranin. Thus gram-positive cellls are purple, while gram-negative cells are pink/red.

Requirements:

- Crystal violet (0.5%)
- Gram's Iodine (1%)
- Acetone (100%) or Ethanol (95%)
- Safranine (0.5%)

Procedure:

- Cover the slide with crystal violet solution and allow to act for about 30 seconds.
- Pour off stain and holding the slide at an angle downwards pou on the iodine solution so that it washes away the crystal violet ; cover the slide with fresh iodine solution and allow to act for 1 minute.
- Wash off the iodine with ethanol and treat with fresh alcohol, tilt the slide from side to side until colour ceases to come out of the preparation. This is easily seen by holding the slide against a white background.

  Or

  Decolorize with 100% acetone. First, tip off the iodine and hold the slide at a steep slope. Then pour acetone over the slide from its upper end , so as to cover its whole surface. Decolorization is very rapid and is usually complete in 2-3 decond. After this period of contact, wash thoroughly with water under a running tap.
- Apply the counterstain (0.5% safranine) for 30 seconds.
- Wash with water and blot dry

When to use this procedure and what you expect to see

Y.pestis appears as a fat, short, gram-negative coccobacilli about 1 /um by 0.5 /um. Gram stains are typically done on cultures/subcultures, buboe aspirates, spleen, liver and sputum smears.

Critical value/ Action to be taken:

When gram stained material reveal small coccoid gram-negative bacilli. Material should be further worked up with culture isolation and identification. No notification is needed at this time.

Interpretation: *Y. pestis* appears as a fat short, gram negative coccobacilli about 1 um by 0.5 /um.

4.4.3 Wayson stain for visualizing Yersinia pestis:

Wayson stain is a polychromatic differential stain used as a presumptive test for the presence of *Yersinia* and *Pasteurella* spp.

Principle

Basic fuschin and methylene blue in the Wayson stain bind to bacterial cells which appear morphologically under light microscopy as bipolar, closed safety pin-shaped cells. The differential polychromatic mrophology can be visualized with many differenttypes of organisms therefore Wayson stain alone is not diagnostic for *Y.pestis*.

Critical values/Action to be taken:

When stained unknown material has a characteristic "safety pin" morphology, it is Wayson stain positive. Further work-up by culture isolation and identfiication must follow No notification is needed-unless submittor specifically requests notification.

If Wayson bi-polar organisms known to have "safety pin" morphology cannot be visualized after staining, check reagents and check for possible technical problems. Repeat stain until characteristic morphological results are obtained with control cultures.

Materials needed for this test:

Wayson stain:

- Dissolve 0.2 grams of basic fuchsin and 0.75 grams of methylene blue in 20 ml of 95% ethanol. Filter solution through Whatman #1 paper (or equivalent).
- Pour dissolved, filtered stain into 200 ml of 5% aqueous phenol. Store at room temperature. Avoid exposure to light.

Procedure:

- Prepare smear of tissue or culture on slide, air dry.
- Heat fix smear or fix in absolute methanol for 3 minutes, air dry slide. (Methanol fixation yields more contrasting staining than heat fixation).
- Flood smear with Wayson stain for 5-10 seconds.
- Wash slide in tap water, blot gently or air dry.
- Examine slide under light microscope.

Interpretation:

Consistent, striking bipolar "safety pin" morphology of small, fat bacilli are characteristic of the *Yersinia* and *Pasteurella* spp. Other bacteria may exhibit bipolar appearance as well, especially if the specimen is taken from areas with a wide variety of normal flora (nasal, pharyngeal, and fecal).

"All *Y.pestis* are Wayson positive, but all Wayson positive stains are not *Y.pestis*".

Quality control measures:

Test each lot of Wayson stain using known Yersinia/Pasteurella spp. (positive control) and with Escherichia coli or other enteric bacteria as negative controls. When examining tissue smears, controls slide prepared with plague bacilli infected and uninfected tissue smears should also be examined.

4.4.4 Methylene Blue Staining;

Material required:

- Air dried smear.
- Methylene blue stain.

Procedure:

- Fix the smear by dipping the slides in a jar containing pure Methanol for 5 minutes.
- Cover the smear with methylene blue stain.
- Leave the stain for 3 minutes.
- Wash with tap water. Air dry .
- Examine under the oil immersion of microscope.

Observation:

Characteristic bluish bacilli showing bipolar staining. Suggests presence of Y.pestis organisms.

4.5 Malaria

Malaria is a parasitic disease caused by Plasmodium species. In India, the disease is commonly caused by P.vivax and P.falciparum. The laboratory diagnosis is based on demonstration of different stages of the parasite in the peripheral blood film of the patient.

4.5.1 Collection of sample:

Peripheral blood smear:

Time for taking blood:

- Collect blood either during or 2-3 hours after the peak of temperature.
- Sample should be taken before administration of antimalarial drugs.

4.5.2 Preparation of blood smear:

Both thick and thin films should be made on the same slide.

Blood sample should be collected from the tip of the ring finger of the left hand. However in small children, sample should be collected either from the heal or the tip of the big toe of the foot taking all aseptic precautions using a sterile needle or a lancet (see also page.... under "Filter paper method").

Apply gentle pressure to the finger and collect a single small drop of blood on to the mdidle of the slide. This is for the thin film. Apply further pressure to express more blood and collect 2 or 3 large drops on the slide about 1 cm from the drop intended for the thin film. Wipe the remaining blood away from the finger with cotton wool.

Thin film: Using another clean slide as a 'spreader' and with the slide with the blood drops resting on a flat firm surface, touch the small drop with the speader and allow the blood to run along its edge. Firmly push the spreader alongwith the slide away from the largest drop keeping the spreader at an angle at 45oC. Make sure the spreader is in even contact with the surface of the slide all the time the blood is being spread.

> Thick film: Always handle slides by the edges or by a corner to make the thick film as follows:
>
> Using the corner of the spreader, quickly join the larger drops of blood and spread them to make an even thick film. The blood should not be excessively stirred but can be spread in a circular or rectangular form with 3 - 6 movements.

> Allow the thick film to dry in a flat level position protected from flies, dust and extreme heat . Label the dry film with a pen or marker pencil , by writing ross the thicker portion of the thin film the patient's name , or number and the te. Do not use a ball pen to label the slide.

> Wrap the dry slide in clean paper and despatch with the patient's record form the laboratory as soon as possible.

> The slide used for spreading the blood films must be disinfected and could en be used for the next patient, another clean slide from the pack being used as a spreader.

4.5.3 Staining of Blood smears:

GEIMSA STAIN

Materials and Reagents:

1. Geimsa stain powder/ Ready Giemsa Stain solution.
2. Alcohol
3. Methanol
4. Marking pen
5. Staining jars
6. Boric acid Borax buffer - pH 7.2.

Preparation:

- Dissolve the stain powder in alcohol as per the manufacturer s instructions.
- Prepare Borax Acid - Boric buffer as below:

  a) Dissolve 12.4 gms of Boric Acid in l lit. of Distilled water (I)

  b) Dissolve 19.05 gm Borax in l lit of Distilled water (II)

Take 50 ml of solution I and adjust the pH to 7.2 using appropriate volume of solution II. Then make up the volume to 200 ml with distilled water.

Staining Technique:

- Prepare thick and thin smear from malaria case on a glass slide.
- Deaemoglobinize the thick smear by placing the film in a vertical position in a ass Jarontaining distilled water for 5 minutes. When film becomes white, take t and dry in upright position.
- Fix the thin smear in methanol for 15 minutes.
- Dilute the Giemsa's stain solution, one part with 9 parts of Boric buffer pH 7.2.
- Immerse the smears in this stain for 1 hour.
- Wash the smears in buffer solution.
- Blot and dry.
- Examine the slide under oil immersion of microscope.

4.5.4 J.S.B. Stain

Materials and Reagents Required:

- Eosin yellow (water soluble)
- Methylene Blue
- Potassium Dichromate
- Di-sodium hydrogen phosphate (dihydrate)
- 1% sulphuric Acid.
- Round bottom flask (2 lit.)
- Healing mantle
- Distilled water
- Staining jars.

Preparation:

J.S.B. II

Dissolve 2 gms Eosin Yellow in 1 lit. of distilled water and store in the dark for 4 weeks before use.

J.S.B.

⇒ Dissolve 1 gm of Methylene blue in 600 ml of

⇒ distilled water and mix well.

⇒ Add 1% sulphuric acid (6.0 ml) drop by drop and shake well.

⇒ Add 1 gm of potassium dichromate and shake well till precipitation occurs.

⇒ Dissolve the precipitate by adding 7 gms. of Di-sodium hydrogen phosphate dihydrate.

⇒ Make up the volume to 1 lit.

⇒ Boil the stain in round bottom flask over a heating mantle for one hour.

⇒ Cool the stain and re-adjust the volume to 1 lit by adding distilled water.

⇒ Store in dark for 4 weeks before use.

Staining technique:

⇒ Prepare thin and thick smears from malaria cases on micro slides.

⇒ De-haemoglobinise the thick smear.

⇒ Fix the thin smear in methanol for few minutes.

⇒ Take 3 staining jars for J.S.B. I, J.S.B.II and tap water.

⇒ Dip the smears in J.S.B. II for few seconds and immediatedly wash in water.

⇒ Drain the slides free of excess water.

⇒ Dip the smears in J.S.B.I for 30–40 seconds.

⇒ Wash well in water and dry.

⇒ Examine the smears under oil immersion.

4.5.5 Observation:

Examine thin film first. If no parasite is found then only examine thick film. If parasites are seen in the thick film but the identity is not clear, the thin film should be reexamined more thoroughly so as to determine nature of infection.

Thin film examination:

o Area of the film examined should be along the upper and lower margins of tail end f ilm as parasites are concentrated over there.

o A minimum of 100 fields should be examined in about 8-10 minutes.

c) The following stages of the parasite can be observed in a peripheral blood thin smear.

I. Ring, trophozoite, schizont and the gametocytes in case of *Plasmodium vivax*.

2.The infected erythrocytes is usually enlarged in *P.vivax* infection.

3.However, in case of P.falciparum infection, it is mainly the ring stages which are een and occasionally schizonts and trophozoites. During the late stages of the disease ven crescent shaped gametocytes can be seen in the peripheral blood.

Observation on thick smear:

o Only elements seen are leucocytes andmalarial parasites.

O Mrphology of malarial parasites is distorted.

o secies of parasites cannot be identified.

Appearance in thick film

o Trophozoites appear as streaks of blue cytoplasm with

detached nuclear dots. The ring forms rarely seen.

o Schizonts and gametocytes, however, retain their normal appearances (although the pigments are seen more clearly) are seen if present in smear.

4.6 **Examination of blood for Microfilaria**

Filariasis is a disease of the lymphatics caused mainly by the nematode *Wuchereria bancrofti* and rarely *Brugia malayi*.

Laboratory diagnosis:

Is based on the demonstration of the larval stages of the parasite in the peripheral blood of the cases.

4.6.1 Collection of blood:

The blood should be preferably collected between 10 PM and 2 AM specially in areas where microfilaria shows nocturnal periodicity.

4.6.2 Examination of unstained preparation:

- Take 2-3 drops of blood on a clean glass slide. Put a coverslip on it.
- The rim is then smeared with vaseline to prevent drying up of the blood.
- Examine the slides under low power microscope immediately or within 24 hours of ollection of blood.
- Wriggling microfilaria present in the blood can be seen.

4.6.3 Examination of stained smear:

Thick film:

- Prepare a thick blood film as per the instruction given in the Chapter on Malaria.
- Dehemoglobinise the smear by putting the slides in a jar containing water.
- Air dry
- Fix the smear with methyl alcohol.
- Stain with Geima's stain as described earlier.
- Examine the smear under the oil immersion of the microscope.

Thin film:

- o Prepare as described for malaria.
- o Fix it with Methanol by dipping the smear in a jar containing methanol for 15 minutes.
- o Stain it with Giemsa stain as described earlier.

Observation:

Microfilaria of *Wuchereria bancrofti* are seen.

- Size - 290 /u in length and 6-7/u in breadth.
- It has blunt head and pointed tail and has smooth curve.
- Structureless sack called Hyaline Sheath seen where it projects beyond the extremites f embryo.
- Somatic cells/nuclei seen as granules in central axis from head to tail end except the erminal 5 percent area. At the anterior end there is a space devoid of granules called ephalic space.
- The granules are broken at definite space serving as the landmarks for identification of the species.
  - Nerve ring, a oblique space.
  - Antereior V spot, represents the rudimentary excretory system.
  - Posterior V spot or tail spot, represents the terminal part of the alimentary canal.

Microfilaria of *Brugia malai*.

- Smaller then Wucheraria bancrofti (230 /u x 6 /um)
- Possess secondary Kinks instead of smooth curved.
- Cephalic space is broader.
- Tail tip is not free of nuclei and nuclei are blurred.
- It lies folded with head close to tail.

CHAPTER - 5

SEROLOGICAL TESTS

CHAPTER- 5

5. SEROLOGICAL TESTS:

5.1 LATEX AGGLUTINATION TEST FOR MENINGITIS

The ideal immunological test, which is also a rapid test and easy to perform in a district laboratory, is the latex agglutination (LA) test. test is done to detect the bacterial antigen (Capsular polysaccharide) in CSF samples collected from patients.

The available comercial kits are designed to provide diagnosis for meningitis caused by:-

- *N. meningitidis* serogroup A
- *N. meningitidis* serogroup C
- *Streptococcus pneumoniae*
- *H.influenzae* type B

The general procedure for performance of the test is given below, however, the laboratory personnel are advised to go through the instructions provided by the kit manufacturer, carefully, and strictly adhere to the same.

5.1.1 Equipments required: (but not supplied with the kit)

- Pasteur pipettes (sterile)
- Rubber teats
- Container with disinfectants (for discard)

5.1.2 Procedure

- Systematically heat all CSF specimens for 5 minutes at 80-100°C.
- Centrifuge the CSF samples at 2000 rpm for 10 minutes, preserve the supernatant for further use.
- Shake each latex suspension well.
- In the corresponding fields of the slide, dispense one drop of each of the latex suspension fllowed by one drop of the CSF supernatant.

- o Mix with a stirring stick; use separate stick for each combination of CSF and latex suspension.

- o Rotate the slide. and read within 2 minutes.

3.1.3 Controls: Periodically check:

a) thatnone of the four latex reagents agglutinate in presence of 0.15 mol/l NaCl solution.

b)that each of the four latex reagents do agglutinate with positive control.

3.1.4 Reading:

- o Negative reaction: The CSF latex suspension mixture remains a "milky suspension" (disregard any granules that may occur with *S.pneumoniae*).

- o Positive reaction: Distinct rapid agglutination occuring within 2 minutes

(normally 30 seconds).

3.1.5 Interpretation:

Agiutination with one of the latex reagents indicates presence of the corresponding antigen in the CSF sample.

Advantages of LA test:

- o Most sensitive method available

- o Rapid

- o Good field applicability

- o Can diagnose the disease even in antibiotic treated patients.

- o No special equipment/instrument required.

Disadvantages of LA test:

- o Commercial kits not produced in India; to be imported.

- o Expensive

- o The test does not yield any bacterial isolate; other prameters cannot be tested.

5.2 DIAGNOSIS OF HEPATITIS B VIRAL INFECTION:

Diagnosis of Hepatitis B viral infection is very important, not only in case of chronic hepatitis and liver cirrhosis patients, but also in the screening of donor blood samples, to ensure safe blood transfusion and to control or check the spread of hepatitis B infection through unsafe bloodt ransfusion.This is achieved by detection/demonstration of 'Hepatitis-B surface Antigen" (HBsAg) or the 'Australia Antigen in the patient/donor blood samples.

A simple latex agglutination test for rapid detection of HBsAg, which is very much feasible in the district laboratories, is described below:

LATEX AGGLUTINATION TEST FOR RAPID DETECTION OF HBsAg

(AUSTRALIA ANTIGEN)

5.2.1 PRINCIPLE:

A distinct agglutination occurs, when serum sample containing HBsAg is mixed with latex particles coated with purified and highly reactive anti-HBsAg antibodies: there would be no agglutination when the serum sample does not contain HBsAg".

5.2.2 MATERIALS AND REAGENTS:

Commercial kits for this test are available in India. They contain the following reagents and accessories.

Reagent 1:	HBsAg Latex Reagent	- 1 vial
Reagent 2:	Positive control serum	- 1 vial
Reagent 3:	Negative control serum	- 1 vial
Accessories:	Disposable plastic slides	
	Disposable applicator sticks	
	Disposable plastic droppers	
	Rubber teats.	

All the reagents are stable and active, till the expiry date mentioned, provided they are stored in a refrigerator at 2-8°C. Do not freeze the reagents.

5.2.3 SPECIMEN:

- The test is performed on serum harvested from the patient's/donor's blood.

- Do not heat inactivate the test or the control sera samples.

- If delay in testing, store test sera samples in a refrigerator or deep freezer, takingcare to avoid repeated freezing and thawing of the specimens.

5.2.4 TEST PROCEDURE

- Allow the reagents to attain room temperature, and shake the vials gently to make sure that the latex reagent is completely in suspension.

- Place one drop (50 /ul) of undiluted serum in one of the circles on the slide. More circles to be filled if more than one test sera samples are to be tested. Use separate droppers for each specimen.

- Add one drop (50 /ul) of latex reagent on to each specimen drop in circles, using a disposable dropper.

- Mix the content of each circle, using separate disposable applicator sticks for each circle, and spread the mixture uniformly over the entire area of the circle.

- Rock the slide gently, to and fro, for 5 minutes, and watch for agglutination.

Precautions:

1. To avoid contamination of reagents, make sure that the cap of each vial is properly and promptly applied to the same vial. Interchanging of caps and droppers lead to contamination and erroneous results.

2. Improper mixing and interchange of applicator sticks also lead to erroneous results.

3. Vigourous rocking of slides may lead to impaired agglutination.

Use of Controls: Positive and negative controls are not always required, when reagents are in continuous use. However, the performance of the kits needs checking, occasionally, using the controls.

5.2.5 Interpretation:

- Visible agglutination within 5 minutes - **HBsAg Positive**

- No agglutination - **HBsAg Negative**

5.2.6 LIMITATIONS:

- Probability of **FALSE POSITIVITY** = 1% of all samples, due to presence of other antigens (RF).

- **FALSE NEGATIVE results** may be encountered with specimens containing very high titres of HBsAg (Prozone effect). In such cases the characteristic syndrome (severe jaundice, GPT/GOT elevation) will be apparent. In that case repeat the test after diluting the specimen 1:40, with normal saline.

5.3 VDRL SLIDE FLOCCULATION TEST FOR SYPHILIS:

This is a test with high sensitivity and specificity and can be used for rapid and exact quantitative titration of the reactive sera samples.

5.3.1 PRINCIPLE:

The VDRL antigen particles, which are seen as small fusiform needles under the microscope, floculate into clumps (small, medium and large), when they comein contact with a reactive (+ve) serum.

5.3.2 MATERIALS:

VDRL Antigen:

It consists of a mixture of Cardiolipin, lecithin and cholesterol in definite proportions and is commercially available. Each sealed glass ampoule contains 0.5 ml (with sufficient excess for convenient withdrawal). Antigen amouples should be stored in a cool, dark place. Ampoules showing precipitate should be discarded.

Buffered Saline Solution:

10 ampoules containing 5 ml each are supplied with each package of VDRL antigen. Buffered saline is required for preparing the antigen emulsion for the test.

SLIDES:

Glass slides, 2"x3", with 12 paraffin rings of 14 mm inner diameter are used for the test. Slides of same size, with permanently fixed ceramic rings are also available commercially and may be used. The following points regarding the slides are to be noted.

- New slides, as well as the used slides should be cleaned thoroughly.

- Slides should be handled by the edges only, to avoid any greasy finger prints.

- Serum within the circles will spread evenly, within the rings, only if the slides are absolurtely clean.

- Parafin rings can be made on slides by transferring molten paraffin on to slide using a suitable mould or threaded wire rings.

5.3.3 PROCEDURE:

A. Preparation of serum:

- Inactivate serum by heating at 56°C for 30 minutes.

- On removal from water bath, centrifuge the serum sample if it shows particulate debris.

- Test sera sample need to be reheated (at 56°C for 10 min.), if they are >4 hr. old since original inactivation.

- 0.05 ml of each sample is required for testing.

B. PREPARATION OF ANTIGEN EMULSION:

- Pipette out 0.4 ml of buffered saline on to the bottom of a 1 oz.reagent bottle with flat or concave inner bottom surface.

- Add 0.5 ml of VDRL antigen, drawn out from an ampoule, using a graduated pipette, directly on to saline in the reagent bottle, while rotating the bottle on a flat surface.

- The antigen should be added drop by drop, but rapidly, so that it takes approximately 6 seconds to complete the delivery of antigen.

- Blow the last drop of the antigen and continue rotation of the bottle for 10 more seconds.

- Add 4.1 ml. of buffered saline, using a graduated 5 ml. pipette.

- Stopper the bottle and shake it vigorously for about 10 seconds.

- Take care to see that the temperature of buffered saline solution and that of VDRL antigen is maintained within the range of 23-29°C, during preparation of the antigen emulsion.

- Maturation of antigen is important for increased sensitivity, maturation is complete in 15-30 minutes, after preparation.

o Store the antigen emulsion in a refrigerator, if necessary. It should be brought to room temperature and shaken gently before use.

o 5.0 ml of antigen emulsion would suffice for 250 serum tests.

o Each batch of antigen emulsion prepared must be pre-tested with known ractive and non-reactive sera samples, in order to confirm that exact pattern of distribution of antigen particles, typical of reactive and non-reactive sera samples, would result on testing.

5.3.4 TEST PROCEDURE:

o Qualitative Test:

* Pipette out 0.05ml of inactivated serum into one paraffin/ceramic ring on the glass slide; serum should spread.

* Add one drop (1/60 ml) of antigen emulsion on the serum within the ring.

* Rotate the slide for 4 minutes, by hand on a flat surface (+ or -120 times per minute covering circle of 2" dia.)

5.3.5 READING AND REPORTING OF RESULTS:

o Read the test results immediately after rotation.

o Observe the slide under micrscope, using low power objective (100 x magnification)

o Antigen particles appear as small fusiform needles, they are more or less evenly spread in case of a non-reactive serum sample, and aggregated into clumps (flocculation) in the case of a reactive serum. Grade the observations as under:

No clumps or very slight roughness	.....	NON-REACTIVE (N)
Small clumps	.....	WEAKLY REACTIVE (W)
Medium and large clumps	.....	REACTIVE (R)

Zone reactions are possible; they are recognizable by irregular clumping. The clumps are not compact and very small and large clumps may be seen within the same microscopic field. In such cases, the results are reported on the basis of quantitative test done on the same serum.

O Quantitative test:

Quantitative test is performed on all positive (reactive) serum samples and on samples which show weak(W) or "rough" reaction in the qualitative tests.

* Prepare successive two-fold dilution (1:1, 1:2, 1:4, 1:8, 1:16, 1:64 etc.) of serum sample to be tested, using 0.9% saline.

* Each serum dilution sample thus prepared is treated as an individual sample and tested as described under "qualitative" test.

* Results are read and graded under the microscope as before.

Reporting of results:

Results are reported in terms of the highest dilution of the serum that produces a definite positive (or Reactive, R) reaction as below. Weakly reactive is not acceptable.

Serum Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	Report
	R	W	N	N	N	N	N	R1 dil
	R	R	W	N	N	N	N	R2 dils.
	R	R	R	W	N	N	N	R4 dils.
	W	W	R	R	W	N	N	R8 dils.
	N	W	R	R	R	N	N	R16 dils.
	W	N	N	N	N	N	N	WO dils.
	W	W	N	N	N	N	N	R1 dils.

5.4 RAPID PLASMA REAGIN (RPR) TEST FOR DIAGNOSIS OF SYPHILIS:

This test detects antibodies formed, in the blood of syphilitic patients, against Cardiolipin. These antibodies are called "Reagin". Two advantages of this test over the previously described VDRL Slide flocculation test are - (a) It does not require a microscope to read the test results; (b) The test sera/plasma sample need not be inactivated prior to testing.

5.4.1 PRINCIPLE:

"Reagin formed in the blood of syphilitic patients cause flocculation of the antigen, which co-agglutinates with the charcoal particles, giving small black clumps that are readily visible without a microscope".

Commercial "Rapitest" kits, designed for carrying out 50 tests per kit, are available in India.

5.4.2 REAGENTS AND MATERIALS:

a) Provided in the Kit:

RPR Antigen	...	1 vial
Positive Control serum	..	1 vial
Negative control serum	...	1 vial
RPR Antigen dropper	...	1
Specimen droppers (disposable)		
Rubber Teats		
Mixing sticks (disposable)		
Plastic test cards	..	9

b) Materials required, but not provided in the kit:

Micropipette (capable of delivering 0.05 ml of test sample)

Stop watch

Saline solution (0.9%) - Only for quantitative test.

Container with disinfectant (for discard)

Storage: The RPRantigen and control sera will remain stable and active, till the expiry date printed on the label, provided they are stored in a refrigerator between 2-8°C. They should not be freezed.

5.4.3 THE SPECIMENS:

a) Serum:

o Use fresh serum harvested from patient's blood sample.

o If the test cannot be conducted immediately due to some reason, store the serum sample between 2-8°C in a refrigerator, BUT NOT LONGER THAN 48 hr., after collection.

b) Plasma:

o Collect patient's blood into a tube/vial containing one of the anticoagulants (EDTA, Heparin, Oxalate, Sodium Flouride etc.) Avoid excess of coagulant.

o Centrifuge the blood sample, to separate the cells.

o Use the plasma sample within 18 hr. of collection.

o Inactivation of serum/plasma samples is not necessary.

PRECAUTIONS:

o Blood samples should be collected from fasting patients, since very lipaemi samples may give false +ve reactions.

o Do not use grossly haemolysed samples.

o Discard contaminated samples.

5.4.4 TEST PROCEDURE

A. Qualitative test:

o Allow all reagents to attain room temperature.

o Place one drop of (0.05 ml) test serum or plasma, positive control and negative control sera on to separate circles on the plastic test card, using disposable specimen droppers provided.

o Shake the RPR antigen suspension gently, to resuspend the particles.

o Place one drop (0.015-0.02 ml) of the antigen suspension, on each of the circles containing test samples and the positive and negative control sera drops, using the antigen dropper provided.

o Mix the contents of each circle, using the disposable mixing sticks provided, and spreading the reagent mixture over the entire area of the circle.

o Gently rock the card, to and fro, for 6 minutes, either manually or on a mechanical shaker at 100 rpm, to ensure thorough mixing.

o Read the results at the end of 6 minutes, using a high intensity light source.

Interpretation of Results:

POSITIVE (REACTIVE	-	Development of clearly visible clumps of black particles, within the test circles.
NEGATIIVE (NON-REACTIVE)	-	No development of clumps, the charcoal particles remain in a HOMOGENEOUS GREY SUSPENSION.

A quantitative estimation is further recommended for all samples positive in the qualitative test.

3. Quantitative test:

o Dispense 0.05 ml (50 /ul) of saline solution on to each of the circles (No.1-5) on the test card, using a micropipette.

o Dispense 0.05 ml (50 /ul) of the specimen (test serum/plasma) onto circle 1 and mix the two (saline and test sample) thoroughly by drawing the mixture into the micropipette, up and down several times.

o Transfer 0.05 ml (50/ul) of the mixture in Circle-1, on to the drop of saline in Circle-2. Repeat the mixing action, several times, as explained above.

o Repeat transferring and mixing actions from Circle-2, through circle-5.

o Discard 0.05 ml (50 /ul) from Circle-5, after mixing.

o The dilutions of specimen obtained in different circles on the test are as under:

CIRCLE	1	2	3	4	
SALINE (ml)	0.05	0.05	0.05	0.05	
SPECIMEN (Serum / Plasma) ml	0.05	-	-	-	
MIX & TRANSFER	0.05				
DILUTION	1:2	1:4	1:8	1:16	1:32

o Using the disposable mixing sticks, spread the specimen dilutions in the circles to cover the area of the circle. Start with circle 5 and end with Circle 1. Wipe the sticks clean between circles.

o Gently shake the RPR antigen vial to resuspend the particles, and add one drop (0.15-0.20 ml.) of antigen, on to each circle, using the antigen dropper.

o Gently rock the card to and fro for 6 minutes (manually or on a mechanical shaker), to ensure thorough mixing.

o Read results at the end of 6 minutes, as described above under Qualitative testing.

5.4.5 Interpretation:

The highest dilution of the sample, giving a definite positive reaction, is considered as the titre of the specimen. In case the titre exceeds 1:32, continue with double dilutions beyond that point,till the titre is obtained.

LIMITATIONS OF THE TESTS (RPR and VDRL Slide Flocculation):

Both these tests are considered as "non-treponemal antibody tests", which are primarily meant as screening tests. If the tests are positive when there is no clinical evidence of syphilis, they must be repeated; if positivity persists, verifications by more specific tests (for anti-Treponemal antibody) would be necessary to confirm syphilis. In RPR and VDRL slide flocculation tests, false positive results may be obtained in diseases such as leprosy, malaria, toxoplasmosis, infectious mononucleosis and lupus erythematosis, and also in specimens having bacterial contamination.

5.5 WIDAL TEST FOR DIAGNOSIS OF ENTERIC FEVER:

(TYPHOID AND PARATYPHOID)

Widal test is an agglutination test for detection of antibodies against *Salmonella typhi* and *Salmonella paratyphi*, the common causal agents of enteric fevers.

5.5.1 PRINCIPLE:

"When serum sample containing antibodies against *S.typhi* and *S.paratyphi* AB are mixed with respective antigens, agglutination will take place".

In *S.typhi* and *S.paratyphi* AB, two types of antigens are recognised as diagnostically important:

(a) 'O' antigen or 'Somatic' antigen.

(b) 'H' antigen or 'Flagellar' antigen.

'O' antigens of various species have components in common and hence only one 'O' antigen i.e. that of *S.typhi* is employed; the 'H' antigens of *Salmonella* spp. are species specific, and hence the 'H' antigens of all three, viz. *S.typhi*, *S.paratyphi* A and *S.paratyphi* B, are employed in the test.

Commercial test kits for WIDAL test are available in India, and using them both quantitative and quantative tests can be put up on suspected sera samples.

5.5.2 MATERIALS AND REAGENTS:

(a) Test kit: contains the following reagents and materials.

Reagent 1: *S.typhi* ('H')	-	5 ml
Reagent 2: *S.typhi* ('O')	-	5 ml
Reagent 3: *S.paratyphi* A ('H')	-	5 ml
Reagent 4: *S.paratyphi* B ('H')	-	5 ml
Reagent 5: Positive control	-	1 ml
Glass slide	-	1 No.

Product Insert - 1 No.

(b) Materials required, but not supplied in the kit:

Small, dry and clean glass tubes ... 8/specimen

(for quantitative tube test)

Normal saline solution

Water bath

Micropipette / dropper

5.5.3 SPECIMEN:

Fresh serum (patient) free from contamination should be used. In case of delay in testing, store the sera samples at 2-8°C in a refrigerator.

Note: Specimen is used undiluted.

Do not use haemolysed specimen.

Do not heat or inactivate the specimen.

5.5.4 TEST PROCEDURE

A. Qualitative slide test for screening.

- Clean the glass slide provided and wipe it dry.
- Place a drop of undiluted serum sample to be tested in each of the first four circles.
- Add one drop of Reagent 1, Reagent-2, Reagent-3 and Reagent 4, on to the specimen drop in Circles 1-4 respectively.
- Mix the contents of each circle with separate mixing sticks, and spread the mixture to cover the whole circle.
- Rock the slide gently for 1 minute.

- Read the results at the end of one minute.

Interpretation:

A positive reaction shows agglutination, visible to naked eye, in the respective circle. Then proceed for quantitative slide test or quantitative tube test for the appropriate antigen.

B. Quantitative slide test:

- Clean the glass slide supplied in the kit and proceed as follows:

Circle No.	Serum volume	Appropriate antigen	Titre
1	0.08 ml	1 drop	1:20
2	0.04 ml	1 drop	1:40
3	0.02 ml	1 drop	1:80
4	0.01 ml	1 drop	1:160
5	0.005 ml	1 drop	1:320

- Mix the contents of each circle, starting with circle 5 and through Circle-1, wiping the mixing stick clean between circles.
- Rotate the slide for one minute and observe for agglutination.

Interpretation: Titre of the serum is the highest dilution of the serum giving a positive reaction.

C. Quantitative tube test:

- Take a set of 8 clean glass tubes, per specimen, per antigen.
- Prepare dilutions of serum specimen and add appropriate antigen as below:

TUBE	1	2	3	4	5	6	7	8
Serum dilution	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Saline control
Normal saline	1.9ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0 ml	1.0 ml
Patient serum	0.1ml							
Transfer diluted serum		1ml 	1 ml 	1 ml 	1 ml 	1 ml 	1 ml 	1 ml (discard 1 ml) 
Appropriate antigen	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop

- Mix well and incubate at 37oC for 16-20 hr. and observe for agglutination.

- Repeat steps (ii) and (iii) with all antigens which showed agglutination in the screening test.

- Note the highest dilution showing clearly visible agglutination with naked eye.

  'O' antigen shows granular agglutination.

  'H' antigen shows flocular appearance.

- Saline control should remain unchanged as it is a negative control.

Interpretation: Agglutination titre of >1:80 is suggestive of infection.

Factors affecting WIDAL Test:

Effect of antibiotic administration:

There is evidence that early treatment with antibiotics suppresses the antiboduy response by suppressing the multiplication of organisms. This may result in a low titre in WIDAL test.

Effect of past infection or typhoid vaccination:

It has been seen that th 'H' antibodies persist for a long time upto many years after typhoid vaccination. Also, many years after recovering from enteric fever, any grave negative bacterial infection can trigger a Salmonella 'H' antibody production, thereby giving a false positive result in WDAL test.

Time of collection of blood sample:

This is a very important parameter affecting the results of the WIDAL test. A single blood sample collected during the first week of the illness may give a negative WIDAL result, whereas in the same patient, a sample collected during the third week of illness may show a very high titre. Accordingly, paired samples should be collected; the first sample being taken as early as possible and the second, 10-14 days later, for optimum results.

CHAPTER - 6

BACTERIOLOGICAL ANALYSIS OF WATER

CHAPTER- 6

6. BACTERIOLOGICAL ANALYSIS OF WATER:

Although it is not possible to lay down fixed standards, as various types of water are examined, from a public health point of view it is generally sufficient to say that no faecal contamination has occured. Coliform bacteria present in water may not be harmful, but they indicate that water supply is contaminated with faecal matter and water is, therefore, liable to contamination with more dangerous organisms. The coliform bacilli of human origin are the most reliable indicators of faecal pollution.

The method of quantitative test for all coliform bacilli known as the 'presumptive coliform count is described below.

6.1 COLLECTION OF SPECIMENS:

Collect water in bottles of 230 ml capacity with ground glass stoppers, having an over hanging rim. Sterilise the bottles by autoclaving.

Tap water:

When water is taken from tap, flame the mouth of the tap and allow the water to run for five minutes before filling the bottle.

Stream, river and lake water:

Insert the bottle with its mouth closed with the stopper, a foot below the surface of water and fill with water. Bring the bottle to the surface and replace the stopper. Avoid the collection of surface water as it contains organic matter.

Precautions:

- During collection of water, avoid the contamination of the sample.
- Test the water samples as soon as possible after collection. If delay is expected, pack the water sample in ice for transport to laboratory.
- When sampling chlorinated water, add a quantity of sodium thiosulphate to the sample bottle before sterilising. This will neutralize the chlorine present in the water.

Presumptive Coliform count:

Requirements:

- Sample of water

- Sterilized test tubes.
- Quarter strength Ringer solution.
- 1 ml and 50 ml pipettes.
- Double strength MacConkey's fluid medium.
- Single strength MacConkey's fluid medium.

Method:

- o Invert the water sample 25 times to mix.
- o Flame the mouth of the bottle and discard 1/3 at the contents and mix thoroughly,
- o Using sterile graduated pipettes, the following amounts of water are added.
- One 50 ml quantity of water to 50 ml double strength MacConkey medium in a flask.
- Five 10 ml quantity each to 10 ml double strength MacConkey medium in test tubes.
- Five 1 ml quantities each to 5 ml single strength MacConkey medium.
- o Incubate all tubes at 37°C for 18-24 hours.
- o All tubes showing acid and gas are regarded as presumptive positives. Reincubate negatives for further 24 hrs.
- o Using McCrady's statistical tables the probable number of coliform organisms present in 100 ml of sample can be calculated.

Interpretation:

Water samples are classified based on the presumptive count in the following way:

Class	Preumptive coliform count 100 ml.
1. Excellent	0
2. Satisfactory	1-3
3. Suspicious	4-10

4. Unsatisfactory >10

Faecal Coliform Count:

From the tubes showing acid and gas in presumptive coliform count, subculture into fresh single strength MacConkey's broth or Incubate at 44 C in a water bath. Tubes showing both acid and gas should be taken as positive for Faecal coliform. Using McCrady's tables compute the number of faecal coliform as in presumptive test. Water showing even one faecal coliform is unfit for human consumption.

Most Probable Number (MPN) values/100 ml of sample, for a set of tests of one 50 ml, five 10 ml, and five 1 ml volumes. (McCrady's Statistical Table)

No. Of tubes giving positive reactions			
1x50 ml	5x10 ml	5x1 ml	MPN/100 ml
0	0	0	<1
0	0	1	1
0	0	2	2
0	1	0	2
0	1	1	2
0	1	2	3
0	2	0	2
0	2	1	3
0	2	2	4
0	3	0	3
0	3	1	5
0	4	0	5

1	0	0	1
1	0	1	3
1	0	2	4
1	0	3	6
1	1	0	3
1	1	1	5
1	1	2	7
1	1	3	9
1	2	0	5
1	2	1	7
1	2	2	10
1	2	3	12
1	3	0	8
1	3	1	11
1	3	2	14
1	3	3	18
1	3	4	21
1	4	0	13
1	4	1	17
1	4	2	22
1	4	3	28
1	4	4	35

1	4	5	47
1	5	0	24
1	5	1	35
1	5	2	54
1	5	3	92
1	5	4	161
1	5	5	>180

6.1 **H_2S-Strip method**:

In recent years a simple, reliable and easy-to-perform (by even untrained personell), 'Yes-No' test for bacteriological quality of water has been devised. This test, which is currently under field evaluation and quality standardization is expected to be adopted as the field test for water quality monitoring in the hands of peripheral health workers and community participants.

Principle:

Presence of coliform bacteria in drinking water is associated with hydrogen sulphide (H_2S)- producing organisms, and faecal pollution of water can be established by demonstration of H_2S production.

It has been claimed, by various workers, that the H2S-strip method shows 80% agreement with the conventional MPN test described above.

Description of the test device (kit):

It simply consists of a pre-calibrated 20 ml glass bottle (McCartney bottle) with a screw-cap lid, from which a strip of specially treated/coated tissue paper hangs down, internally. The whole system is sterile and needs to be opened at the time of water testing.

The paper strip inside the glass bottle (80 cm^2,folded) is pre-soaked in a concentrated medium containing peptone (20g), dipotassium hydrogen phosphate (1.5g), ferric ammonium citrate (0.75g), sodium thiosulphate (1g), Teepol (1ml) and water (50 ml); 1 ml of

the concentrated medium is absorbed on to the folded tissue paper strip and dried at 50°C under sterile conditions. It is then introduced into the sterile bottle.

Test procedure:

- Pour the water sample to be tested for faecal pollution into the bottle, upto the precalibrated level (20 ml).
- Incubate at 37°C or allow to stand at ambient temperature (30-37°C); no incubator is necessary under field conditions, as the bottles can be held in the pockets and body temperature can be made use of.
- Faecal pollution is indicated if the contents of the bottle turn black.

Advantages of H_2S-Strip Test:

- No need to measure the volume of water to be tested:
- No need to dechlorinate the water sample, since it instantaneously dechlorinates thr sample:
- The end point (reading) is very clear, due to development of black colour:
- No incubator is necessary:
- The test starts immediately on collection into the bottle, unlike other methods which start after the sample is transported to the laboratory.

CHAPTER - 7

LABORATORY DIAGNOSIS OF CHOLERA

CHAPTER- 7

7. LABORATORY DIAGNOSIS OF CHOLERA:

Cholera is characterized by sudden onset of effortless and profuse watery diarrhoea. The watery stools with flakes of mucus and sweet fishy odour are characteristic of cholera. These are also popularly known as rice water stool. Cholera is caused by the organism by the name of *Vibrio cholerae* which are Gram negative usually curved by shape of coma and motile by a single polar flagellum. They are oxidase positive.

The laboratory diagnosis is based on demonstration of *Vibrio cholerae* in the stool specimen.

7.1 Collection of samples

Materials required:

- Wide mouth container
- Swabs sticks (sterile)
- Carry blair transport medium
- Case investigation form.

Stool:

(a) Voided stool:

- Most preferred specimen if available.
- Should be collected before antibacterial therapy.
- Should not be collected from bed pan so as to avoid interference from outside bacteria or disinfectant used to clean bed pan.
- Patient may be instructed to void stool in wide mouth container e.g. ice cream cup and transfer 3-5 gm. stool into a sterile screw cap bottle.

(B) Rectal swabs:

Whenever it is not possible to collect stool, a rectal swab specimen may be collected. It is a very useful and convenient sample under field condition and in cases of young babies. By this methodology 0.1-0.2 ml of liquid faeces can be collected.

- Moisten the swab in sterile normal saline, if available.

- Introduce the swab into 4 cm deep into rectum through anal sphancter. Rotate by 90oC and withdraw the swab.

- Store the swab in stoppered container or in transport medium e.g. Carry blair so as to avoid drying.

7.2 Storage and Transportation:

- Store the specimen at 2-8oC.

- Transport to the laboratory at the earliest and in case of delay use to Cary Blair transport medium and send to the nearest laboratory.

Cary Blair transport medium:

It is a semi solid transport medium usually supplied in small bijou bottles. It should be stored in air tight container so as to avoid drying.

Inoculation of Cary Blair Transport Medium:

- Insert one/two rectal swabs taken from the same patient into the medium so that the whole swab is dipped into the medium.

- Break off the extra portion of sticks and replace the screw cap.

- Label the bottles.

7.3 PROCESSING OF SAMPLES IN THE LABORATORY:

Microscopic examination and Culture:

Materials required:

- Enrichment medium

  - Alkaline peptone water.

- Plating media

  - Bile Salt agar

  - Thiosulphate-citrate-

    -Bile Salt-Sucrose (TCBS) agar

- MacConkey Agar

- Inoculating wireloop
- Gas supply/burner
- Incubator at 37°C.
- Hand lens.

After the specimen arrives in the laboratory, it should be given laboratory code number and entered in the register before processing.

Demonstration of motility by direct microscopy:

It can be done using either the direct stool sample or using 4-6 hr. growth in alkaline peptone water.

- Prepare a hanging drop using culture growth in alkaline peptone water or direct stool suspension.
- Examine under the high power of a binocular microscope.

Observation: A darting motility is suggestive of presence of *Vibrio cholerae*.

Culture:

- Directly streak over BSA and TCBS media and also inoculate alkaline peptone water (an enrichment medium).
- Incubate overnight at 37° C in ordinary incubator.
- Examine the plates after overnight incubation while APW is to be examined after 4-6 hours.

Colony morphology on culture plates:

BSA (Bile Salt Agar): Small translucent raised flat colonies are characteristic of *Vibrio cholerae*.

TCBS: Yellow, flat smooth colonies with pale yellow periphery are suggestive of *Vibrio cholerae*.

APW: Subculture growth in alkaline peptone water after 4-6 hour onto BSA/TCBS plates. Incubate plates and APW overnight. Look for the characteristic colonies of *V.cholerae* as described earlier.

7.5 Slide agglutination test for Confirmation of *Vibrio cholerae*:

Materials required:

- Glass slides (clean)
- Normal Saline
- Platinum wire loop
- Growth on BSA/TCBS
- Discarding jars with disinfectant
- Gas supply
- *V.cholerae* 01 antisera.

Procedure:

- Put a small drop of normal saline on a clean glass slide.
- Using a wire loop pick a colony from a culture plate.
- Emulsify the growth in a drop of Normal saline using wire loop.
- Add a loopful of cholera 01 non-differential antisera on to the suspension mix.
- Look for agglutination (i.e. immediate clumping of organism within 30-60 seconds.

Observation: A positive agglutination is indicated by immediate clumping of organism and suggests presence of *Vibrio cholerae* O1 and rest are labelled as non-agglutinating vibrios.

7.6 Stock culture and Referral to Reference Laboratory:

- Make a stab into the nutrient agar slope, the cultures resembling *Vibrio cholerae*. (Agglutinating as well as non-agglutinating strains).

- Transport the stab cultures to the reference laboratory for further testing.

7.7 Composition and source of culture media/reagents necessary for cholera laboratory:

Bile Salt Agar (BSA)

Use: Used for isolation and enumeraton of enteric bacilli.

Readymade dehydrated media is available from Hi-Media Laboratories Pvt. Ltd., Bombay.(Product code M-739 500 gms pack)

Directions for use:

- Suspend 43 gms of media in 1000 ml of distilled water in a flask.
- Plug flask with cotton.
- Autoclave at 15 lbs pressure 121oC for 20 minutes.
- Cool to 60-70°C.
- Pour in sterilised Petridishes.
- Remove air bubbles using flame.
- Let it solidify
- Store in refrigerator.

T.C.B.S. Medium:

(Thiosulphate Citrate Bile Salt Medium)

Use: Recommended for the selective isolation and culture of *Vibrios* causing cholera and vibrios which cause food poisoning.

Readymade dehydrated medium available from Hi-Media Laboratories Pvt. Ltd. Bombay (Product Code No. M-189).

Directions for use:

- Suspend 89 gms of Media in 1000 ml of distilled water.
- Boil to dissolve completely. Do not autoclave.
- Cool at 50°C.
- Pour into sterilised petridishes.
- Allow to solidify.
- Store in refrigerator.

Alkaline Peptone Water:

Use: At pH 8.4 it is suitable for the cultivation and enrichment of *Vibrio cholerae* from infected material.

Readymade dehydrated medium available from Hi-Media Laboratories Pvt. Ltd. Bombay (Product code No: M-82 - Peptone water).

Directions for use:

- Suspend 15 gms in 1000 ml distilled water.
- Mix well.
- Adjust pH to 8.4 with NaOH.
- Dispense 2-3 in test tubes.
- Plug tubes, using cotton plugs.
- Autoclave at 15 lbs pressure at 121oC for 20 minutes.

Cary Blair Medium

(Transport Medium w/o Charcoal)

Use: Recommended for collection and shipment of clinical specimens.

Readymade dehydrated medium available from Hi-Media Laboratories Pvt.Ltd., Bombay (Product Code - M-202).

Directions for use:

- Suspend 12.6 gms of media in 99% ml of distilled water.
- Boil to dissolve completely.
- Cool to 50°C.
- Add aseptically 9 ml of 1% aqueous calcium chloride solution.
- Adjust pH 8.4.
- Distribute 5-7 ml in screw capped small bottles.
- Steam bottles for 15 minutes.
- Cool, allow to solidify.
- Bottles are kept at room temperature.

Nutrient Agar Stabs:

Use: A general culture medium.

Readymade dehydrated media supplied by Hi-Media Laboratories Pvt.Ltd., Bombay (Product Code M-012 Nutrient Agar W/1% peptone).

Directions for use:

- Suspend 35 gms of media in 1000 ml of distilled water.
- Boil to dissolve medium completely.
- Pour 2-3 ml in sugar tubes.
- Plug tubes with cotton plugs.
- Autoclave at 15 lbs pressure at 121°C.
- Cool to solidify.

CHAPTER - 8

SAFETY PRECAUTIONS IN THE LABORATORY

CHAPTER- 8

8 SAFETY PRECAUTIONS IN THE LABORATORY:

Biosafety in a microbiological laboratory is very essential and basically depends on three components:

- o Basic standard of laboratory design, operation and equipment.
- o Selection and use of essential biosafety equipment.
- o Safe labooratory procedures.

An exhaustive review of each component is beyond the scope of this manual but practical and easily achievable safe laboratory rules are listed below:

⇒ Avoid mouth pipetting

⇒ Avoid eating, drinking, smoking and storing eatables in the laboratory.

⇒ Decontaminate the working area at least once a day and more frequently after the spillage of potentially infective material.

⇒ Wash your hands after handling the infectious material.

⇒ Wear laboratory coats/Gowns in the laboratory and these should not be taken outside.

⇒ Use gloves for all those procedures that may involve accidental, direct contact with blood or infectious materials.

⇒ Decontaminate all liquid or solid waste before disposal.

⇒ Perform all technical procedures in a way that minimises the aerosol formation.

⇒ Provide adequate training to the staff in laboratory safety procedures.

⇒ As far as possible actively immunize the workers against the diseases the materials of which are handled by them.

⇒ Employ only medically fit staff to work in clinical laboratories.

⇒ Report accident and illness promptly to the concerned officials.

⇒ Provide ample space and illumination for safe conduction of laboratory rpocedures.

⇒ Design smooth easily cleanable walls, ceilings and floors which should be impermeable to liquids and resistant to chemicals and disinfectants.

⇒ Ensure a dependable and good quality water supply.

⇒ Make suitably equipped 'first aid' rooms readily accessible.

⇒ Provide the staff safe laboratory equipments e.g.pipetting aids, safety cabinets, screw cap tubes and bottles, loop, incinerator if possible, and autoclaves, etc.

⇒ Carry out periodic health and medical surveillance of the workers to exclude the highly susceptible individuals.

⇒ Provide safety systems covering fire and electrical emergencies.

⇒ Control rodents and insects in the laboratory.

⇒ Don't permit the entry of the experimental animals which are not to be used in the laboratory.

⇒ Immunize your staff handling blood and blood products against Hepatitis B.

CHAPTER - 9

COMMON LABORATORY EQUIPMENTS

CHAPTER- 9

9. COMMON LABORATORY EQUIPMENTS:

9.1 INCUBATOR:

Incubator is an apparatus having a desired temperature. The heating device used can be gas, oil or electricity. Maintenance of uniform temperature within the incubator is essential and is achieved by fan, blower or a waterjacket containing heated water.

Ideal temperature for most of the medically important bacteria is 35°+2°C. However for some organisms different temperatures are necessary e.g. atypical mycobacteria (22oC-45°C), fungi (22°C) etc. Some organisms may need extra gaseous element e.g. 5-10% CO2 incubator for Brucella. The size of the incubator may vary from a small table top to a large walkin type rooms. A proper temperature recording thermometer and a small tray of water inside the incubator to prevent excessive drying of air are the two other essential requirements.

9.2 HOT AIR OVEN

It is used for sterilisation of the following materials:

a) Dry glass materials like test tubes, Petridishes, flasks, pipettes, syringes.

b) Instruments like forceps, scalpels, throat swabs, etc.

c) Sealed materials which can stand heat and when penetration of steam is not possible.

The instrument is electrically operated and should be equipped with a fan to have unfirom temperature inside, the required temperature for sterilisation is generally l60oC for l hour.

Operation of Hot air Oven

- Arrange the material to be sterilised loosely and evenly on the racks of the oven allowing free circulation of air and thereby even heating of the load.
- Air is poor conductor of heat so do not pack the load tightly.
- Switch on the power supply and control the temperature of the oven by adjusting thermostat. When the desired temperature is reached, note the time. Time taken for the oven to reach the desired temperature is called 'heating up period'.

- Hold the load in the oven at this temperature for a definite period of time. This period known as 'holding up period' is dependent upon the temperature employed. At 160oC the holding up period is 60 minutes, at 170oC for 18 minutes, at 180oC 7.5 minutes and at 190oC it is 90 seconds.

- The most common temperature for hot air sterilisation is 160oC for one hour. When the temperature is raised further, cotton plugs and paper wrappings get charred.

- On the expiry of holding up period, switch off the power supply and allow the load to cool.

- Open the oven door only when the temperature fall below 80oC, otherwise it may result in breaking up of glassware and also cause injuries to the operator.

- Dry up the instruments before placing them in the hot air oven.

- Do not place the heat sensitive materials inside.

9.3 WATER BATH:

Water bath is a water container having an electrically operated heating device to provide a fixed and uniform temperature. A thermometer is inserted inside the water bath for recording temperature. A mixer immersed inside water is also desired to maintain uniform temperature throughout the water bath.

A few applications of water bath are:

37°C Water bath - required during performance of WIDAL test;

44°C Water bath - required in faecal coliform count (water bacteriology) test.

56°C Water bath - for inactivating complement in the serum.

9.4 CENTRIFUGE:

For an average laboratory a small table top centrifuge with a maximum revolutions per minute of 6000 and capable of accommodating 10-12 tubes of 15 ml capacity is sufficient. The tubes should be placed exactly opposite to each other, should be of the same weight and should contain same amount of fluid. The speed is adjusted by a rheostat and should be allowed to rise slowly. A timer for fixed duration of centrifugation is preferred.

A few common uses are:

- Sediment examination of urine - 1500 rpm for 5 minutes.
- Separation of serum from clotted blood - 1500 rpm for 15 minutes.
- Concentration of microfilaria from blood - 2000 rpm for 2-5 minutes.

9.5 pH METER

A pH meter consists of an electrode pair which is sensitive to hydrogen ion concentration due to the development of an electrical gradient which is directly proportional to the hydrogen ion concentration. The electrodes commonly used are one of glass for the unknown and other of colomel to be used as a standard precautions while using pH meter are:

- The electrodes specially the glass ones should be handled carefully to prevent breakage due to contact with hard surface.
- Sufficient time should be given to warm up the instrument before use.
- Frequent standardizations of the pH meter should be made using standard buffer solution.
- Electrodes are to be washed with a stream of distilled water between measurements.
- The electrodes should never be removed from the solution when the measuring circuit is closed.
- When not in use, the electrodes must be kept immersed in water or electrode solution.

9.6 REFRIGERATOR:

Refrigferators are essential for storage of degradable laboratory substances like media, reagents, antisera, antibiotic discs etc. Refrigerators can vary in their capacity ranging from table top to a large walk-in-type. The usual temperature needed is 4o-2oC which is maintained comfortably by household use refrigerators. Substances to be kept at frozen state like sera may be kept in the freezer units of the same. Proper recording of the temperature is very important to avoid deterioration of biological materials.

9.7 MICROSCOPE:

o Place a slide on the stage, specimen side up and the centre of the section to be examined as accurately as possible over the hole in the centre of the stage.

o Adjust the mirror until it reflects the maximum amount of the light through the specimen with the low objective in position, lower the body tube by means of the coarse adjustment until the objective is about 1/4" from the slide.

o Look through the eye piece and slowly raise the objective with the coarse adjustment until the specimen is in approximate focus. Never focus downward while looking through the eye piece. Bring the specimen to sharp focus with the fine adjustment. Adjust the iris diaphgram and substage. Condense until the light intensity is optimum.

o After examining the specimen with the low power objective shift to the high dry objective by rotating the nose piece until the objective clicks into place.

o Look through the eye piece and slowly raise the body tube with the coarse adjustment until the specimen comes into approximate focus. Then bring the image into final accurate focus by using the fine adjustment. Once the specimen is in focus adjust the mirror and the iris diaphgram to give the clearest possible image.

o Focussing of the oil immersion objective:- First use the low power objective to locate the portion of the specimen to be examined. Raise carefully the body tube, and then rotate the nose piece until the oil immersion objective clicks into the position. Now place a drop of immersion oil on the portion of the slide directly under the objective watching the object from the side carefully lower it into the oil. Do not allow the objective to touch the slide. Look through the occular and slowly focus upward with the fine adjustment until the age appears. Once it appears do the fine adjustment and adjust the mirror and iris diaphragm to obtain optimum illumination.

Maintenance:

o Never touch the lenses if they become dirty, wipe them gently with lens paper.

o Always remove oil from the oil immersion objective after its use. If by accident, oil should get on either of the low power, wipe of the objective immediately with the lens paper. If oil becomes dry or hardened on a lens, remove it with lens paper lightly moistened with xylol.

o Keep the stage of the microscope clean and dry.

o Do not tilt the microscope when working with the oil immersion system.

- When the microscope is not in use, keep it covered in a microscope compartment. Never apply force to the microscope. Never allow the objective lenses to touch the cover glass or the slide. Never lower the body tube with the coarse adjustment while looking through the microscope. Never exchange the objective or occulars of different microscopes.

- Store the microscope in its cabinet when not in use.

9.8 AUTOCLAVE

Principle

Water boils when its vapour pressure equals the pressure of surrounding atmosphere. The temperature at sea level is 100°C. When water is boiled within a closed vessel at increased pressure, the boiling point of water is increased and so is the temperature of steam produced. This principle is employed in sterilising material by steam at temperature higher than 100oC and the process is called autoclaving.

For autoclaving in the laboratory, the most agreeable and commonly used method is to use steam at 121°C for 15 to 30 minutes depending upon the particular material to be sterilised.

Items to be sterilised:

Autoclaving is most suitable for culture media, aqueous solutions, decontamination of discarded cultures and specimens, rubber items such as gloves, stoppers with rubber

liner, glass ware with attached rubber tubings such as transfusion sets, glass metal syringes, etc. Autoclaves designed for laboratory work and capable of handling mixed loads should be used.

9.8.1 Types of autoclave

Only autoclaves designed for laboratory work and capable of dealing with a 'mixed load' should be used. 'Porous load' and 'bottled fluid sterilizers' are rarely satisfactory for laboratory work. There are two varieties of laboratory autoclave:

- **Pressure cooker** types; and

- **Gravity displacement models** with automatic air and condensate discharge.

Pressure Cooker type Laboratory autoclaves:

The most common type is a device for boiling water under pressure. It has a vertical metal chamber with a strong metal lid which can be fastened down and sealed with

a rubber gasket. An air and steam discharge tap, pressure gauge and safety valve are fitted in the lid. Water in the bottom of the autoclave is heated by external gas burners, an electric immersion heater or a steam coil.

Diagram

Operating Instructions:

There must be sufficient water inside the chamber. The autoclave is loaded and the lid is fastened down with the discharge tap open. The safety valve is then adjusted to the required temperature and the heat is turned on.

When the water boils, the steam will issue from the discharge tap and carry the a from the chamber with it. The steam and air should be allowed to escape freely until all of the air has been removed. This may be tested by attaching one end of a length of rubber tubing to the discharge tap and inserting the other end into a bucket or similar large container of water. Steam condenses in the water and the air rises as bubbles to the surface; when all of the air has been removed from the chamber, bubbling in the bucket will cease. When this stage has been reached, the air-steam discharge tap is closed and the rubber tubing removed. The steam pressure then rises in the chamber until the desired pressure, usually 15 lb/in2, is reached and steam issues from the safety valve.

When the load has reached the required temperature the pressure is held for 15 min.

At the end of the sterilizing period, the heater is turned off and the autoclave allowed to cool.

The air and steam discahrge tap is opened very slowly after the pressure gauge has reached zero (atmospheric pressure). If the tap is opened too soon, while the autoclave is still under pressure, any fluid inside (liquid media, etc.) will boil explosively and bottles containing liquids may even burst. The contents are allowed to cool. Depending on the nature of the materials being sterilized, the cooling (or 'run-down') period needed may be several hours for large bottles of agar to cool to 80oC, when they are safe to handle.

Autoclaves with air discharge by gravity displacement

These autoclaves are usually arranged horizontally and are rectangular in shape, thus making the chamber more convenient for loading. A palette and trolley system can be used.

The jacket surrounding the Gravity displacement autoclave consists of an outer wall enclosing a narrow space around the chamber, which is filled with steam under pressure to keep the chamber wall warm. The steam enters the jacket from the mains supply, which is at

high pressure, through a valve that reduces this pressure to the working level. The working pressure is measured on a separate pressure gauge fitted to the jacket. This jacket also has a separate drain for air and condensate to pass through.

The steam enters the chamber from the same source which supplies steam to the jacket. It is introduced in such a way that it is deflected upwards and fills the chamber from the top downwards, thus forcing the air and condensate to flow out of the drain at the base of the chamber by gravity displacement. The drain is fitted with strainers to prevent blockage by debris. The drain is usually fitted with a thermometer for registering the temperature of the issuing steam. The temperature recorded by the drain thermometer is often lower than that in the chamber. The difference should be found with thermocouple tests. A 'near to steam' trap is also fitted.

The autmatic steam trap or 'near-to-stream' trap is designed to ensure that only saturated steam is retained inside the chamber, and that air and condensate, which are at a lower temperature than saturated steam, are automatically discharged. It is called a 'near-to-steam' trap because it opens if the temperature falls to about 2oC below that of saturated steam and closes within 2oC or near to the saturated steam temperature. The trap operates by the expansion and contraction of a metal bellows , which open and close a valve. The drain discharges into a tundish in such a way that there is a complete airbreak between the drain and the dish. This ensures that no contaminated water can flow back from the waste-pipe into the chamber.

Operation of a gravity displacement autoclave:

If the autoclave is jacketed, the jacket must first be brought to the operating temperature. The chamber is loaded, the door is closed and the steam-valve is opened, allowing steam to enter the top of the chamber. Air and condensate flow out through the drain at the bottom. When the drain thermometer reaches the required temperature a further period must be allowed for the load to reach that temperature. This should be determined initially and periodically for each autoclave. Unless this is done the load is unlikely to be sterilized. The autoclave cycle is then continued for the holding time. When it is completed the steam valves are closed and the autoclave allowed to cool until the temperature dial reads less than 80oC. Not until then is the autoclave safe to open. It should first be 'cracked' or opened very slightly and left in that position for several minutes to allow steam to escape and the load to cool further.

9.9 BALANCE:

For measurement of mass, we employ an instrument known as balance. With this instrument, we determine the mass in comparison with some standard weights. The instrument primarily consists of the following parts. (Fig.)

Figures

A vertical pillar H is fixed on a base provided with levelling screws L, L. Through the vertical pillar passes a rod which can be raised or lowered by means of a key K at the base. At the end of the vertical rod, there is a small piece of agate plate. On it rests an agate or hard-steel knife-edge C, called the fulcrum, rigidly attached at the middle of a horizontal beam AB of the balance. The beam is a light but rigid frame-work of thin metal rods. At the two ends of the beam, there are two adjustable screw-weight.

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Operation of Hot air Oven

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- Switch on the power supply and control the temperature of the oven by adjusting thermostat. When the desired temperature is reached, note the time. Time taken for the oven to reach the desired temperature is called 'heating up period'.

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- Open the oven door only when the temperature fall below 80oC, otherwise it may result in breaking up of glassware and also cause injuries to the operator.

- Dry up the instruments before placing them in the hot air oven.

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CASE DEFINITIONS

OF

EPIDEMIC PRONE DISEASES

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
22-SHAM NATH MARG, DELHI - 110 054

initiated with high priority in known endemic areas. The help of the community could be sought for this purpose.

5. The guidelines include 'trigger-events' which should serve as early warning signs or alarm signals warranting immediate investigations. All medical and health personnel should be aware of these events and should report them to the nodal officer on telephone or fax with complete details. Name of the nodal officer, telephone, fax number and address must be widely circulated.

6. The trigger events include information about any acute infectious severe illness requiring hospitalization of unknown etiology. Each state and district should have a team of experts including an epidemiologist/ public health specialist, microbiologist, entomologist (for vector borne diseases) and clinician as a 'Rapid Response Team' for investigation of such reports. This will ensure adequate follow-up investigations, including collection of clinical samples for confirmation of diagnosis and avoid delay in initiating field investigations and outbreak containment measures where indicated. It will also ensure that panic and chaos is avoided due to unsubstantiated rumours.

7. If rare diseases, such as plague, are suspected, appropriate investigations should be started on high priority. However, cases of the following diseases will require laboratory confirmation and cannot be diagnosed on the basis of clinical symptoms alone:

   a. Plague
   b. Japanese encephalitis
   c. Dengue fever
   d. Leptospirosis
   e. Cholera
   f. Yellow fever
   g. Typhoid fever

CASE DEFINTIONS OF
EPIDEMIC PRONE DISEASES

1. Chicken pox
2. Cholera
3. Dengue fever
4. Diarrhoea, acute
5. Diphtheria
6. Dysentry (Shigellosis)
7. Food Poisoning
8. Hepatitis, viral
9. Japanese encephalitis
10. Leptospirosis
11. Malaria
12. Measles
13. Meningitis
14. Pertussis (whooping cough)
15. Plague
16. Poliomyelitis
17. Tetanus
18. Tuberculosis
19. Typhoid fever
20. Visceral leishmaniasis (Kala azar)
21. Conjunctivitis

Classification	Suspect	Presumptive	Laboratory confirmed
Personnel	Lay public/MPWs	Medical officers	Medical officers
Method	History	History + Clinical Investigations	Diagnostic Tests
Acute Diarrhoea	• Three or more loose or watery stools, OR • Mother's opinion during infancy	• Three or more loose or watery stools, OR • Change in consistency and character of stools, OR • Mother's opinion during infancy	• None
Cholera	• Acute watery diarrhoea in older children (>5 years) and adults leading to severe dehydration, OR • Profuse watery diarrhoea with or without vomiting in an area where outbreak is occurring	• Acute watery diarrhoea in older children (>5 years) and adults leading to severe dehydration, OR • Profuse watery diarrhoea with or without vomiting in an area where outbreak is occurring, OR • Other cases of similar illness reported from the area, OR • Positive microscopic immobilisation test for *V.cholerae* in stool sample of patient having clinically compatible illness **Note:** Mild cases are clinically indistinguishable from non-specific acute diarrhoea	• Isolation of V.cholerae O1 or O139 from stool samples
Dysentery	• Acute bloody diarrhoea • Fever and pain abdomen	• Acute bloody diarrhoea	• Isolation of Shigella sp. from stool
Viral Hepatitis	• Yellow colouring of the eyes and skin (*Pilia*)	• Acute onset • Jaundice • Malaise, anorexia,	• Hepatitis A - IgM HAV +ve • Hepatitis B - IgM HBc with or without HBsAg positive

		• Fever at present or preceding jaundice • Hepatomegaly • Right upper quadrant abdominal tenderness • ALT ≥ 8 times of normal and Serum Bilirubin > 2 mg% in clinically compatible illness.	• Hepatitis C - anti-HCV positive • Hepatitis D - HBsAg & anti HDV +'ve • Hepatitis E - IgM HEV positive
Typhoid fever	• Sustained fever of more than one week duration with gradual onset with headache, malaise and loss of appetite usually with gastrointestinal symptoms	• Suspected case, **AND** With two or more of the following:- • Toxic look • Coated tongue • Relative bradycardia • Splenic enlargement • Non productive cough	• Isolation of *S.typhi* from blood, stool or other clinical specimens.
Dengue Fever (DF)	• Acute onset, and • High fever of less than 7 days duration, and • Severe headache, and • Joint and muscle pain and pain behind eyes , and • Outbreak of dengue fever in the area • With or without rash	• Suspected case of dengue fever, AND • Blood slide negative for malarial parasite and patient does not respond to anti-malarial drugs.	• 4-fold rise of antibodies in paired serum samples, OR • Serological test for IgM antibody in single serum samples, OR • Isolation of virus from blood in early phase
Dengue haemorrhagic fever (DHF)	• Suspected case of dengue fever , AND • Bleeding tendencies	• Suspected case of DHF with bleeding tendencies evidenced by one or more of the following:- * Positive tourniquet test * Petechiae, ecchymoses or	• above tests as in dengue Fever

		purpura * bleeding from the mucosa, gastrointestinal tract, injection sites or other locations * haematemesis or melena AND • Thromobocytopenia (100,000 cells per mm^3 or less) AND • Evidence of plasma leakage due to increased vascular permeability, manifested by one or more of the following: ⇒ A rise in the haematocrit equal to or greater than 20% above average for age, sex and population ⇒ A drop in haematocrit following volume replacement treatment equal to or greater than 20% of baseline ⇒ Signs of plasma leakage such as pleural effusion, ascitis and hypoproteinaemia.	
Dengue shock syndrome (DSS)	• Suspected case of Dengue/DHF with signs of shock including cold clammy skin, restlessness or sleepiness, excessive thrust	• Suspected case of Dengue shock syndrome with • Low blood pressure (Systolic less than 90 mm Hg) • Narrow pulse pressure (≤ 20 mm of Hg.)	• As above.
Japanese encephalitis	• High grade fever of acute onset with atleast two of the following:	• Suspected case of Japanese Encephalitis, and	• Serology for JE antibodies.

	• Decrease in level of consciousness independent of convulsions • Significant change in mental status either in behaviour or personality • Convulsions	• Usually not more than a few cases (1-2) in one village. • With or without signs of meningeal irritation and varying degree of neurological deficits • Pleocytosis in CSF of more than 10 cells/mm^3 in clinically compatible illness.	• Demonstration/isolation of virus/antigen from CSF, brain tissue or rarely blood.
Visceral leishmaniasis (Kala-azar)	• Persistant, irregular fever of more than two weeks duration not responding to anti malarial and anti microbial. • Patient belonging to an area known to be endemic for kala-azar or adjacent areas.	• Suspected case of kala-azar • Progressinve weakness and anemia • Splenomegaly, hepatomegaly, lymphadenopathy • Clinically compatible illness in endemic area with thrombocytopenia and leukopenia makes the presumptive diagnosis. • Direct agglutination test (DAT) OR Postive aldehyde test in clinically compatible case is also highly suggestive of kala-azar.	• Demonstration of L-D bodies in stained smears from bone-marrow, spleen, liver, lymphnode or blood is highly suggestive but not conclusive. • Presence of anti-leishmanial antibody in significant titre as detected by IFAT or ELISA. • Culture of the organism from a biopsy material or aspirated material confirms the diagnosis.
Diphtheria	• Sore throat (with or without difficulty in swallowing and/or breathing) • Mild fever • Exposure to a case of diphtheria in the previous 1 week or epidemic of diphtheria in the area	• Suspected case of diphtheria, AND • Greyish-white membrane in throat (with or without difficulty in breathing), AND • Acute pharyngitis, naso-pharyngitis or laryngitis, OR • Myocarditis or neuritis (paralysis) one to six weeks after onset of	• Positive culture of Corynebacterium diphtheriae (demonstration of toxin production recommended but not required)

		symptoms • Staining of throat swabs by Albert stain showing organism resembling *C.diphtheriae* in a clinically compatible case is also sufficient to make presumptive diagnosis.	
Measles	• Generalised blotchy rash lasting 3 or more days • Fever • At least one of: Cough, runny nose or red eyes	• Generalised maculopapular rash lasting 3 or more days, and • Fever 38°C. (101°F) or more, and • At least one of: Cough, coryza, conjunctivitis. • Koplik's spots in a clinically compatible illness confirm the diagnosis. **Note:** A child may come with post measles complications like pneumonia and diarrhoea. Mother should be interviewed for any past history of measles.	• Positive serology (4 fold or greater rise in serum antibody titre). **Laboratory confirmation is not required at present.**
Neonatal tetanus	• Normal suck or cry for first 2 days, and • Onset between 3-28 days of birth, and • Inability to suck, followed by stiffness or convulsions	• A suspected case of neonatal tetanus, and • Trismus often leading to death	• None
Poliomyelitits	• Fever • Abrupt onset of weakness or paralysis of the leg(s) or arm(s) • No progression of paralysis after first three days • Paralysis not present at birth or associated with serious injury or	• A suspected case of poliomyelitis, and • Flaccid paralysis • No sensory loss • Muscle tenderness • Absent or depressed deep tendon reflexes	• Isolation of wild polio virus from cases or contacts of AFP.

	mental retardation	• Asymmetrical findings • Wasting of affected muscles (late findings) • Residual paralysis 60 days after onset of illness makes the presumptive diagnosis. • Death or unknown follow-up in AFP cases also makes the presumptive diagnosis.	
Tetanus	• History of Injury, ear infection or child birth or abortion, AND • Difficulty in opening mouth, and/or • Acute stiffness or convulsion	• A suspected case of tetanus • Trismus	• None
Tuberculosis (childhood)	• Fever or swelling neck/axilla/ groin with history of tuberculosis in the family	• Tubercular meningitis - Any case of meningitis not responding to conventional treatment but responding to anti TB drugs. • Tubercular lymphadenitis - lymphadenitis in a clinically compatible or radiologically suggestive case not responding to conventional treatment but responding to anti TB drugs.	• Microscopy or culture of tubercle bacilli, identified as Mycobacterium tuberculosis, from secretions or tissues • Suggestive histological findings in biopsy material
Whooping cough (Pertussis)	• Cough persisting 2 weeks or more, and one of the following: • Fits of coughing which may be followed by vomiting	• Prolonged coughing followed by apnoea, cyanosis or vomiting • Typical whoop • Subconjunctival haemorrhages	• Culture of nasopharyngeal secretions for *Bordetella pertussis* bacteria.

	• Typical whoop • Exposure to a case in previous 2 weeks or epidemic of whooping cough in the area	may or may not be present • White blood cell count with 15000 lymphocytes/cu mm or more in a clinically compatible illness is suggestive of whooping cough.	
Chicken pox	• Acute onset of fever and generalised vasico-pustular eruptions on trunk and face but less on limbs.	• Fever, bodyache • Rash from 3rd-4rd day • Rash on trunk and face, less on limbs • Lesions have irregualr oval shape, are not homogenous and are generally unilocular. Never indented • Crops of spots appear so that lesions are at different stages. • A clinically compatible case epidemiologically linked to another probable case confirms the diagnosis.	• Not required.
Food poisoning	• Occurrence of diarrhoea and/or vomiting within a short but variable period of time, and • Consumption of food from a common source.	• Suspected case of food poisoning, and • 2 or more persons experience a similar illness after ingestion of a common food and the epidemiological analysis indicates the food as the source of illness. (Exception: botulism or chemical poisoning may affect only one person).	• Isolation and/or identification of causative organisms and/or toxins from the clincal samples (faeces or vomitus) and incriminated food.
Meningitis	• Sudden onset of high grade fever • Severe headache with or without altered conciousness • Stiff neck	• A suspected case of meningitis • Nuchal rigidlity • Kerning and Brudzinski sign +ve	• Latex agglutination kits can detect meningococci, pneumococci and *H.influenzae* in CSF in field conditions.

		• Staining of CSF may reveal organisms; Meningococci are Gram negative diplococci which may be intracellular also. • Biochemical features	• Culture for isolation and confirmation of pathogens.
Leptospirosis		• An illness charaterised by fever, headache chills, myalgia conjunctival suffusion, and less frequently meningitis, rash, jaundice or renal insufficiency. Symptoms may be biphasic. AND • Supportive serologic findings i.e., a leptospira agglutination titer of ≥200 in one or more serum specimens.	• Isolation of leptospira from clinical specimen • 4 fold or greater increase in agglutination antibody titre in paired samples obtained ≥ 2 weeks apart. • Demonstration of leptospira in a clinical specimen by immunofluorescence.
Plague	• Contact with a confirmed case of Plague or presence of plague outbeak in the area. AND • Bubonic plague: Sudden high fever , chills and painful lymph node swellings in the groin or underarm region. Patient looks extremely ill. OR • Pneumonic plague: Sudden high fever, chills, cough, chest pain with or without haemoptysis (blood in sputum). Patient looks extremely ill.	• A clinically compatible illness, and contact with a confirmed case of plague or presence of plague outbeak in the area should be taken as a suspect case. • Clinically compatible illness with identification of a *Y.pestis* like organism by light microscopy in the clinical material should be taken as a probable case. • Clinically compatible illness and demonstration of *Y.pestis* like organism with fluorescent microscopy gives presumptive diagnosis.	• Isolation of *Yersinia pestis* from bubo aspirate or sputum. • 4 fold rise in specific F1 antibody titre by PHA test in paired serum samples. • Clinically compatible illness with one serum specimen tested by PHA test for F1 antigen giving titre ≥ 1:8 also gives presumptive diagnosis.

SURVEILLANCE

OF

EPIDEMIC PRONE DISEASES

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
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AUGUST 1997

CONTENTS

1. Introduction

Surveillance of diseases is the continuing scrutiny of all aspects of the occurrence and spread of a disease that are pertinent to effective control. Communicable diseases constitute a significant disease burden and are major causes of morbidity, mortality and long-term severe mental and physical disabilities. Many of the diseases are epidemic prone.

The vulnerability of an area to an outbreak increases with increase in population density and high migratory habits of the population. The receptivity of an area to outbreaks is related to inadequate drinking water facilities, poor sanitary conditions and adverse environmental events conducive for increase in vector density and natural disasters such as earthquakes, floods etc. Developmental activities such as large constructions, irrigation, industries can also increase the risks of epidemics unless adequate preventive and precautionary measures are inbuilt in the planning and implementation stages.

Epidemics are public health emergencies. Epidemics disrupt routine health services and are a major drain on resources. Besides direct costs in outbreak control measures and treatment of patients, the indirect costs due to negative impact on domestic and international tourism and trade can be significant. The avoidable human misery resulting from illness and death cannot be quantified in economic terms.

Not all outbreaks can be predicted or prevented. However, precautionary measures can be taken within the existing health infrastructure to reduce risks of outbreaks and to minimize the scale of the outbreak if it occurs. The effectiveness with which national programmes are implemented and monitored, the alertness for identification of early warning signals and the capacity for initiating recommended specific interventions in a timely manner are important to achieve the above objectives.

The course of an epidemic is dependant on how early the outbreak is identified and how effectively specific control measures are applied. The epidemiological impact of the outbreak control measures can be expected to be significant only if these measures are applied in time. Scarce resources are often wasted in undertaking such measures after the outbreak has already peaked and the outcome of such measures in limiting the spread of the outbreak, and in reducing the number of cases and deaths, is negligible.

When outbreaks occur or when the risk of such outbreaks is high, the co-operation of other government departments, non-governmental agencies and the community often becomes necessary. Such help will be more forthcoming if mechanisms for interaction have been developed before the onset of an outbreak.

The frequency of the occurrence of the epidemics is an indication of the inadequacy of the surveillance system and preparedness of the district to identify and control outbreaks in a timely manner.

The purpose of the training programme is for capacity building at district level to detect the outbreak in its early rising phase and to respond effectively to control its further spread.

2. Surveillance

Surveillance is **data collection for action.** Surveillance data are required for planning disease control activities and for evaluating impact. Disease surveillance data are also required to identify high risk areas or high risk age specific and other groups who require special attention. Early warning signals will be missed in the absence of an effective surveillance system.

To plan any disease control programme and to identify and control outbreaks, it is important to know the following:

- **who** get the diseases
- **how many** get them
- **where** they get them
- **when** they get them
- **why** they get them

There are five steps in the surveillance procedure which must be carried out at each level, starting from the PHC. Each level must have the capacity for analysing and using surveillance data for it to be effective for outbreak prevention and control. The five recommended steps are:

- collection of data
- compilation of data
- analysis and interpretation
- follow up action
- feedback

3. Prioritisation of diseases for surveillance

There are many communicable diseases which are widespread and are the major causes of morbidity and mortality. Many of these diseases also have the potential of causing large outbreaks. Most of the diseases with an epidemic potential are either water-borne, vector borne or are transmitted through contact.

Besides the epidemic prone diseases, the surveillance system must also include diseases which are covered under the national programmes. These diseases account for a significant disease burden, lead to lifelong severe mental and physical disabilities and are usually amenable to control through cost effective means.

The existing health infrastructure can be effectively used to introduce a system of early warning signals or 'trigger events', to detect an outbreak in the early rising phase and to control it effectively. Orientation of the medical officers and the paramedical personnel will be needed to make the programme a success.

4. Prerequisites for effective surveillance

The manual includes diseases and formats which are already in use. Some districts may have special problems or needs. Additional information may be collected if necessary. However, all information collected at PHC or district is not required to be transmitted to the state or central levels unless a special request is received. Only data that is used should be collected otherwise it will clutter and overburden the system. Irrespective of what data is collected, the basic principals of surveillance would remain the same:

- use standard case definitions
- maintain regularity of the reports
- take action on the reports

For developing an effective disease surveillance system, the district health officer/PHC medical officer must also be clear about:

- what information to gather
- how often to compile and analyse the data
- how often and to whom to report
- what proforma or formats to use
- what action to take

The data collected should be uniform, regular and timely. Standard case definitions are important to ensure uniformity in reporting so that all reporting units use the same criteria for reporting cases. It is also important to have a list of all reporting units so that the regularity and timeliness of the reports is checked. If no cases are seen, a nil report should be submitted. All levels in the system must:

- have the standard case definitions
- have a list of all reporting units
- monitor receipt of reports in time
- monitor completeness of reports

The standard case definition of diseases is given in a separate manual. The cases have been classified as:

- lay (suspect) - diagnosis made on the basis of history by paramedical personnel and members of the community

- presumptive - diagnosis made on typical history and clinical examination by a medical officer

- confirmed - clinical diagnosis by a medical officer and/or positive laboratory identification

4.1 Regularity of reports

Monitoring the regularity of surveillance reports is an important function of the surveillance system. A list of all reporting units in the area must be kept. The Chief Medical Officer of Health of the district must identify the reporting units. Besides the PHCs and CHCs, hospitals, large dispensaries and clinics should be included as reporting units.

4.2 Frequency of reporting

4.2.1 A system of monthly reporting of disease and programme specific data already exists in the districts. The routine reporting of cases and deaths should continue.

Many epidemic prone communicable diseases have short incubation period. If a review of the data is made only on a monthly basis, it might delay the timely identification of an outbreak in the early rising phase. Reporting units may consider the weekly plotting of cases seen in their institutions in the simple format. If the number of cases

is doubled for two consecutive weeks, it should serve as a warning signal for investigations. The area of residence of the patients should be checked and if these cases are clustered with respect to time and place, an immediate field visit is indicated. An epidemic can be averted by taking appropriate control measures in time. If an outbreak is suspected or identified, the next level should be notified immediately.

4.2.2 Daily reports are necessary once an outbreak has been identified so that the situation can be monitored. Neighbouring areas would need to step up surveillance activities also to rule out the spread of the outbreak. After the outbreak has subsided to pre-outbreak levels, weekly reports should be continued for at least double the maximum incubation periods of the disease.

5. Methods of data collection

Several methods can be used for collecting data. While routine reporting (passive surveillance) is universalised, other methods are need and area specific. These include:

- sentinel surveillance
- active surveillance (active search for cases)
- vector surveillance
- laboratory surveillance
- sample surveys
- outbreak investigations
- special studies

5.1 Routine reporting (institutional based or passive reporting)

All the national health programmes require that the cases and deaths recorded in the out-patient or in-patient departments of hospitals, dispensaries, community health centres, primary health centres and other health facilities manned by a medical officer, report these cases to the local health authority on a monthly basis. The consolidated report of the district is forwarded to the state health officer. For some national programmes, a copy of the district report is also forwarded to the concerned officer at the central level.

At each level in the system, the report is required to be analysed and appropriate action taken as indicated. The reports should be checked for completeness and regularity as these factors can influence the analysis of the reports.

Each reporting unit must have the following details available in their records for each case. The details of the cases are essential for outbreak investigations. Each reporting unit is advised to 'spot-map' cases at least for a few select diseases so that high-risk pockets could be identified. The line listing is, however, required to be submitted on a regular basis only for a few select diseases for which goals for eradication have been set.

- name of the patient
- name of the mother or father
- full postal address of the residence (the address must be complete to facilitate home visit, if required. It might be helpful to record the name of the nearest sub-centre and PHC, for example, if the patient can provide this information)
- age
- sex
- date of onset
- immunization status (if child under five years of age and vaccine is available for public health use)
- out come
- date of collection of clinical sample (if any) and lab. result

5.2 *Sentinel surveillance*

A sentinel surveillance system is developed to obtain more reliable and extensive disease information than is available from the routine reporting centres. A hospital, health centre, laboratory or a rehabilitation centre which attend to a relatively large number of cases of the disease can be considered as a sentinel centre. A sentinel centre can provide information on one or more diseases.

Since the sentinel centres are carefully selected and because the number of the reporting units is much smaller, it is easier to maintain the quality and regularity of the reports.

There should be a close liaison between the sentinel centre and the local health office. The sentinel centre can help in providing:

- line lists of selected diseases which is essential for epidemiological analysis
- early warning signals which should trigger action for outbreak investigations

The sentinel centre data will not include all cases in the area. However, if one or more sentinel centres have been carefully selected, it will include sufficiently large number of cases for epidemiological analysis and for reliably determining trends in the incidence of the reported disease.

The district hospital, infectious diseases hospital, medical college hospital (if located in the district) and other large hospitals or laboratory should be included as sentinel centres and reports from these centres should be analysed separately. These centres would also be submitting the routine monthly report under the passive surveillance system.

5.3 *Active surveillance*

However good the routine reporting system there will still be cases that will not be recorded under this system as patients with mild or moderate severity may not seek treatment. Some may go to private practitioners. It is also possible that patients in severe condition are taken directly to a large hospital in another district for specialized care. Some cases may die within a short period of onset of symptoms without receiving care at a health facility such as cases of neonatal tetanus.

Active surveillance or active search for cases is resource intensive. The decision to start active surveillance depends on many factors and ground situations. Active search may be called for under the following circumstances:

- during outbreaks to determine the extent of the outbreak and keep mortality rates low by initiating early treatment. Active surveillance is carried out to know the magnitude of the problem which will help in planning logistics for control. In addition it will give baseline data to evaluate control strategy. It also helps in understanding the genesis of the outbreak.

- as the number of cases of a disease decline to negligible levels and it becomes important to receive information on every single case as quickly as possible so that further transmission is interrupted by initiating outbreak control measures. For example, active surveillance is recommended for acute flaccid paralysis (AFP) and guinea worm disease.

- trace cases and contacts over a limited period of time for selective interventions to interrupt transmission. This strategy is recommended in the yaws affected districts. In selected districts, similar strategies are being tried for filariasis and leprosy control

- to confirm the absence of even a single case. This is done during the pre-certification phase for disease eradication as 'zero' incidence has to be maintained for a period of three years. Special measures, such as announcement of cash awards are also introduced at this time. The last case of guinea worm disease was reported in July 1996. If the zero status is maintained, eradication of guinea worm disease will be certified in India in 1999.

During field visits by the supervisors, absence of disease can be confirmed by contacting few key persons such as a school teacher, gram pradhan, anganwadi worker and others.

Since active surveillance is usually recommended when it is important that not even a single case is missed, follow up on the report of a case must be prompt, preferably not later than 48 hours.

The health personnel, outreach personnel of other government departments, non-governmental organizations and the members of the community must be encouraged to report cases. The lay case definition of the disease should be widely circulated for this purpose. **The health personnel should not be punished or discouraged in any way from reporting cases as this will lead to suppression of vital information.**

5.4 *Vector surveillance*

Vector surveillance is important to identify the existing vectors in the area. Increase in the density of the vectors is a high risk factor for vector borne outbreaks.

District health officer should have linkage with the Zonal entomologist of his district. Data on entomological survey carried out in the past should be fully utilised for planning of anti-vector measures. If no entomological studies have been carried out, these can be planned in consultation with the zonal/ state entomologist.

Depending on the resources available, surveillance of vector density and their bionomics should be done in selected epidemic prone pockets such as areas with seepage from irrigation canals, dam projects, flood prone areas and others so that anti-vector measures can be initiated as a precautionary measure to prevent an outbreak.

Sample checks will also document the impact of the routine measures for vector control under the National Malaria Eradication Programme (NMEP).

5.5 *Laboratory surveillance*

Testing water samples for coliform organisms is a measure to determine the risk of water borne outbreaks. Water quality monitoring is recommended in vulnerable pockets and from sources supplying drinking water to a large population.

Checking the chlorination levels of the water is also important, especially during the monsoon and post monsoon periods. These measures by the health department are precautionary measures in addition to the mandatory requirements of the concerned departments.

Laboratory surveillance must be stepped up in anticipation or in the event of an outbreak. Serological and other laboratory based surveys are sometimes conducted as research projects to collect baseline, prevalence rates or identify high risk factors, age-groups or population sub-groups.

The identification of new agents and changes in the behaviour of micro-organisms especially in relation to susceptibility to anti-microbial are also important components of laboratory surveillance.

Laboratory also plays a very important role in surveillance of certain diseases like dengue fever (monitoring of fever cases for antibodies against dengue), cholera (isolation of *V.cholerae* from stool sample of acute diarrhoea leading to dehydration in persons five year and older) and poliomyelitis (isolation of virus from stool samples of AFP cases).

Clinical samples should be collected and transported properly to the identified laboratories for appropriate tests. The samples should be labelled properly and accompanied with requisite epidemiological information.

5.6 *Sample surveys*

Surveys give reliable epidemiological information. These are particularly useful to collect baseline data prior to the launch of a large control programme, especially if such data are lacking through other sources.

Surveys are, however, difficult to conduct and are relatively expensive. The sample size, sampling procedure,

methodology, questionnaires and forms must be well designed to avoid bias and misinterpretation of data. Moreover, the results of the surveys are **area and time specific**. The results of the surveys are relevant only to the area in which these were conducted and the period when these were conducted.

Not all surveys are, however, difficult and standard guidelines are available. These surveys can be conducted after a period of 3 to 5 years or more to document impact. Such surveys include multi-indicator studies on ORT and API in children. Stool surveys of school children for assessing the prevalence of helminthic infestation can also be organized.

It is expected that if a survey is conducted in a district, it will be in collaboration with the state and central health authorities. Since this activity is not recommended as a routine, further information on the subject can be obtained from the concerned state officer directly.

5.7 Outbreak investigations

Outbreaks investigations provide a rich source of epidemiological information. The outbreaks should be investigated to understand why they occurred and to identify high risk areas and groups. The data collected as a result of outbreak investigations must be utilised for improving programme activities and the surveillance system as well as for filling gaps identified as a result of these investigations.

The results should be shared with other district officers and other states so that the experience gained could be effectively used for preventing such outbreaks in these areas.

5.8 Special studies

Special studies are sometimes required to study problems that are not addressed through the methods of data collection listed above. Some districts, for example, may have a high prevalence of cases linked to or suspected to be due to environmental pollution; other districts may have problems related to multi-drug resistant micro-organisms.

6. Data to collect

Usually only the number of cases and deaths are reported under the routine reporting system. If the number of cases of a disease is large, it is neither practical nor necessary to collect detailed information on each case. However, as mentioned earlier, each treatment centre must keep a record of all cases.

Relevant information may be obtained for selected epidemic prone diseases from selected centres (sentinel hospitals) so that high risk areas and groups (for example related to age and immunization status) can be identified. Besides the name of the patient and name of father or mother so that she/ he could be traced for further investigations if necessary, the following information is required for making policy decisions and to evaluate impact:

- age
- sex
- date of onset of symptoms
- outcome
- immunization status (for vaccine preventable diseases in children under five years of age)
- residential address
- date of collection of sample and lab. report (if any)

6.1 Age

Age is important to identify age-groups at highest risk. This information is important in understanding disease dynamics and formulation of control strategies. Data on age of cases for vaccine preventable diseases helps to determine the immunization schedule and to have cut-off age limits during outbreaks which can be controlled by vaccination. Some diseases are more severe in the higher age-groups. Some diseases are seen more in one age-group than another and this might help in differential diagnosis. Age, for example, is one of the criteria in the differential diagnosis of acute flaccid paralysis in children.

6.2 Sex

Gender is recorded to determine the sex differential which is common in some diseases with either males or females being more prone depending on the risk factors. Hospital records may however also reflect the medical attention given to male members of the family as compared to the females. For example, while almost all cases of neonatal tetanus admitted in hospitals are males, this is due to the fact that the female child is not brought for treatment.

6.3 Date of onset of symptoms

The date of onset of symptoms is important for determining the course of the outbreak and to undertake epidemiological investigations. Information regarding the onset

of symptoms is also necessary for interpretation of laboratory tests.

6.4 *Outcome*

One of major objectives of the national programmes and outbreak control measures is to minimize mortality and case fatality rates. Recording the outcome of the diseases in terms of whether the patient recovered fully or has long-term complications as a result of the disease or died.

Recording the outcome and case fatality rates will also indirectly give information on the quality of the management of cases if there are wide variations in the clinical outcome in different hospitals.

High case fatality rates may trigger further investigations and identify other underlying cause (s) in the area.

6.5 *Immunization status*

Immunization is one of the most cost effective public health programmes and no child should be denied its benefits. Contacts with the health care delivery system should be used to check the immunization status of all children under five years of age so that they could be immunized as appropriate for the age. Recording the immunization status is particularly important if the case is of a vaccine preventable disease.

Occasionally a child who is fully vaccinated at the correct age with potent vaccine may get the disease. This is because the vaccine efficacy is usually less than 100%. Even if a potent vaccine is used at the right age in the right dose, some children will not be fully protected. If a large proportion of cases occur in fully vaccinated children, it should be investigated.

While the immunization status of a child may not have a direct relevance for diseases other than the vaccine preventable, high immunization coverage would indicate a healthy public health system with a good outreach and IEC efforts. On the other hand, if a large number of children are non-immunized, it reflects poor contact with the health system or other problems of access to health care.

6.6 *Residential address*

Residential address of the patients helps in identifying high risk area. It is not only essential to apply control measures in the affected areas, but is also useful to understand

the natural history of diseases. Some diseases are more prevalent in rural areas (for example, JE, neonatal tetanus) whereas the others affect mainly urban population (Dengue fever). Diseases like kala-azar are confined to certain geographical area (Bihar, some districts in West Bengal and U.P.).

7. Compilation of Data

Data should be compiled to describe the diseases in terms of time, place and person. There are two methods of compiling data.

1. Tabulation
2. Drawing
   a) Graphs
   b) Diagrams

7.1 Tabulation

Usually the data collected is large in amount and therefore, should be classified and presented in the form of frequency distribution table. This is very important step before statistical analysis of data. It groups large number of observations and presents the data very concisely giving all information at a glance.

Example 1: Table 1 shows the geographic distribution of laboratory confirmed cholera cases in Delhi during 1988.

Table 1
Laboratory confirmed cholera cases in Delhi by Zone, 1988

Zone	Cases	Percent
CITY ZONE	10	0.7
S.P.ZONE	45	2.9
KAROL BAGH	43	2.8
WEST ZONE	50	3.3
CIVIL LINES	508	33.1
NARELA	48	3.1
NAJAFGARH	79	5.1
SOUTH ZONE	69	4.5
N.D.	26	1.7
SHAHDARA	583	38.0
NDMC	15	1.0
CANTONMENT	11	0.7
ZONE UNKNOWN	49	3.2
Total	1536	100.0

Note: 113 cases were from outside Delhi.
Records were not available for 59 cases.

It is evident from Table 1 that most of the cholera cases occurred in Shahdara (38%) and Civil Lines (33.1%) zones.

Example 2: Table 2 describes the vaccination status of measles cases which occurred in 7 districts of Uttar Pradesh during the epidemic of 1996.

Table 2
Immunization status of measles cases in Uttar Pradesh, 1996 *

District	No. of measles cases examined	No. immunized	% immunized
Bareilly	173	5	3.0
Baharaich	41	2	4.8
Hamirpur	108	6	5.6
Maharajganj	363	64	17.6
Kheri	315	66	20.9
Lucknow	102	24	23.5
Almora	31	9	29.0
All	1133	175	15.4

* More than 9 months of age.

It is clear from Table 2 that almost 85% of the cases had not been vaccinated against measles.

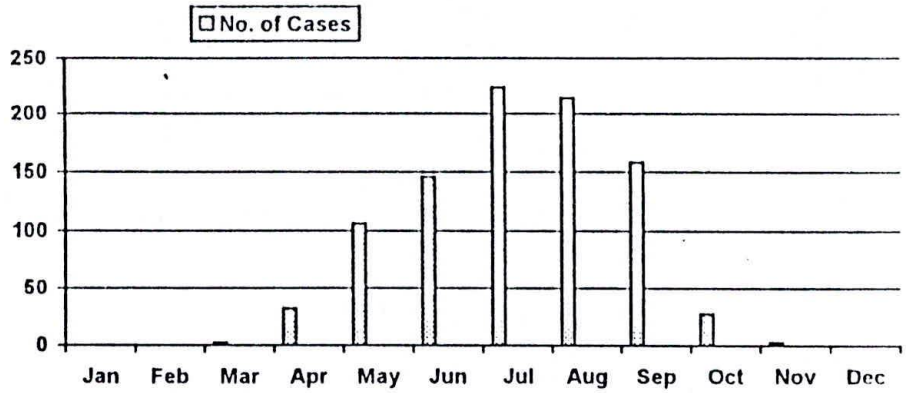
7.2 Drawing

After group-wise tabulation, the frequencies of characteristics can be presented by graphs and diagrams. They are useful for quick eye reading of data.

Line graph, bar diagram, pie diagram and map diagram are used frequently in disease surveillance. For example, in order to monitor the incidence i.e. number of new cases in a defined population during a specified period of time, it is necessary to maintain diagram (charts) or graphs. These charts and graphs show the number of cases of the diseases for each reporting period. With charts and graphs it is easy to visualise the number of cases which occurred in each reporting period.

7.2.1 Disease Diagrams

Figure 1
Seasonality of cholera in Delhi, 1996
(Bar Diagram)



Example 3 **Figure 1** is a sample bar diagram for the number of laboratory confirmed cases of cholera in Delhi in 1996.

By looking at the chart it is easy to determine how many bacteriologically proved cases of cholera occurred each month in Delhi. For example, most of the cases occurred from May to September, while Dec-Feb were completely free from the disease.

7.2.2 Line Graphs

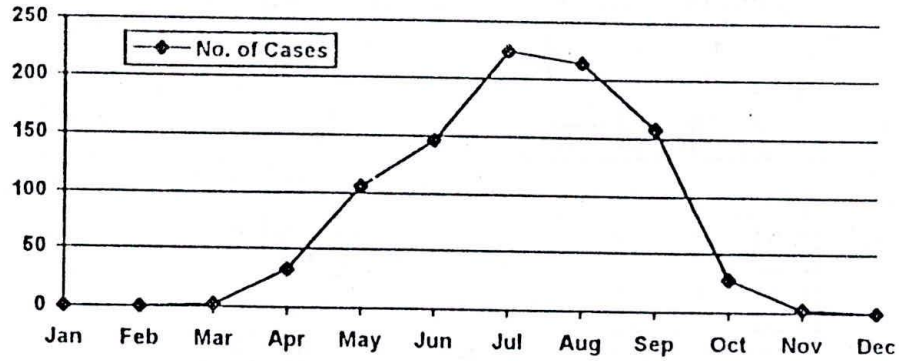
In order to maintain a disease graph, perform the following tasks:

On the first day of each reporting period, count the number of cases of disease diagnosed during the previous reporting period.

Place a dot on the graph directly above the mark for that reporting period.

Draw a line from the previous dot to the new one, so that you will have a clear picture of the trend in the incidence of disease. If the line goes down from left to right, the number of cases is decreasing. If the line goes up from left to right, the number of cases is increasing.

Figure 2
Seasonality of cholera in Delhi, 1996
(Line Graph)



Example 4: Fig 2 drawn from the same data as in Fig 1. You can see that cholera appeared in March, had peaks in July-August and almost disappeared in November-December.

During ongoing disease surveillance of epidemic prone diseases, compile the data weekly rather than monthly (Table 3).

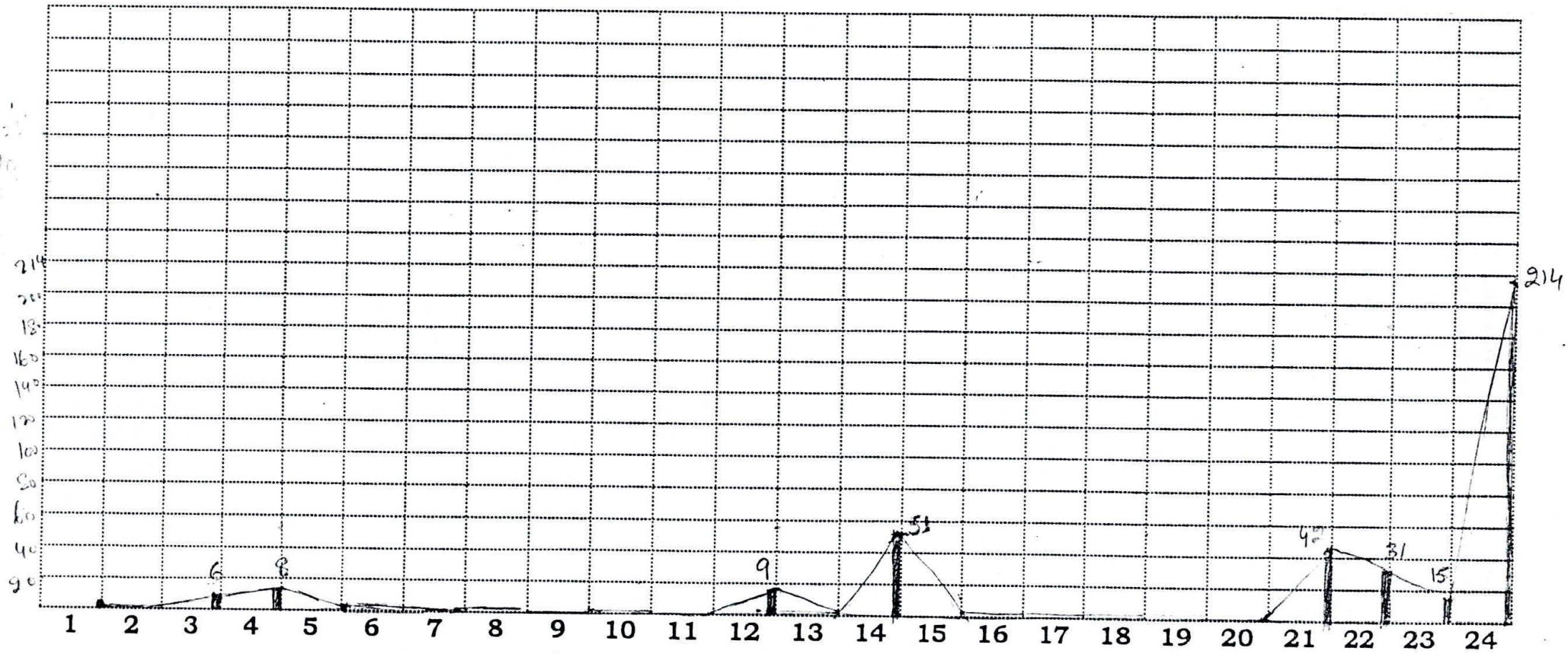
Exercise 1

Table 3
Weekly distribution of cases of epidemic-prone diseases attending a health institution

Diseases	Weeks 1	2	3	4	5	6	7	8	9	... 52
Diarrhoea										
Jaundice										
Malaria										
Measles										

- Draw Bar diagram and line graph using data in table No.4.

1996



Imm. Survey. Coverage Survey
30 cluster @ 7 Children each = 210

Table 4
Week-wise reported cases of Measles in District Maharajganj, Uttar Pradesh, 1996

Period ending week	No. of cases	Period ending week	No. of cases
1	0	13	0
2	0	14	51
3	6	15	0
4	8	16	0
5	0	17	0
6	0	18	0
7	0	19	0
8	0	20	0
9	0	21	42
10	0	22	31
11	0	23	15
12	9	24	214

7.2.3 Map Diagram

A map is commonly used to monitor the location of diseases during investigation. You can tell exactly where acute diarrhoea cases are occurring by just looking at the map. The map will also help you in determining how serious the disease situation is in different reporting units.

Place pins or draw dots on the map to indicate the areas where cases of target disease are recorded.

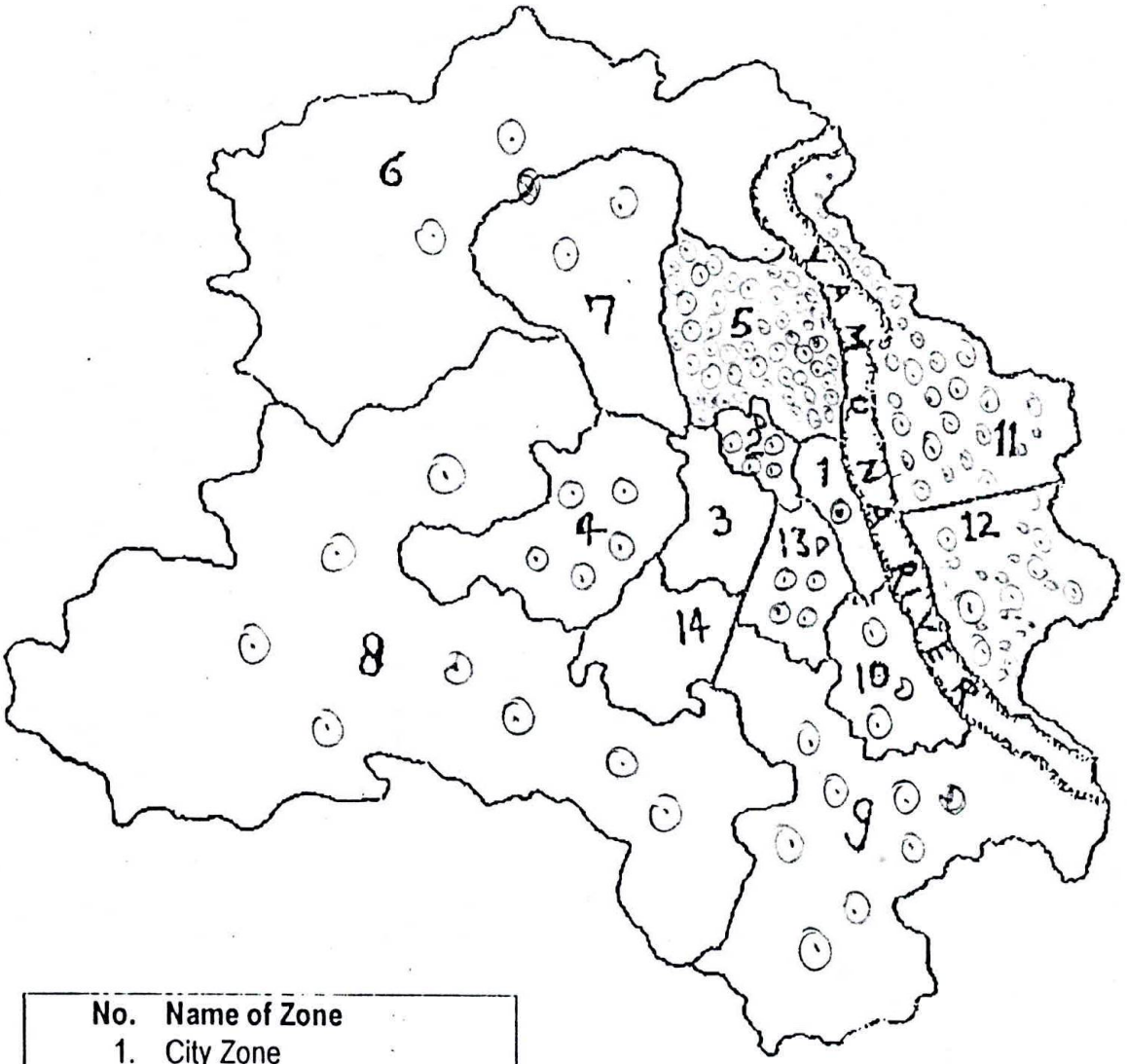
You can monitor locations of many diseases by using different coloured pins or dots. Each colour represents a different disease.

If the number of cases of a disease reported in an area is very large, you can use a pin or dot to represent more than one case. However, be sure to place the pins at the area where the cases occurred (and not where the cases were diagnosed).

If you have a computer, EPI.MAP can be used.

Exercise 2

- Draw the map diagram using data in Table 1. Map of Delhi is being provided.



No.	Name of Zone
1.	City Zone
2.	S P Zone
3.	Karol Bagh Zone
4.	West Zone
5.	Civil Lines Zone
6&7.	Narela Zone
8.	Najafgarh Zone
9.	South Zone
10.	New Delhi
11&12.	Shahdara
13.	NDMC
14.	Cantonment

① Use same type of ch
② 10 – to 1 dd-
③ Use pencil only

8. ANALYSE DATA

After the data have been compiled, it needs to be analysed and interpreted to find out useful information. For proper analysis of data, compare the morbidity or mortality in two different areas or in the same area over a period of time. Data should be compared in different groups also which relate to the characteristics of the host viz age, sex, immunization status etc.

Rates rather than absolute numbers should be used to solve the problem of change in population. Cases should be analysed by age, sex and residence status. The problem of completeness of reports can be solved by utilization of sentinel sites.

For comparing the data in the same area over a period of time, the number of cases reported during the period under review should be compared with the data reported during the previous week/month and with corresponding period of previous years. Is the number higher, lower or nearly same? Whatever the answer the analysis is not complete until you have explained the most probable reason for it.

Exercise 3

Table 1 shows the cases of cholera in Delhi by zone in 1988.

- Name the zone that suffered most in 1988 epidemic of cholera.

Do you want more information ? ग्रे

If yes

What information ?

_____
_____
_____
_____
_____
_____
_____

The best way to define the extent of problem in an area is by rate rather than absolute number of cases. Rates take the denominator (population) in account.

Table 5 gives the incidence of cholera per 100,000 population. Now it is clear that the rate was maximum in Civil

line Zone rather than Shahdara Zone. Therefore, when you are comparing the morbidity or mortality in two different areas or in the same area over a period of time, the attack rate rather than absolute number should be taken into account.

Table 5
Zone-wise incidence of cholera in Delhi, 1988

Zone	Cases	Percentage	Incidence per 100,000 population
City Zone	10	0.7	1.9
S.P.Zone	45	2.9	8.7
Karol Bagh	43	2.8	5.6
West Zone	50	3.3	5.2
Civil Lines	508	33.1	85.6
Narela	48	3.1	16.9
Najafgarh	79	5.1	14.0
South Zone	69	4.5	10.8
N.D.	26	1.7	5.3
Shahdara	583	38.0	55.5
NDMC	15	1.0	1.5
Cantonment	11	0.7	9.1
Zone Unknown	49	3.2	

However, when the denominator is not likely to change much during a period under examination, even the absolute numbers can be compared. For example, examine the data shown in Table 4.

Exercise 4

- What inference you draw from Table 4.

- What more information, if available, can make the data more meaningful?

Exercise 5

- How the information available in Fig. 1 or 2 are useful to you as a programme manager?

Table 6
Reported cases of Gastro-enteritis in some districts of UP, 1982-89

District	1982	1983	1984	1985	1986	1987	1988	1989
Agra	192	202	395	139	119	58	23	5
Alligarh	-	-	-	-	-	-	69	1
Mathura	2	10	73	3	-	78	105	10
Jhansi	2	44	75	113	184	29	264	1
Etwah	-	-	121	24	-	15	11	7
Rai Barcilly	-	98	157	96	84	42	24	39
Sitapur	73	177	166	131	-	79	128	88
Saharanpur	-	-	13	-	5	-	274	-
Ballia	-	5	6	44	26	17	1084	216

Exercise 6

Examine table 6 carefully and answer the following questions.

- Do you think that the reported incidence is correct?

If yes: Substantiate your answer with arguments.

If no : Are the incidence overestimated or underestimated?

Exercise 7

- Enumerate the possible reasons of underestimation of acute diarrhoea, measles and malaria in a district?

Deliberate Under reporting
not seeking treatment
not identified
Traditional healers
Diagnosis not mentioned
Cases managed by Pvt personnel
not sent
Carrier less people

Exercise 8

- How can you solve the problem of under-reporting of disease morbidity and mortality.

① Educate community.
② Convince ourselves.
Set right time & right type of person

Exercise 9

Despite limitations of the data reported through routine surveillance system, useful information can be drawn if the data is compiled properly and interpreted cautiously.

While investigating an outbreak of Malaria in PHC Talgram of district Farrukhabad, UP in October 1991, an epidemiologist visited the adjoining PHC Jalalabad which was not reported to be affected during the current outbreak. Table 7 shows data on Malaria from PHC Jalalabad during 1 January 1987-October 1991.

Table 7
Malaria situation in PHC Jalalabad, Farrukhabad, 1987-1991

Year →	1987		1988		1989		1990		1991	
Months ↓	Slides collected	Slides Positive	Slides collected	Slides Positive	Slides collected	Slides Positive	Slides collected	Slides Positive	Slides collected	Slides Positive
Jan	109	1	414	0	276	1	273	0	267	0
Feb.	187	1	337	0	287	0	348	0	334	0
Mar.	203	1	278	0	263	0	341	0	259	0
Apr.	254	0	334	2	408	0	252	0	113	0
May.	200	2	293	0	283	4	229	0	317	0
Jun.	170	0	211	0	324	0	323	0	372	0
Jul.	267	3	326	0	345	1	550	0	183	0
Aug.	292	3	1009	20	1602	8	1440	5	1091	7
Sep.	418	11	830	22	1492	1	941	9	2236	17
Oct.	301	4	650	0	862	0	497	0	31873	104
Nov.	287	0	438	0	333	0	289	0		
Dec.	230	0	353	1	279	0	295	0		
Total	2918	26	5473	45	6754	15	5778	14	86295	130

*1227 slides were still to be examined.

- Do you agree with the local health officials that PHC Jalalabad was not affected during the current outbreak of Malaria?

If yes: Substantiate your answer with arguments.

If no : why the area was not considered to be affected by the outbreak?

- Calculate the SPR for each year. Do you still feel that PHC Jalalabad did not register unusual increase in cases during October 1991?

1987 - 0.87 SPR.
1988 - 0.52
1989 - 0.22
1990 - 0.24
1991 1.50

If SPR is double it can be considered

If no: Why were the local health authorities not aware of the increase in cases in October 1991?

The Medical Officer I/C of the PHC was not aware of the unusual increase in the Malaria cases because the data was not compiled properly. You could appreciate the increase in incidence because the data has been compiled properly for you. This example highlights the importance of compiling the data properly before analysis and interpretation.

Exercise 10

Table 8

Typhoid fever in PHC Galore, district Hamirpur, Himachal Pradesh, June 1991: Distribution of cases by sex and village

Village	Males	Females	Total
Lanjiana	22	31	53
Daswin	17	1	18
Pahal	1	2	3
Halti	2	3	5
Ghirmani	4	0	4
5 other villages	6	12	18
Total	52	49	101

Note: All the villages had almost equal population.

- Provide comments on the data shown in Table 8.

- Were male and females affected differently? Substantiate your answer with arguments.

- You must have appreciated that cases were more in two villages (Lanjiana and Daswin) than other villages. Can you provide some explanation for such geographic & sex distribution of typhoid fever in this PHC?

_____

_____

_____

_____

_____

A marriage function was held in village Lanjiana in May, 1991. The bridegroom belonged to village Daswin and only males accompanied the marriage party. The cases started coming two weeks after this function. In fact, the outbreak occurred due to consumption of contaminated water from a local water body.

Exercise 11

Screening method for estimating vaccine effectiveness is a simple, rapid and cheap surveillance tool. It requires data on proportion vaccinated among the cases and population, which may be available in many circumstances. For example, 26 of 136 (19:1%) cases of acute poliomyelitis in Delhi in 1991 (age group of 12-23 months) were found having received 3 doses of oral polio vaccine. Immunization coverage surveys in J.J. colonies and resettlement colonies of Delhi which contributed most of the polio myelitis cases in Delhi revealed vaccine coverage of three doses of OPV as 64.5%. Using the curves shown in figure 3, the vaccine effectiveness was calculated as around 90%.

$$\frac{ARU - ARV}{ARU} \times 100$$

Percentage of Cases Vaccinated (PCV) per Percentage of Population Vaccinated (PPV), for 7 Values of Vaccine Efficacy (VE)

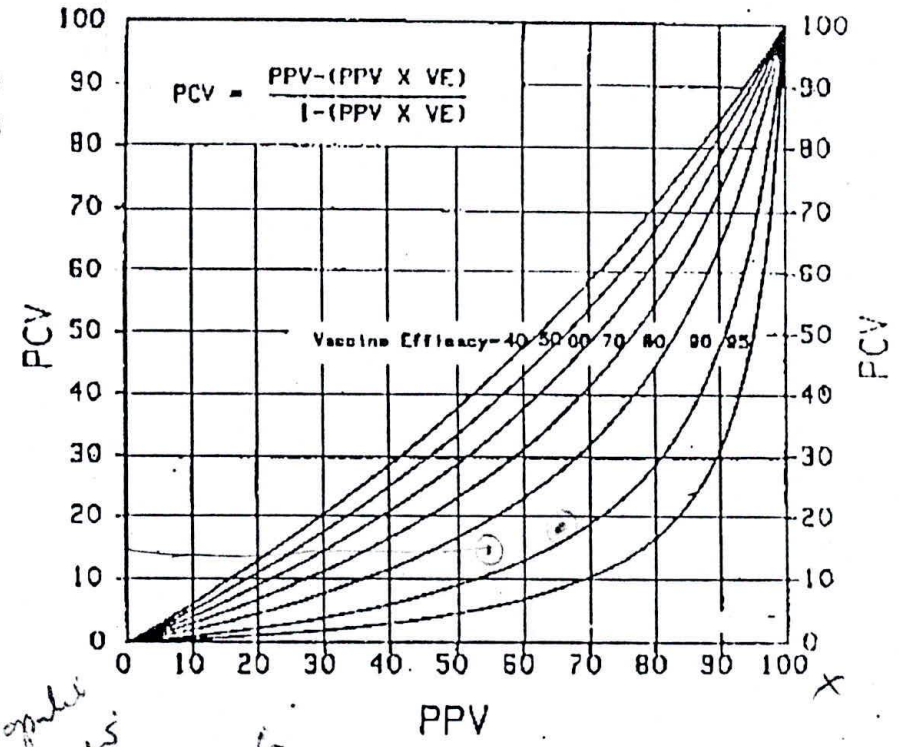


Figure - 3

- Assuming that measles vaccine is 85% effective when administered at 9 months of age, calculate the measles vaccine coverage in UP using the data from Table- 2.

Exercise 12

8.1 Increasing Completeness of Reports

One method to overcome incompleteness of reporting from multiple sites is to chose what is known as a sentinel site - an institution that consistently and completely reports for all cases of the diseases under study. At first glance, Hospital A in Table-9 would seem to fit that criteria.

Table 9
Case of viral hepatitis reported by different hospitals in a Geographical area

	1990	1991	1992	1993	1994
Hospital A	100	110	120	130	140
Hospital B	-	25	30	25	30
Hospital C	-	-	15	20	20
Hospital D	-	-	-	15	20
Hospital E	-	-	-	-	30
Total Cases	100	135	165	190	240

But, once again, it is important to look beneath the surface of yearly compilation of reports. For example, it is possible that the hospital has not completely reported for each month of the year. Again, if only the yearly summary of total cases are plotted, the artefact of increased completeness of reporting in evident. The following data illustrate this point.

Table 10
Cases of Viral hepatitis reported by Hospital A

	1990	1991	1992	1993	1994
January	5	-	15	10	10
February	-	10	10	15	15
March	-	25	-	15	15
April	25	-	20	-	10
May	30	-	30	15	15
June	-	30	-	10	5
July		10	20	-	15
August	-	-	15	20	10
September	15	-	-	20	20
October	-	20	10	10	10
November	-			-	10
December	25	15	-	15	5
TOTAL CASES	100	110	120	130	140

8.1.1 Increasing Number of Non-Residents

Now consider the situation of a sentinel hospital that consistently and completely reports all cases of viral hepatitis. It is still possible that data on cases of Viral hepatitis are confounded. Consider the situation where improved standards of living and transportation make it possible for increasing numbers of persons residing from

outside of the normal catchment area of the hospital to now have access to the referral hospital. The number of cases of Viral hepatitis may be artificially higher due to the increasing number of non-residents. If only the yearly summary of total cases is considered, the artefact of increasing numbers of non-residents is seen. The following data demonstrate this possibility:

Table 11
Increased Non-Residents, Sentinel Hospital

	1990	1991	1992	1993	1994
Residents	90	90	95	95	100
Non-residents	10	20	25	35	40
TOTAL CASES	100	110	120	130	140

8.1.2 Increasing Size of Population in Catchment Area

A final consideration of possible confounding of the true trend of Viral hepatitis in the community is a changing population base in the community. This may be especially important if there is large migration of persons into the area. The best way to compensate for a changing population in the hospital's catchment area is to convert the absolute number of cases into rates. Rates take the changing population denominator into account and correct for changes in the population over time. The following data illustrate this point and now it is finally possible to demonstrate an impact of the control programme for Viral hepatitis through incidence falling rates.

Table 12

Increased Population in Viral Hepatitis in Catchment Area under Hospital A

	1990	1991	1992	1993	1994
Resident Cases	90	90	95	95	100
Catchment Popu.	10000	12000	14000	16000	18000
Rate in Residents	9	7.50	6.79	5.94	5.56

9. SUMMARY

The discussion so far demonstrates the concept of disease surveillance, the methods commonly used, the changing needs of surveillance in different stages of programme development and some of the problems in interpretation of data.

The problems of changing population can be solved by using rates rather than absolute numbers. The problems of increasing referrals from outside the usual catchment area can be solved by keeping information on the residency status of cases. The problems of completeness of reporting can be solved by utilisation of sentinel sites. Further analysis may be done by analysing the age-specific incidence rates.

The important point to realize is that surveillance data require thoughtful analysis and simply adding up absolute numbers of cases reported from various institutions in the area may not give a true picture of disease trends in the community.

10. Conduct Investigation

There are various situations requiring investigations such as:

1. Clustering of cases/deaths in time and/or space.
2. Unusual increase in cases or deaths.
3. Unusual increase in bacteriologically proved cases even if total cases are not increased.
4. Unusual decrease in cases.
5. Even one case of AFP, plague, DHF.
6. Even one case of a disease which is not known to be present in an area.
7. Cases of measles from remote areas.
8. Shifting in age distribution of cases.
9. Patients older than 5 years of age with severe dehydration from acute diarrhoea with or without vomiting.
10. Occurrence of two or more epidemiologically linked cases of meningitis.
11. Unusual isolates.
12. High vector density.
13. Disasters.

First try to confirm the diagnosis of cases. Visit the areas from which the cases are being reported. During the visit see as many cases as possible and involve laboratory to the maximum possible extent.

11. ACTION

Action has to be taken to correct any problem uncovered during routine reporting or epidemiological investigation or a survey. If increased rates of morbidity or mortality are documented, you have the responsibility for determining the methods and feasibility required to control the situation. The purpose of investigations and analysis is thus not only to determine what happened, but also to decide how to control current outbreaks and how to prevent further attacks in the future. Action, where possible and feasible, is the logical follow up of every investigation and analysis.

12. FEEDBACK

To ensure that the reporting units at the various levels remain motivated and involved in the surveillance process, there must be regular communication back from higher levels of programme managements to lower levels. This can be done through communication in staff meeting and publication of news letters. The list of various reporting units would show how many regular reports have been received. The number of cases reported during a particular reporting period may be compared with data of the corresponding period of previous years. The feed-back should include comments on the performance in recording and reporting of cases and suggestions in solving problems in collection of data. The feed back will keep the staff motivated by helping them to understand that the information they collect is important.

The news letter should be sent not only to reporting units but also to all involved in the programme.

Other method of feed-back can be discussion of reports during routine visits to the health centres.

Exercise 13

- Now you have understood all the steps to surveillance. Can you design an ideal surveillance system for viral hepatitis in your district?

- Under what circumstances you will like to get the assistance from other agencies for investigation of an epidemic.

INVESTIGATION & CONTROL OF OUTBREAKS

VIRAL HEPATITIS

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
(DIRECTORATE GENERAL OF HEALTH SERVICES)
22-SHAM NATH MARG, DELHI - 110 054

JULY 1997

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1. Notification of cases

1.1. Hepatitis is an acute infection of the liver caused by hepatitis virus. There are six types of hepatitis virus - A, B, C, D, E and G. Although clinical picture is similar and the type of virus can only be confirmed by laboratory tests, there are differences in modes of transmission, incubation period, long-term complications and mortality rates.

1.2. Based on clinical diagnosis, around 100,000 cases of viral hepatitis are reported annually. The numbers reported is a gross under-estimate of the actual annual incidence of the disease. It is important that the surveillance system is improved as this is necessary for early identification of an outbreak (viral hepatitis E) or potential high risk areas, groups, behaviour and practices such as use of unsterile syringes and needles (hepatitis B & C).

1.3. Cases of viral hepatitis must be reported monthly to the Central Bureau of Health Intelligence through the concerned state health officer. A report may be endorsed to the Director, National Institute of Communicable Diseases (NICD), 22 Shamnath Marg, Delhi - 110 054 (Phone:-2521272, 2521060, 2913148; FAX:-2922677; Telegram:- COMDIS, DELHI).

1.4. If there is a sudden increase or clustering of cases or deaths due to viral hepatitis, information must be notified immediately (particularly by telephone or fax) to the next higher level. The district officer must similarly inform the concerned state officer immediately. If an outbreak is confirmed, NICD should be notified.

2. Causative agent

There are six viruses which are hepatogenic and lead to a similar clinical syndrome of hepatitis. These viruses are hepatitis virus A, B, C, D, E and G.

3. Reservoir

Man seems to be the only natural host for HAV to HDV. However, these viruses can be transmitted experimentally to chimpanzees. Reservoirs of HEV and HGV are unknown. Humans as well as nonhuman animals are possible reservoirs for HEV; HEV is transmissible to chimpanzees, pigs, tamarins and cynomolgus macaques.

Chronic carriers are absent in hepatitis A and Hepatitis E. Hepatitis B, C, D, and G can lead to persistent infections

(chronic carriers). These chronic carriers can transmit the disease to others.

4. Mode of Transmission

HAV and HEV are transmitted by faeco-oral route through contamination of water and food. Important mechanisms of transmission of HBV are mother to infant in perinatal period, parenteral (through infected needles and syringes, blood transfusion), and sexual routes. Intimate physical contact especially in infants and children has also been postulated to cause transmission of HBV infection. HCV is usually transmitted by parenteral route; the risk of transmission by household contact and sexual activity appears to be very low. HDV which needs the presence of HBV for its multiplication is transmitted like HBV. Only limited information is available for HGV; this virus can certainly be transmitted by parenteral route.

MODE OF TRANSMISSION
Hepatitis A & E • Faecal-oral
Hepatitis B • contaminated blood and blood products • contaminated (unsterile) syringes and needles • unsafe sex • perinatal • intimate physical contact between children or mother & child
Hepatitis C, D & G • contaminated blood and blood products • contaminated (unsterile) syringes and needles • ?

5. Incubation period

Agent	Range	Average
HAV	15-50 days	28-30 days
HBV	15-180 days	60-90 days
HCV	15-180 days	45-60 days
HDV	20-140 days	15-60 days
HEV	15-65 days	25-45 days

CHARACTERISTICS OF DIFFERENT TYPES OF VIRAL HEPATITIS

VIRUS	HAV	HBV	HCV	HDV	HEV
Transmission	Faecal-oral	Parenteral	Parenteral	Parenteral	Faecal-oral
Average Incubation Period (days)	28-30	60-90	45-60	15-60	25-45
Epidemics	Occasional	Recently documented	No	No	Yes
Homologous immunity	Yes	Yes	?	Yes	?
Chronic carrier	No	Yes	Yes	Yes	No
Chronic hepatitis	No	Yes	Yes	Yes	No
Liver Cirrhosis	No	Yes	Yes	Yes	No
Liver Cancer	No	Yes	Yes	?	No

6. Clinical manifestation and case definition

6.1. In majority of the cases, especially in young children, the infection is mild with no overt signs or only mild jaundice. In moderate to severe cases, the onset is usually acute with fever, loss of appetite, feeling of general ill-health and abdominal discomfort, followed within a few days by jaundice. Fever is present or precedes jaundice. Right upper quadrant abdominal tenderness and hepatomegaly are important findings. Liver function tests include a raise in ALT to 8 times or more than normal levels and serum bilirubin to more than 2mg%.

6.2. Hepatitis B, C and D can lead to persistent infection (chronic carriers). The sequelae of persistent infection include chronic active hepatitis, liver cirrhosis and liver carcinoma. Around 3 to 5% of our population are estimated to be chronic carrier of hepatitis B. This data is mainly based on institutional studies.

6.3. The public and the paramedical personnel can assist in reporting cases whom they can recognise by the yellow colouring of the eyes and skin (*pilia*). Active case finding is particularly important during the investigation of an outbreak.

6.4. Definitive diagnosis by type of hepatitis virus can be made only by laboratory tests.

CASE DEFINITION
SUSPECT • yellow colouring of the skin and eyes (*pilia*) PRESUMPTIVE/CONFIRMED • acute onset • jaundice • fever usually precedes jaundice. May be present when jaundice appears • malaise, anorexia • hepatomegaly and abdominal tenderness in the right upper quadrant • increase of ALT >8 times and serum bilirubin >2mg% in clinically compatible illness • epidemiological link or outbreak in the area of residence of case Serological test are necessary for confirmaiton of types of viral hepatitis viz. A,B,C,D,E & G.

6.5. It would also be useful to know if:

- there are any other laboratory confirmed cases in area

OR

- there is a clustering of cases clinically compatible with viral hepatitis

Any one of the two above will support the presumptive diagnosis of viral hepatitis and type of virus. It would also facilitate better understanding of the epidemiology of disease and institution of appropriate control measures.

6.6 Clinical features in specific types of viral hepatitis

6.6.1 Hepatitis A is usually mild in children, but can be very severe in adults. However, almost all the children in India develop immunity following sub clinical repeated exposure to this virus before 10 years of age. The disease is hence rare in adults. Hepatitis A characteristically has an acute, sudden, influenza-like onset with a prominence of myalgia, headache, fever, and malaise. This type of onset is less common with other forms of viral hepatitis, which tend to have a more gradual, insidious onset. Hepatitis A usually is not as severe or as long lasting as type B hepatitis. Hepatitis A does not lead to a chronic hepatitis or a carrier state; chronic hepatitis with persistence of HAV infection for more than 12 months has not been observed. Morbidity from Hepatitis A is not higher in pregnant women than in non-pregnant women of the same age, nor is the course of Hepatitis A during pregnancy usually different, and foetal malformations due to Hepatitis A have not been reported.

6.6.2 Onset of hepatitis B is usually insidious with anorexia, vague abdominal discomfort, nausea and vomiting, sometimes arthralgias and rash, often progressing to jaundice. Fever may be absent or mild. Hepatitis B has a more prolonged course than HA. Chronic liver disease due to HBV (chronic hepatitis, liver cirrhosis, and liver carcinoma) occur only in persons who become chronic HBsAg carriers. Protective immunity follows the infection if antibody to HBsAg (anti-HBs) develops and HBsAg becomes negative.

6.6.3 Hepatitis C clinically resembles hepatitis B. However, it tends to be milder during the acute phase and tends to progress to chronicity much more frequently than hepatitis B. Subclinical HCV infections predominates. There is usually episodic fluctuation of ALT.

6.6.4 Clinically HDV infection, whether acute or chronic, tends to be a severe illness. Although only some of HBV infections cause

icterus, most of the HDV infections (co-infection or super infection) cause an episode of clinical acute hepatitis with jaundice. The risk of fulminant disease may reach 10% for clinical HDV-HBV coinfections and 20% in HDV superinfections.

6.6.5 The clinical course of Hepatitis E is similar to that of Hepatitis A. More than 50% of HEV infections may remain anicteric. The expression of icterus appears to increase with increasing age. There is no evidence of a chronic form. A majority of Hepatitis E cases occur in young adults. Secondary household cases during the outbreaks are uncommon. The case fatality rate is also similar to that of HA, except in pregnant women where the CFR may reach 20% among those infected during the 3rd trimester of pregnancy.

6.6.6 HGV has been recently discovered. Its circulation in India has been demonstrated. HGV has been linked to acute and chronic hepatitis. The majority of individuals infected with HGV do not have clinical evidence of liver disease.

6.7. Case fatality rates for hepatitis A and E infections are generally low. Viral hepatitis may cause serious illness in pregnant women. High CFR (up to 20%) has been seen in pregnant women having hepatitis due to HEV. Case fatality rate for hepatitis B infection for hospitalised cases is around 1%; higher in those over 40 years of age. The case fatality rate increases if there is concurrent infection of hepatitis B and D viruses.

7. Laboratory confirmation of diagnosis

7.1. Acute viral hepatitis is such a sufficiently distinct clinical syndrome that it usually poses no difficulty in diagnosis. Clinical diagnosis is supported by:

(i) Elevation of ALT and AST (≥8 times of normal), and mild elevation of alkaline phosphatase (usually only 3 times of normal).

(ii) Exclusion of nonviral causes of acute hepatitis (e.g. Bacteria- Leptospirosis; Drugs- Anti-tubercular drugs, acetaminophen; Toxins and Non-specific Injury).

7.2. The type of virus causing hepatitis can be identified by laboratory tests only.

LABORATORY DIAGNOSIS OF SPECIFIC TYPES OF VIRAL HEPATITIS BY SEROLOGY
• Acute Hepatitis A virus infection - IgM HAV positive • Acute Hepatitis B virus infection - IgM HBc and HBsAg positive • Chronic hepatitis B virus infection (chronic carrier) - HBsAg alone positive • Acute Hepatitis E virus infection - IgM HEV positive • Acute or chronic Hepatitis C virus infection - Anti HCV positive

7.3 Most of the diagnostic kits are imported, expensive and require an ELISA reader. The cost of some of the kits (especially for hepatitis C, hepatitis D and hepatitis E) is around Rs 30,000 and unit cost of each test may vary from Rs 200 to 600. Carrier rates for hepatitis B can be determined by testing the blood using a similar technique and the reagents are less costly. Unit cost varies from Rs 15 to Rs 35.

7.4. Treatment of viral hepatitis does not depend on the results of laboratory examination. However, laboratory analysis of specimens from the first few suspected cases during the outbreak is important to confirm diagnosis and to determine the characteristics of the organism. Once the hepatitis virus is confirmed, ***it is not necessary to examine specimens from all cases or contacts***. In fact, this should be discouraged since it places an unnecessary burden on laboratory facilities and is not required for effective treatment.

7.5. Serum specimens should be sent to the laboratory as early as possible, preferably in cold chain. If delay is inevitable in transporitng the samples to laboratory, they should be stored at +2 to +8° C. Specimens should be stored and transported in sterile screw capped vials.

7.6. Full particulars of the patient(s) from whom samples have been collected must be sent along with the samples as many factors can influence the results of the laboratory tests. The information that should accompany each serum sample is given below:

- name
- name of mother or father
- sex
- date of onset of symptoms
- provisional diagnosis
- clinical outcome (recovered, under treatment, dead, not known)
- date sample collected
- full address

7.7. The conditions of collection and transportation of samples can influence laboratory tests. The recommended practices and precautions to be taken to minimise deterioration in the quality of the sample is given in the box.

7.8. Keep an inventory of the referral laboratories which can undertake laboratory tests for all markers of viral hepatitis.

COLLECTION AND TRANSPORTATION OF SAMPLES
• collect the blood sample from a few patients with clinical/frank jaundice
• use a sterile syringe and needle for drawing blood. ✯ Practise universal infection control safety precautions.
• Separate serum and keep in a sterile screw capped vial.
• Serum specimens should be sent to the laboratory as early as possible, preferably in cold chain. If delay is inevitable in transporitng the samples to laboratory, they should be stored at +2 to +8° C. Specimens should be stored and transported in sterile screw capped vials.
• bottles should be placed in separate plastic bags each to prevent leakage of the potentially contaminated material.
• each sample should be labelled. Detailed information as indicated at 7.6 should be sent for each sample.

7.9. The National Institute of Communicable Diseases (NICD), Delhi has the facilities for testing all markers of viral hepatitis. This support is available for investigation of outbreaks provided samples are accompanied by comprehensive details of outbreak and information indicated at 7.6.

8. Clinical Management

- There is no specific treatment. Patient requires only supportive and symptomatic management.
- Ensure nutritious diet and adequate rest. However, strict bed rest is not required unless patient is acutely ill.
- Corticosteroids are not indicated.

9. Health Education

Outbreaks of viral hepatitis are usually caused by hepatitis E virus which spreads through contaminated water and food products. Outbreaks of hepatitis A are not frequent because HAV causes very mild or sub-clinical infection in children, and

most of the persons in our country are exposed to this virus during childhood. However, we may see such outbreaks in future as many people may escape exposure during childhood due to improvement in hygiene and sanitation. The risk of outbreaks due to HEV is higher in the summer, monsoon and post-monsoon periods.

Since transmission of hepatitis A and E is faecal-oral, measures of personal hygiene and environmental sanitation that apply to control of other enteric infections are the basis for their control in any settings. There is no vaccine available for hepatitis E. Vaccine available against hepatitis A is not cost-effective due to epidemiological considerations and very high costs.

Hepatitis B, C and D viruses spread through body fluids of patients or carriers. General measures to prevent hepatitis B, C, D, and G include sterilisation of needles and instruments that penetrate the skin, screening of blood for hepatitis B and hepatitis C markers and promotion of safe sex behaviour. Vaccine is available against hepatitis B only.

Health education and public awareness and co-operation are important to prevent and control viral hepatitis. If the community knows how the disease spreads and what measures they can take in their own families, the risk can be considerably reduced. While the key messages will essentially remain the same for all areas, the language and style may be adapted to local needs. Suggested material or health education messages which can be adapted for local use is given at Annex 2.

10. Prevention and control of an outbreak

10.1. Almost all outbreaks of viral hepatitis in India are due to faeco-orally transmitted hepatitis E. An occasional outbreak of hepatitis A has also been reported. Although an unusual increase in viral hepatitis cases due to hepatitis B have been recognised in hospital setting, it had not been documented as the cause of an outbreak in community setting. However the NICD investigated 3 outbreaks of hepatitis B in 1997 which occurred due to the use of inadequately sterilised needles and syringes by unqualified medical practitioners in the concerned areas.

10.2 Outbreaks of hepatitis E are due to contamination of water supply. Measures to prevent and control these outbreaks should be directed towards ensuring safe water supply.

10.3. Majority of the outbreaks have occurred due to contamination of pipe water supply. Clustering of cases in some area linked to a

common water supply will help in identifying the points where the contamination is taking place. Repair of water pipe lines at these points will prevent the further spread of Hepatitis E.

10.4 Alternate arrangement for safe water supply should ideally be made during the outbreak. Simultaneously, source of contamination should be removed on priority. If the local water sources are still used, the community should be advised to boil the water. **Hepatitis E virus is not destroyed by chlorination in usual doses; the virus is killed by boiling. Hepatitis A virus is however, destroyed by chlorination.**

10.5 All human excreta should be disposed of promptly and safely.

10.6 Whether the outbreak is due to hepatitis A or E, the measures for prevention and control are similar. These measures should be applied immediately.

10.7. If an outbreak of hepatitis B occurs, identification and removal of risk factor/s will control the outbreak.

10.8. Ensuring safe injections and screening of all blood donations for hepatitis B (and if possible hepatitis C) will largely prevent all the parenterally transmitted infections including hepatitis B, C, D and G. National AIDS Control Programme takes measures to promote safe sex behaviour to prevent HIV infection. These measures will also contribute towards the prevention of hepatitis B and other parenterally transmitted viral hepatitis.

ACTION TAKEN FOR PREVENTION OF HEPATITIS B INFECTION
• mandatory testing of blood in all blood banks
• promotion of safe sex behaviour under the National AIDS control programme
• health awareness campaigns regarding the dangers of using unsterile syringes and needles under the immunisation programme and National AIDS Control Programme
• guidelines have been issued to the state health authorities for the use of a separate sterile syringe and needles for each injection
• immunization of health personnel in hospitals against hepatitis B

PREVENT WATER-BORNE DISEASES BY
• provision of safe water
• adopting safe practices in food handling
• sanitary disposal of human waste

10.9 Hepatitis B vaccine

There are two types of hepatitis B vaccines available - the recombinant DNA vaccine and the plasma derived. Both are safe and effective. They should be administrered as per recommendations by manufacturers. These vaccines are available in single and multi-dose vials.

10.10 When an outbreak occurs or when the risk of such outbreaks is high, the cooperation of other government departments, non-governmental agencies and the community often becomes necessary. Such help will be more forthcoming if mechanisms for interaction have been developed before the onset of an outbreak. It might be useful to convene a meeting of the concerned departments, community representatives and the NGOs before the expected seasonal increase of cases of diarrhoeal diseases. Some mechanism for briefing the press should also be established. Some suggested areas in which the government departments and NGOs can assist may be seen at Annex 1.

11. Preparatory action in anticipation of an outbreak

11.1 Maintain surveillance for acute diarrhoea, typhoid fever, and jaundice cases. Viral hepatitis E has a long incubation period of usually 1-2 months (not less than 15 days). There are other diseases which have shorter incubation period and are also transmitted by faeco-oral route. They are acute watery diarrhoea (incubation period: a few days and typhoid fever (incubation period: 1-3 weeks). By analogy, viral hepatitis E is likely to follow an increase in acute diarrhoea or typhoid fever following water contamination.

Describe cases by time, person and place to identify any clustering of cases in an area or group.

11.2. Regular monitoring of water quality by examination of water samples for residual chlorine and/or coliform organisms on a random basis.

11.3 An increase in the incidence of diarrhoea and/or typhoid fever, and poor water quality indicate impending epidemic of viral hepatitis. Manage the existing increase in diarrhoea and/or typhoid fever cases, and take corrective measures to prevent the impending outbreak of viral hepatitis.

11.4. Periodic sanitary surveys should be undertaken, especially before monsoon, for sewer overflow, and leakage in water supply system. If any leakage is found, it should be attended to

on priority. In areas where water supply is intermittent, on-line-booster pumps can create negative pressure in the pipeline leading to suction of sewage into the water pipeline.

11.5. Prepare/update inventory of existing and required resources if outbreak strikes.

11.6. IEC activities for minimising the risk of faeco-orally transmitted diseases should be undertaken in the community.

11.7 To prevent faeco-oral transmission, supply of safe water, environmental sanitation, and personal and domestic hygienic practices should be applied.

12. Investigation of an outbreak

12.1 Establish the existence of an outbreak:

Surveillance for jaundice cases (institutional surveillance-supported by surveys if necessary) to find out the existence of outbreak - rule out any seasonal increase.

12.2 Confirmation of diagnosis:

Acute viral hepatitis is such a sufficiently distinct clinical syndrome that it usually poses no difficulty in diagnosis. However, specific blood tests are needed to diagnose different types of viral hepatitis. See Box at page 8.

Collect and transport the blood samples as given in the box under-7.

12.3 Characterise the outbreak in terms of time, person and place.

Geographical distribution-Identify the area or group affected by the outbreak-attack rates in different areas will indicate the points where contamination is occurring.

12.4 Carry out a sanitation survey-find out the leakage points.

12.5 Institute the control measures

(i) Repair of the water and sewer lines - close co-ordination with public health engineering department - safe disposal of human excreta.

(ii) Disinfection of water - boiling (chlorination does not inactivate HEV). Chlorination of water sources to prevent

the transmission of agents of acute diarrhoeal and typhoid fever. Frequent water quality monitoring.

(iii) IEC to prevent the spread of infection.

(iv) Standard measures to prevent faeco-oral transmission.

(v) Arrangement for management of the existing cases.

(vi) Supply of ORS for acute diarrhoea, and antibiotics for typhoid fever.

12.6 In case the CFR is very high hepatitis B should also be suspected. Data should be analysed for the risk factors of viral hepatitis B. All the 3 outbreaks of hepatitis B investigated by the NICD during the first half of 1997 were due to the use of unsafe injections by unqualified medical practitioners. Removal of the risk factor/s will prevent the further spread of the infection.

12.7 Notify the state nodal officer about the outbreak.

12.8 Write outbreak investigation and action taken report. Send a copy to the concerned state authorities as well as to NICD.

ACKNOWLEDGEMENT

The manual has been edited by Dr. Jotna Sokhey, Director NICD; Dr. Rajesh Bhatia, Consultant (Microbiology); and Dr.Jagvir Singh, Deputy Director (Epidemiology).

Annex 1

INTER-DEPARTMENTAL COMMITTEE
SUGGESTED AREAS OF RESPONSIBILITY AND ACTION

District administration

- mobilize resources by organizing meetings with
  - concerned government departments
  - non-governmental agencies
  - community leaders
- ensure adequate quality monitoring of water samples
- repair of leakage in water pipe lines
- arrange safe water supply
- ensure supplies of ORS packets and other essential items
- ensure adequate facilities for transportaion of serious patients to district hospital, if necessary
- strengthening of existing provision under the Drug & Cosmatic Act to curtail over the counter sale of parenteral drugs
- provide relevant information to the press
- monitor status of control activities

District Health Office / Municipal Health Office

- arrange repair of leakage in water pipe lines
- alert health personnel to report cases and to monitor trends
- arrange active surveillance in affected area
- ensure that treatment guidelines are followed in hospitals and other health facilities
- ensure availability of ORS packets and other essential items
- strengthening of existing provision under the Drug & Cosmatic Act to curtail over the counter sale of parenteral drugs
- arrange health educational camps and distribution of health educational material
- arrange chlorination of water sources if possible
- arrange water quality monitoring
- convene meeting under distrist administrator to seek cooperation of other government departments and NGOs
- check sterilisation practices of medical practitioners for syringes, needles and sharp instruments

Concerned Department (s) responsible for water suppy

- repair of leakage in water pipe lines
- arrange potable water supply, including water tankers if necessary
- arrange chlorination of water
- ensure water quality monitoring

Other government departments such as social welfare, education, tribal welfare and NGOs

- dissemination of relevant information
- promotion of oral rehydration therapy
- check sterilisation practices of medical practitioners
- reporting cases of jaundice (*pilia*)

Panchayat members, village pradhans, community leaders

- dissemination of relevant information
- promotion of oral rehydration therapy
- check sterilisation practices of medical practitioners
- reporting cases of *pilia*
- monitoring chlorination of water sources such as wells
- arranging transportation of serious cases to hospital

PROTECT YOURSELF FROM VIRAL HEPATITIS BY

- drinking water from a safe source
- not eating cut fruit and vegetables from the market
- cleaning thoroughly fruits and vegetables which are eaten raw
- storing drinking water in a clean, covered and narrow-mouthed container
- eating cooked food while it is still hot
- washing hands before eating and after using the toilet
- avoiding use of on-line-booster pumps which can suck sewage in water line
- reporting to municipal authorities broken water pipes
- reporting cases(s) of JAUNDICE (*PILIA)* to the nearest health centre or local health officer

HOW CAN YOU PROTECT YOURSELF FROM HEPATITIS B

AVOID

- unsterile syringes and needles
- other unsterile sharp instruments
- transfusion with unscreened blood
- unsafe sex

PROTECT YOURSELF FROM PARENTERALLY TRANSMITTED VIRAL HEPATITIS BY

- ensuring that a separate sterile syringe and needle is used for each injection. Insist that a glass syringe and needle are boiled for 20 minutes before reuse OR a sealed disposable syringe and needle is used
- taking injections only when medically indicated. Take treatment from qualified personnel only
- using condoms. Avoid unsafe sex and extra-marital sexual contacts
- getting blood from a registered blood bank. Promote voluntary blood donation and donate blood
- reporting JAUNDICE (*PILIA*) case (s) to the nearest health centre or local health officer
- hepatitis B vaccine is available in the market. Contact your physician for further guidance.

PROTECT YOURSELF FROM UNSAFE INJECTIONS BY

- ensure that a separate sterile syringe and needle is used for each injection. Insist that a glass syringe and needle are boiled for 20 minutes before reuse OR a sealed disposable syringe and needle is used
- take injections only when medically indicated
- take treatment from qualified personnel only

Your life is precious. Say No to Injections

Annex - 3

OUTBREAK INVESTIGATION REPORT

General Information

State :_____

District :_____

Town/PHC :_____

Ward/Village :_____

Population :_____

Background Information

Person reporting the outbreak :_____

Date of report :_____

Date investigations started :_____

Person(s) investigating the outbreak _____

Details of Investigation

Describe how the cases were found (may include: (a) house-to-house searches in the affected area; (b) visiting blocks adjacent to the affected households; (c) conducting record reviews at local hospitals; (d) requesting health workers to report similar cases in their areas, etc.):

_____
_____
_____
_____
_____

Descriptive Epidemiology

- Cases by time, place and person (attach summary tables and relevant graphs and maps).
- Age-specific attack rates and mortality rates
- High-risk age-groups and geographical areas.

Description of Control Measures Taken

_____

Description of Measures for Follow-up Visits:

_____
_____
_____

Brief Description of Problems Encountered

_____
_____
_____

Factors Which, in Your Opinion, Contributed to the Outbreak

_____
_____
_____

Conclusions and Recommendations

_____
_____
_____

Date (Name and Designation)

Annex 4

PROFORMA FOR SURVEY FOR JAUNDICE CASES

Name of Surveyor______________________ Date of Survey________

City/Village__________________ Street/Mohalla_________________

House No.	Name of persons	Age/Sex	History of jaundice in past 6 months Yes/No	Currently having jaundice Yes/No	Case No. for jaundice cases

Annex 5

PROFORMA FOR JAUNDICE CASES

Surveyor's Name_____ Date of Survey_____ Case No. _____
Name of case_____ Father's/Husband's Name_____ Sex: M/F
Age of Patient (in complete Years)_____ Ward No/Village._____
Address_____
If female, history of pregnancy when she had jaundice: Yes/No/do not know

If case is a child <15 Years then ask :
Education of Mother_____ Education of Father _____
Occupation of Mother_____ Occupation of Father_____

Information about Family for all Jaundice cases :
No. of Family Members_____ No. of members had jaundice in past 6 months (excluding index case)___
Source of drinking water: Tap/Hand pump/Well/other (specify)
Storage of drinking water: Bucket/Pitcher/Surahi/Vessel with Tap/other
Method of drawing drinking water: Tap/Ladle/other
Type of Latrine: Open field/Service/Sanitary/Community latrines
Hand washing after defecation: Soap/Ash/Earth/Water alone/did not wash
Hand washing before meals: Soap/Ash/Earth/Water alone/did not wash

Information about Illness for all Jaundice cases:
Date of Onset of Jaundice_____ Outcome: Still Ill/Recovered/Died
Symptoms Present (circle): Dark Urine, Yellow Eyes, Anorexia, Nausea, Vomiting, Pain abdomen, Fever, Clay coloured stool, Malaise, Itching, Others (Specify)_____
Liver palpable: Yes/No/not examined
Any Treatment taken for Jaundice: Yes/No
If Yes, the type of treatment: Allopath/Ayurved/Homeopath/Other (specify)
Past history of jaundice before this episode : Yes/No

History within 6 months but 15 days prior to onset of jaundice for the following points:

Hospital Admission due to any cause:	Yes/No	Nose/ear pricking:	Yes/No
Multiple Injections:	Yes/No	Shave from a barber:	Yes/No
Surgical Operation:	Yes/No	Drug addiction:	Yes/No
Dental Surgery:	Yes/No	Alcoholism:	Yes/No
Blood Transfusion:	Yes/No	Extramarital sexual contact (adult):	Yes/No
Dialysis:	Yes/No	Movement outside the village:	Yes/No
Intravenous Drip:	Yes/No	Contact with a case in the family:	Yes/No
Blood Donation:	Yes/No	Contact with a case in the Community:	Yes/No
Vaccination (Specify):	Yes/No	Contact with a case outside the village:	Yes/No
Tattooing:	Yes/No		

Any chronic illness_____
Drug history_____
Diagnosis by a Doctor_____

Investiagtions already done:
S.bilirubin_____ SGOT_____ SGPT_____ Urine_____HbsAg_____

Blood Sample collected: Yes/No
(Collect Blood Sample if the patient had jaundice in the last 3 months)
Blood sample No._____

Remarks

INVESTIGATION & CONTROL OF OUTBREAKS

DENGUE & DENGUE HAEMORRHAGIC FEVER

NATIONAL INSTITUTE OF COMMUNICABLE DISEASES
(DIRECTORATE GENERAL OF HEALTH SERVICES)
22-SHAM NATH MARG, DELHI - 110 054

JULY 1997

CONTENTES

Now - being reported from Rural areas also.

Now it is Rural Broadcast also.

1. Introduction

1.1. Dengue virus infections are significant causes of morbidity and mortality in many parts of the world, including India. The dengue virus is believed to cause two forms of clinical syndrome, namely, classical dengue fever (DF) and dengue hemorrgahic fever/dengue shock syndrome (DHF/DSS).

1.2. Dengue fever is a self - limiting disease and represents the majority of cases of dengue infection. In some situations, it manifests in severe forms as haemorrhagic (DHF) and shock syndrome (DSS).

1.3. In India, dengue infection is known to exists in endemic proportions for a very long time. The first major outbreak of dengue fever accompanied by dengue haemorrhagic fever was reported in Calcutta in 1963. About sixty outbreaks have been reported during the period 1956 to 1996. Because dengue infections have the potential of rapid spread leading to an acute public health problem, special attention is required to be paid for its surveillance, prevention and control.

2. Notification of cases

2.1. Cases of DF/DHF must be reported monthly to Directorate of National Malaria Eradication Programme (NMEP) through the concerned state nodal officer. A report may be endorsed to the Director, National Institute of Communicable Diseases (NICD), 22-Shamnath Marg, Delhi 110054; Phone:-2521060, 2521272, 2913148; FAX: 2922677; Telegram: COMDIS, DELHI.

2.2 If there is a sudden increase or clustering of cases or deaths due to DF/DHF, it must be reported immediately to the district health office. The district health office must similarly inform the concerned health officer by the quickest mode of communication, preferably through telephone, Fax or e-mail with details of the outbreak including investigation and control measures initiated. The National Institute of Communicable Diseases is expected to be kept informed of the action taken.

3. Causative agent

DF/DHF is caused by a group B arbovirus (Flavivirus) and include serotypes 1,2,3 and 4 (Den-1, Den-2, Den-3 and Den-4).

4. Incubation period

The incubation period is usually 5-6 days but may vary from 3 to 10 days.

5. Mode of transmission

5.1 The infection is transmitted by the bite of an infected female mosquito- *Aedes aegypti*. The mosquito usually bites during day time. The mosquito becomes infected by biting a patient with dengue infection. Once the mosquito becomes infected, it remains so for life. The female mosquitoes can survive upto 3 weeks under normal temperature and humidity.

5.2 Female mosquitoes get infected after feeding on a viraemic host. They can transmit the virus to human host after an extrinsic period of 8-10 days. The ambient temperature range for dengue transmission is 16°C to 40°C. Below 16°C *Aedes aegypti* ceases to bite.

6. Vector of transmission

6.1 *Aedes aegypti* is the main vector of dengue transmission in India. Dengue outbreaks have also been attributed to *Aedes albopictus*. However, *Aedes polynesiensis* and species of *Aedes scutelloris* complex have also been incriminated as vector in other South East Asian countries.

6.2 The mosquito has characteristic white strips on the back and legs. It is also known as Tiger mosquito (figure).

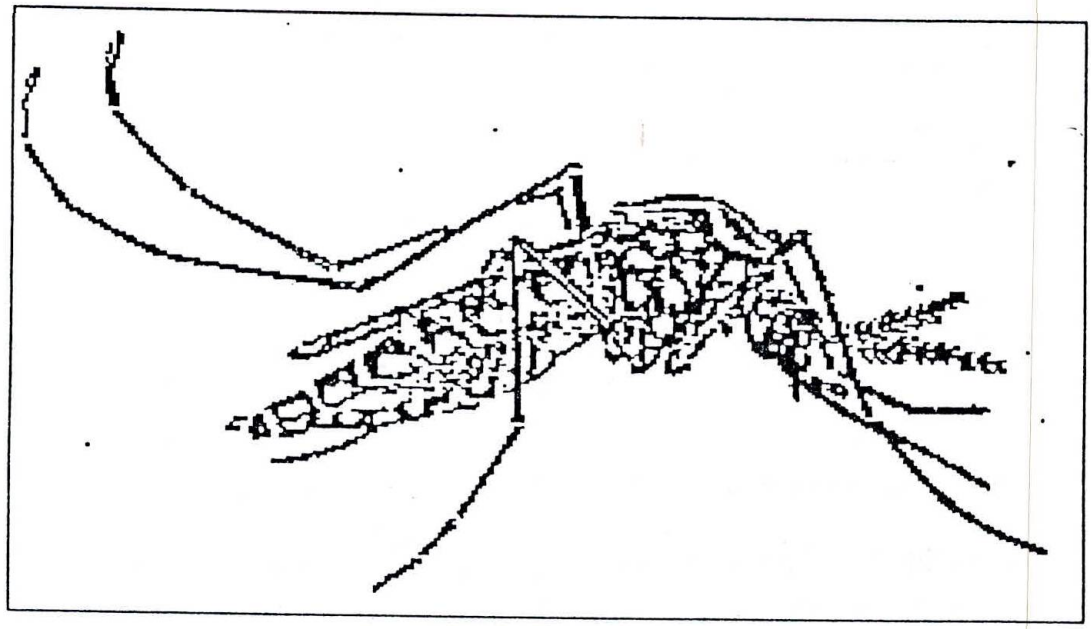

Figure. Aedes aegyptii

6.3 The mosquito rests indoors, in closets and other dark places. Outside, they rest where it is cool and shady. The female mosquito lays eggs in clean water containers in and around homes, schools and work places. The larvae hatch from the mosquito eggs, and live in the water for about a week; they then change into a round pupal stage for one or two days after which the adult mosquito emerges, ready to bite.

6.4 The mosquito is a domestic breeder. It breeds in any water catching or water storage container in shady or sunny places. The water coolers and overhead water tanks are major places of breeding of this mosquito. The mosquito can also breed in any container which has water for several days such as unused tyres, broken plastic containers and discarded cups, glasses and other utensils, flower vases etc.

6.5 *Aedes* mosquito can fly upto a limited distance of 400 meters.

6.6 The outbreaks of dengue fever/DHF are most likely to occur in the post-monsoon period when the breeding of the mosquitoes is highest.

7. High risk areas

7.1 Usually, urban areas, having high population density, poor sanitation and large number of desert coolers, overhead tanks, discarded buckets, tyres, utensils etc. which promote mosquito breeding, are at high risk.

7.2 Dengue fever /DHF can also occur in rural areas where the environment is friendly for mosquito breeding. Mosquito breeding can occur, for example, in large containers used for collecting rain water, which are not emptied and cleaned periodically.

8. Clinical manifestations and case definitions

8.1 Dengue fever (DF)

The symptoms of dengue fever are similar to acute fevers of viral origin. These are sudden onset of fever, headache, bodyache, joint pains and retro-orbital pain. Other common symptoms include anorexia, altered taste sensation, constipation, colicky pain, abdominal tenderness, dragging pains in the inguinal region, sore throat and general depression.

Patient may or may not have rash. Some of the patients may also show signs of bleeding from the gum, nose etc.

CASE DEFINITION OF DENGUE FEVER

SUSPECT CASE

- Acute onset
- High fever of less than seven days duration
- Severe headache, backache
- Joint and muscle pain and pain behind eyes
- With or without rash

PROBABLE CASE

- Suspect case of DF
- High vector density
- Presence of confirmed case in the area
- Blood slide -ve for malarial parasite & patient does not respond to anti-malarial drugs

CONFIRMED CASE

- Isolation of virus from blood in early phase
- Serological test for IgM antibody in single serum samples, or 4 fold rise of antibodies in paired serum samples

8.2 Dengue Haemorrhagic Fever (DHF)

DHF is a severe form of dengue fever. Typically, it begins abruptly with high fever accompanied by headache, anorexia, vomiting and abdominal pain. During the first few days, the illness resembles classical dengue fever, but a maculopapular rash is less common. There are signs of haemorrhage (bleeding), such as easy bruising and bleeding at the venepuncture sites.

The liver is usually enlarged, soft and tender. Approximately 50% of patients have generalized lymphadenopathy.

The critical stage is reached after 2-7 days, when the fever subsides. Accompanying or shortly after a rapid drop in body temperature, varying degree of circulatory disturbances occur. The patient is usually restless and has cold extremities. Sometimes there may be sweating.

Bleeding tendency start after fever stops.
(after 4th to 7th day of illness occur)

CASE DEFINITION OF DHF

SUSPECT CASE

- Acute onset
- High fever of less than 7 days duration
- Severe headache, backache
- Joint and muscle pain and pain behind eyes
- Bleeding tendencies

PROBABLE CASE

- Suspect case of DHF
- Positive tourniquet test

CONFIRMED CASE

- Probable case
- Thrombocytopenia ≤ 1,00,000/mm.^{3}
- Heamoconcentration (haematocrit increased by ≥ 20% or evidence of increased capillary permeability(e.g. pleural effusion in X-ray chest).

DHF is clinically confirmed by the positive tourniquet test (a blood pressure cuff is used to impede venous flow. A test is considered positive if there are >20 petechiae/square inch).

STANDARD TOURNIQUET TEST

- Apply sphygmomanometer cuff to the arm and take Blood pressure.
- Inflate cuff so that it registers a pressure midway between that of systolic and diastolic blood pressure.
- Hold it for five minutes.
- Examine the cubital fossa for petechiae if >20 petechiae in 3 cm diameter circles, the test is positive, if >20 petechiae in 2.5 cm square, the test is positive.

Haematocrit increase by 20% or more of the baseline value

Thrombocytopenia and haemoconcentration are constant findings in DHF. Haemoconcentration- indicating plasma leakage- is always present.

CLINICAL LABORATORY FINDINGS

- **A platelet count of 1,00,000/mm^3 or less between the third and eighth day of illness**
- **Haematocrit increase by 20% or more of the baseline value**

In more severe cases, shock ensues and the patient may die within 12-24 hours. Prolonged shock is often complicated by metabolic acidosis and severe bleeding, which indicate a poor prognosis. If the patient is appropriately treated before the irreversible shock has developed, rapid recovery is the rule.

A major cause of deaths due to DHF is leakage of plasma in the pleural and abdominal cavities leading to hypovolaemic shock. **Determination of haematocrit and platelet is essential for diagnosis and case management.** The time course relationship between the fall in platelet count and a rise in haematocrit level appears to be unique to DHF. These changes occur before the subsidence of fever and before the onset of shock and are correlated with the disease severity.

Encephalitic signs associated with intracranial haemorrhage, metabolic and electrolyte disturbances, and hepatic failure (a form of Reye's syndrome) may occur. They are uncommon but carry a grave prognosis.

9. Differential diagnosis of DHF/DSS

Early in febrile illness, the differential diagnosis includes a wide spectrum of viral and bacterial infections.

The presence of marked thrombocytopenia with concurrent haemoconcentration differentiates DHF/DSS from other diseases such as endotoxic shock from bacterial or meningicoccaemia. In patients with severe bleeding, evidence of pleural effusion and/or hypoproteinaemia may indicate plasma leakage.

10. Laboratory confirmation of diagnosis

10.1 The diagnosis of DF/DHF can be confirmed by serological tests. The tests include detection of IgM antibodies which appear around the end of first week of onset of symptoms and are detectable for 1-3 months after the acute episode.

10.2 A rising titre of IgG antibody in paired sera taken at an interval of ten days or more is confirmatory.

10.3 IgG antibodies indicate previous infection and are useful for conducting sero-epidemiological studies to determine the extent of silent infection and immunity levels in the local population.

10.4 Antigen is produced in limited quantities for operational research and outbreak investigations at the National Institute of Virology (NIV), Pune. With the antigen received from NIV, the National Institute of

Communicable Diseases (NICD) supports outbreak investigations on the request from the state health authorities. The antigen is also commercially available.

10.5 Blood for serological studies should be carefully collected taking due universal precautions from suspected DF/DHF cases (a) as soon as possible after hospital admission or attendance at the clinic (S-1) (b) shortly before discharge from the hospital (S-2) and (c) if possible, 14-21 days after disease onset (S-3). Failure to leave an interval of 10-14 days between collection of S-1 and S-2 samples may prevent the serological diagnosis of primary dengue infection. Specimen containers should be clearly labelled.

10.6 Each specimen should be accompanied with the detailed information about the case as given in the box so that the results could be scientifically interpreted.

INFORMATION WHICH SHOULD ACCOMPANY EACH SAMPLE

- name of the patient
- name of the mother / father
- age
- sex
- complete residential address
- name of the hospital / PHC sending the sample
- registration number of the patient
- date of onset of illness
- date of hospitalisation
- date of collection of sample
- provisional diagnosis
- brief clinical findings
- results of clinical laboratory investigations

11. Clinical management

11.1 Management of Dengue fever

The management of Dengue fever is symptomatic and supportive and comprises of :

- Bed rest is advisable during the acute febrile phase
- Antipyretics or sponging are required to keep body temperature below 39°C. **Salicylates should be avoided.** Paracetamol may be prescribed.
- Analgesics or a mild sedative may be required for those with severe pain

- Home available fluids and ORS solution are recommended for patients with excessive sweating, nausea, vomiting or diarrhoea to prevent dehydration.

11.2 Management of DHF

- Management during febrile phase is similar to that of DF
- Antipyretics may be indicated but **salicylates should be avoided.**
- Increased fluid intake
- Fluid and electrolyte replacement by IV fluids, isotonics etc.
- Plasma expanders, if clinically indicated
- Fresh frozen plasma may be indicated in some cases
- Blood transfusion

As thrombocytopenia and concurrent haemoconcentration usually occurs well before the onset of shock, the use of these criteria can enable the clinician to make early diagnosis at the time the plasma leakage starts and hence early fluid replacement for plasma loss can be administered and disease severity can be modified. Prolonged shock is often complicated by severe massive bleeding and grave prognosis.

Judicious volume replacement is mandatory as the rate of plasma loss is short for 24 to 48 hours and is more rapid around the time of defervescence and /or shock. Haematocrit determination is essential for monitoring the rate of IV fluid infusion and to check overload (which has been recognised as a common problem).

Isotonic solution (0.9% sodium chloride, also known as normal saline) or a compound solution of sodium lactate is preferred. Saline with or without glucose can be used depending upon availability. Glucose solution without saline do not provide the salt required to restore electrolyte balance.

Transfusion of platelets does not change the course of the illness and is not recommended. Blood transfusion may be indicated in patients with severe shock, massive bleeding and DIC.

Amount of fluid given should be constantly monitored. Any evidence of swelling, shortness of breath or puffiness may indicate fluid overload.

12. Pathogenesis and pathophysiology

The pathogenic mechanism of DHF is not clear, but two main pathophysiologic changes occur:

(a) vascular permeability increases which results in plasma leakage, leading to hypovolaemia and shock

(b) abnormal haemostasis, due to vasculopathy, thrombocytopenia and coagulopathy, leading to various haemorrhagic manifestations.

The severity of DHF as compared with dengue fever may be explained by the enhancement of virus multiplication in macrophages by heterotypic antibodies resulting from a previous dengue infection. There are evidences suggesting that cell mediated immune response may also be involved in the pathogenesis of DHF.

Tetravalent vaccine may help

13. Immunity

Infection with one serotype provides life-long homologus immunity but does not provide protection against other serotypes, and instead may exacerbate subsequent infection.

14. Surveillance in DF/DHF

Surveillance is prerequisite for monitoring the dengue situation in the area and should be carried out regularly for early detection of an impending outbreak and to initiate timely preventive and control measures. Surveillance should include epidemiological, entomological and laboratory parameters.

14.1 Epidemiological surveillance

The epidemiological surveillance should include the following:

- Fever surveillance
- Diagnosis based on standard case definition
- Reporting of DF/DHF cases to state health authorities
- During an outbreak situation, samples of about 5% of clinically diagnosed cases should be tested for confirmation of diagnosis by the laboratory.

The peripheral health staff should be alerted to report increase or clustering of acute febrile illness compatible with the case definition of DF/DHF. Such increase of cases should be investigated locally, including entomological investigation to check for vector density in the area.

14.2 Vector surveillance

A number of indices have been described and are currently used to monitor the vector population in a defined area:

1. **House index-** Percentage of houses positive for larvae of *Aedes aegypti.*

2. **Breteau index-** Number of positive containers for *Aedes aegypti* per 100 houses.

3. **Container index-** Percentage of containers positive for *Aedes* breeding.

Epidemiological interpretation of various entomological indices		
Entomological indices	***High risk of transmission***	***Low risk of transmission***
Breteau index	>50	<5
House index	>10%	<1%

Container index :Container index mostly utilised for drawing vector control strategy.

In areas where *Aedes aegypti* is absent or very scarce and dengue outbreaks occur, a special effort should be made to identify the local vector(s) to develop vector surveillance accordingly.

15. Investigation of an outbreak

15.1 The investigation of an outbreak of DF/DHF is similar to the investigation of other epidemic prone diseases. The first principle is to receive early signals, confirm diagnosis and to take prompt measures for control of the outbreak. Control measures are most effective when selective measures are applied early.

Factors increasing the risk of DF/DHF outbreaks

- Increasing urban population
- Expanding mosquito breeding due to:
  - shortage of water supply,
  - traditional water storage,
  - poor garbage collection,

  (create more mosquito breeding places)
- Changing life style (use of water coolers etc.)
- Rapid transportation

15.2 Line list of cases, including age, sex and address and other details should be maintained and also reported to district health office

(**Annexure 1&2**). Active search should be made for more cases. Serum samples should be collected for laboratory confirmation of diagnosis.

15.3 Vector surveillance should be immediately initiated and should include collection of adult mosquitoes, identification of mosquito species and density, assessment of susceptibility of vectors to available insecticides.

15.4 Arrange health educational activities in the community regarding prevention of mosquito bites by use of mosquito nets and mosquito repellent creams, mosquito breeding by drying out water containers at least once a week, residual insecticide spray, wearing of protective clothing and reporting suspected cases at the health facilities early.

15.5 On confirmation of an outbreak of DF/DHF, take precautionary measures in other neighbouring high risk areas.

15.6 After the outbreak is over, a detailed report must be written. Format for outbreak investigation is given at **annexure 3.**

16. Prevention and control of an outbreak

PREVENTION AND CONTROL OF DF/DHF OUTBREAK

- Initiate vector surveillance and control measures
- Conduct IEC
- Community participation
- Assess facilities for case management of patients with haemorrhagic shock
- Alert health personnel to report increase/clustering of cases

16.1 A surveillance system should be established so that DHF is immediately reported to the local health authorities. Necessary field investigations must be carried out in the area of residence of the patient to check for vector.

16.2 The preventive measures are directed at reducing the vector density and in taking personal protection to prevent the bite of mosquitoes.

PREVENTION OF MOSQUITO BITES

- Wear cloths that cover arms and legs during outbreak situations
- Use mosquito nets or insect repellents while sleeping at night to keep away mosquitoes
- Children should preferably not wear shorts and half sleeved cloths
- Keep patients protected from mosquito bite in acute phase

16.3 Immediate measures are called for to reduce the density of mosquitoes by use of insecticides. The public should also be informed to take necessary precautions against mosquito bite such as use of full sleeved clothes, mosquito nets at night and mosquito repellent creams, if necessary.

ELIMINATION OF MOSQUITO BREEDING PLACES

- Empty water containers at least once a week
- Cover and seal septic tanks and soak-away pits
- Removal of rubbish
- Remove water from coolers and other places where water has remained stagnant

16.4 Long term measures include the recommended steps for vector control under the National Malaria Eradication Programme(NMEP).

16.5 Pockets of high risk should be identified so that these areas could be given more attention with regard to control measures, health educational activities and field supervision.

16.6 Isolation of patients and disinfection of secretions and excretions are not required as DF /DHF virus is not transmitted from person to person.

16.7 Patients should be treated in nearby health centre/ hospital where the facilities for platelet count and haematocrit value estimation are available. These parameters are essential to monitor clinical status of the patients. Haematocrit levels are required to know the degree of plasma leakage and determine the impact of fluid replacement to avoid fluid overload.

17. Community participation

17.1 Community participation is essential for the prevention and control of an outbreak of DF/DHF. The community must be encouraged to take steps to protect themselves from mosquitoes by eliminating mosquito breeding sites and taking personal measures such as use of bed nets, mosquito repellents etc. The co-operation of the community is also important during the periodic insecticide spray.

17.2 In pockets of high risk, active surveillance of DF/DHF should be encouraged so that first case(s) is (are) immediately reported to the local health authorities.

17.3 Co-ordinated efforts by government departments such as sanitation, urban development, education etc are essential so that risk factors for mosquito breeding can be reduced and other control measures taken up effectively.

17.4 In an event of an outbreak, the co-operation of other government departments will help to bring it more effectively under control. An inter-departmental committee for outbreak prevention and control should be constituted which should meet more periodically. Panchayat members, key community representatives and NGOs should be included as members of the committee. A meeting of the committee should be convened before the expected seasonal increase of water and vector borne diseases. In districts where risk factors exist, status of control measures for DF/DHF should also be assessed. The suggested areas of responsibilities of the various departments is given in **Annexure 4.**

Annex 1

Format for MPWs for reporting suspected DHF case

Village_____

District_____

Name_____

Age_____Sex_____

Address_____

Fever____Yes/No__

Date of onset_____

Headache__Yes/No__

Bleeding __Yes/No__

Cold extremities ____ Yes/No__

Unconsciousness ____Yes/No__

Recovered/still suffering/death with date_____

House : Kacha/Pacca

Mosquito breeding : Desert coolers/Over head tanks/discarded tyres/Buckets/Others

Signature
(Name and designation)

Date:

Annex 2

FORMAT FOR MEDICAL OFFICERS FOR REPORTING DHF CASE

District_____ Village_____
House No._____ Head of Family_____
Date of Survey_____

Particulars of Patient:

Name_____Age(Years)_____Sex: M/F
Father's Name_____ Date of onset of illness _____
Recovered / Still suffering / Died on_____
Date of investigation_____ Informant_____

Symptoms

Fever (more than 100 ° F): Yes / No
Headache : Yes / No
Haemorrhagic manifestations Yes/No
-Petechiae. Purpura, Ecchymosis
-Epistaxis, Gum bleeding
-Haematemasis and or melena
Tourniquet test: Positive/Negative
Enlarged liver: Yes / No
Shock: Yes / No
Other signs: _____

Similar illness in family/neighbourhood:
Name_____Age_____Sex_____Date of onset_____

Clinical laboratory investigations
Haematocrit (%)
Platelet count

Samples sent for confirmation of diagnosis and result if available
Acute sera sent: Yes/No, Date of collection:
Convalescent sera sent: Yes/No

Signature
Date: (Name and designation)

Name of PHC / Hospital

Annex 3

OUTBREAK INVESTIGATION REPORT

General Information

State :----------

District : ----------

PHC/Town :----------

Village/Ward :----------

Population :----------

Background Information

Person reporting the outbreak :----------

Date of report :----------

Date investigations started :----------

Person(s) investigating the outbreak :----------

Details of investigation

Describe how cases were found (may include (a) house to house search in the affected area; (b) visiting blocks adjacent to the affected area; © conducting record reviews at local hospitals; (d) requesting health workers to report similar cases in their areas etc.):

_____

_____

_____

_____

_____

Descriptive epidemiology

Cases by time, place and person (attach summary tables and relevant graphs and maps)

Age specific attack rates and mortality rates

High risk age groups and geographical areas

Prevalence and density of dengue vectors

Description of control measures

Description of measures for follow-up visits:

Brief description of problems encountered:

Factors which ,in your opinion , contributed to the outbreak

Conclusions and recommendations

Date: (Name and designation)

Annex 4

INTER-DEPARTMENTAL COMMITTEE SUGGESTED AREAS OF RESPONSIBILITY AND ACTION

District Administration

- mobilse resources by organising meetings with
  - concerned government department
  - non-governmental agencies
  - community leaders
- ensure vector control measures
- ensure adequate facilities for transportation of serious patients to district hospital if necessary
- ensure adequate supply of drugs and insecticides
- provide relevant information to the press
- monitor status of control activities

District Health Office/Municipal health office

- alert health personnel to report cases and to monitor trends
- arrange active surveillance in affected area
- arrange health educational camps and distribution of health educational material
- arrange vector control measures
- convene meeting under district administratror to seek co-operation of other government departments and NGOs
- alert hospitals for prompt management of cases

Concerned department (s) responsible for agriculture and sanitation

- ensure measures for reducing factors favouring the breeding of mosquitoes
- suppport measures for vector control
- report suspected cases of DF/DHF

Other government departments such as social welfare, education and NGOs, and Panchayat members, village pradhans, community leaders

- ensure measures for reducing factors favouring the breeding of mosquitoes
- support measures for vector control
- report suspected cases of DF/DHF
- arrange transportation of serious cases to hospital
- ensure community participation

National Institute of Communicable Diseases
22 Sham Nath Marg, Delhi- 110 054
Line List of Suspected Dengue Cases

03-Feb-97

Pat_ID	Hosp_ID	Hosp_N	Name	Age	Sex	Locality	Onset	AdmitDa	Tour	Plat_co	MP_	Type	Died
1	AIIMS	82288	Priyanka Shar	12	F	Malviya Nagar 9	22-Aug-96		Yes	Yes	No	DSS	Yes
2	AIIMS	471440	Bhura Singh	53	M	Sadiq Nagar 9	01-Sep-96		No	Yes	No	DHF	No
3	AIIMS	471866	Tina	9	F	Vinay Nagar 9	05-Sep-96		No	No	No	DHF	No
4	AIIMS	471879	Sachin	17	M	Pitampura	05-Sep-96		No	No	No	DHF	Yes
5	AIIMS	472402	Sanjay	12	M	Dakshinpuri	10-Sep-96		No	No	No	DHF	No
6	AIIMS	472458	Urvashi	11	F	Malviya Nagar 9	11-Sep-96		Yes	Yes	No	DHF	No
7	AIIMS	472424	Aparajita Khosl	11	F	Narela 6	11-Sep-96		No	No	No	DSS	Yes
8	AIIMS	472605	Galib Samuel	9	M	Ashram 10	12-Sep-96		No	No	No	DHF	No
9	AIIMS	120742	Sannah	3	F	Lajpat Nagar 10	12-Sep-96		No	No	No	DHF	No
10	AIIMS	472606	Rakesh Kumar	10	M	Ansari Nagar 9	12-Sep-96		No	No	No	DHF	No
12	SJH	94553	Arun Kumar	24	M	Mehrauli 9	21-Sep-96		No	No	No	DHF	No
15	SJH	146461	Resham Bhadu	30	M	Greater Kailash 9	21-Sep-96		No	No	No	DHF	No
16	SJH	146715	Bhog Singh	45	M	Inderpuri 3	21-Sep-96		No	Yes	No	DHF	No
17	SJH	146749	Lalita	13	M	Pragati Vihar 13	21-Sep-96		No	Yes	No	DHF	No
18	SJH	61522	Shagwani	14	F	Hasanpur —	21-Sep-96		No	No	No	DHF	No
19	SJH	9673	RamKishore	18	M	Badarpur 10	21-Sep-96		No	No	No	DHF	No
20	SJH	146947	Neeta	20	F	Sadiq Nagar 9	21-Sep-96		No	No	No	DHF	No
21	SJH	145985	Vikas Verma	20	M	Palam Colony 8	21-Sep-96		No	No	No	DHF	No
22	SJH	146829	Pinki	28	F	Najafgarh 8	21-Sep-96		No	No	No	DHF	No
23	SJH	146977	Somesh	20	M	Saraswati Vihar 7	21-Sep-96		No	No	No	DHF	No
24	SJH	147058	Vijay Kumar	22	M	Vasant Vihar 9	21-Sep-96		No	No	No	DHF	No
25	SJH	147114	Babu lal	36	M	Delhi Cantt. 14	21-Sep-96		Yes	No	No	DHF	No
26	SJH	147118	Suresh Kumar	30	M	Mayur Vihar 12	21-Sep-96		No	No	No	DHF	No
27	SJH	147121	Harbeshan	25	M	Mehrauli 9	21-Sep-96		No	No	No	DHF	No
28	SJH	61615	Sarvesh Kumar	17	M	Bareilly 15	21-Sep-96		No	No	No	DHF	No
29	SJH	147164	Reena Gupta	21	F	Pushp Vihar 7	21-Sep-96		No	No	No	DHF	No
30	SJH	147177	Subha	20	F		21-Sep-96		No	No	No	DHF	No
31	SJH	147193	Guddy	20	F	Pahar Ganj 2	21-Sep-96		No	Yes	No	DHF	No
32	SJH	147196	Guddu	22	M	farukabad U.P 15	21-Sep-96		No	No	No	DHF	No
33	SJH	60812	Neeraj	22	M	Kidwai Nagar 9	21-Sep-96		No	No	No	DHF	No
35	SJH	61015	Rehmat	22	M	New Delhi 10	21-Sep-96		No	Yes	No	DHF	No
36	SJH	66913	Atul	20	M	Uttam Nagar 8	21-Sep-96		No	No	No	DHF	No
37	SJH	61025	Raj Kumar	25	M	Gautam Nagar 9	21-Sep-96		No	No	No	DHF	No
38	SJH	61040	S.M. Yadav	42	M	Soami Nagar 9	21-Sep-96		No	No	No	DHF	No
39	SJH	60974	Rajinder	20	M	Aliganj 10	21-Sep-96		No	No	No	DHF	No
40	SJH	61024	Afttabuddin	12	M	Garhi 10	21-Sep-96		No	No	No	DHF	No
41	SJH	60975	Ram Wati	45	F	Govindpuri 10	21-Sep-96		No	No	No	DHF	No
42	SJH	C/145499	Rajeev	15	M	Hauz Khas 9	21-Sep-96		No	No	No	DHF	No
43	SJH	67800	Kudan	21	M	Okhla Estate 10	21-Sep-96		No	No	No	DHF	No
44	SJH	6099-	Satnam	40	M	Sarojini Nagar 13	21-Sep-96		No	No	No	DHF	No
45	SJH	61910	Sebawati	31	F	Sangam Vihar 10	21-Sep-96		No	No	No	DHF	No
46	SJH	61022	Kalawati	28	F	Lado Sarai 9	21-Sep-96		No	No	No	DHF	No
47	SJH	60824	Beddgiri	18	F	Old Faridabad 15	21-Sep-96		No	No	No	DHF	No
48	SJH	C/145032	Minucharan	25	M	Sangam Vihar 10	21-Sep-96		No	No	No	DHF	No
49	SJH	C/1452500	Tulocharan	40	M	Sangam Vihar 10	21-Sep-96		No	No	No	DHF	No
50	SJH	61011	Laxmi	65	F	Mohd.pur 9	21-Sep-96		No	No	No	DHF	Yes
51	SJH	61026	Naresh	23	M	Shahpur 9	21-Sep-96		No	No	No	DHF	No
52	SJH	61648	Gopal	14	M	Gautam Nagar 9	22-Sep-96		No	No	No	DHF	No
53	SJH	147269	Ved Raj	40	M	Neb Sarai 9	22-Sep-96		No	No	No	DHF	No
55	SJH	147318	Jai Kishan	24	M	Sewa Nagar 10	22-Sep-96		No	No	No	DHF	No
56	SJH	146365	Anesh	20	M	Gautam Nagar 9	22-Sep-96		No	No	No	DHF	No
57	SJH	147415	Narender	14	M	Masjid Moth 9	22-Sep-96		No	No	No	DHF	No

Pat_ID	Hosp_ID	Hosp_N	Name	Age	Sex	Locality	Onset	AdmitDa	Tour	Plat_co	MP_	Type	Died
58	SJH	147413	Kavita	26	F	R.K.Puram 9	22-Sep-96		No	No	No	DHF	No
59	SJH	147414	Sunita	16	F	Masjid Moth 9	22-Sep-96		No	No	No	DHF	No
60	SJH	147952	Sanjay	26	M	KaleKhan 9	22-Sep-96		No	No	No	DHF	No
61	SJH	95003	Rakesh	27	M	Sadiq Nagar 9	22-Sep-96		No	No	No	DHF	No
62	SJH	147647	Surender	22	M	Dakshinpuri 9	22-Sep-96		No	No	No	DHF	No
63	SJH	C/147799	Ram Bahudar	22	M	Chirag Delhi 10	22-Sep-96		No	No	No	DHF	No
64	SJH	C/47839	V.K. Upadhaya	25	M		22-Sep-96		No	No	No	DHF	No
65	SJH	C/143855	Qayam	13	M	Malviya Nagar 9	22-Sep-96		No	No	No	DHF	No
66		C/14785	Narean Singh	25	M	Hauz Khas 9	22-Sep-96		No	No	No	DHF	No
67	SJH	C/147879	Rajvir	25	M		22-Sep-96		No	No	No	DHf	No
68	SJH	C/147866	Ravi	22	M	Ashram 10	22-Sep-96		No	No	No	DHF	No
69	SJH	C/147893	K.C.Barge	42	M	East Kidwai Nag 9	22-Sep-96		No	No	No	DHF	No
70	SJH	C/147959	Daya Kishan	20	M	New Mubarakpur 10	22-Sep-96		No	No	No	DHF	No
71	SJH	61081	Kali Parsad	18	M	Sarojini Nagar 9	21-Sep-96		No	No	No	DHf	No
72	SJH	61150	Mahavir	0	M	Faridabad 15	21-Sep-96		No	No	No	DHF	No
73	SJH	145815	Arun	30	M	Mehrauli 9	21-Sep-96		No	No	No	DHF	No
74	SJH	61148	Madhu	25	F	Hauz Khas 9	21-Sep-96		No	No	No	DHF	No
75	SJH	C/145913	Ram Parsad	0	M	Lajpat Nagar 10	21-Sep-96		No	No	No	DHF	No
76	SJH	61258	Dayaram	0	M	Nehru Place 10	21-Sep-96		No	No	No	DHF	No
77	SJH	146014	Pravesh	15	M	Gandhi Nagar 12	21-Sep-96		No	No	No	DHF	No
78	SJH	146213	Batul	45	F	Prem Nagar 8	21-Sep-96		No	No	No	DHF	No
79	SJH	146275	Brij Mohan	24	M	Ghaziabad 15	21-Sep-96		No	No	No	DHF	No
80	SJH	61322	Ashok Dass	25	M	Okhla Estate 10	21-Sep-96		No	Yes	No	DHF	No
81	SJH	67035	Nadeem	10	M	Savitri Nagar 9	19-Sep-96		No	No	No	DHf	No
82	AIIMS	473350	Gunjari	20	F	Jaipur 15	20-Sep-96		No	No	No	DHF	No
83	AIIMS	473566	Prapti	8	F	Ansari Nagar 9	20-Sep-96		No	No	No	DHF	No
84	AIIMS	473503	Gaurav	3	F	Kotla Mubarakp 10	20-Sep-96		No	No	No	DHF	No
85	AIIMS	476492	Rinku	8	M	Trilokpuri 12	20-Sep-96		No	No	No	DHF	No
86	AIIMS	473456	Baby Arushi	2	F	A.V. Nagar 9	20-Sep-96		No	No	No	DHF	No
87	AIIMS	121264	Shahid	3	M	Sangam Vihar 10	20-Sep-96		No	No	No	DHF	No
88	AIIMS	473991	Vishvanath	24	M	C.R.Park 9	24-Sep-96		No	No	No	DHF	No
89	AIIMS	473187	Chandan Wadh	14	M	Malviya Nagar 9	24-Sep-96		No	No	No	DHF	No
90	AIIMS	473981	Manisha Rajan	19	F	Masjid Moth 9	24-Sep-96		No	No	No	DHF	No
91	AIIMS	473976	Reetu	7	F	Masjid Moth 9	24-Sep-96		No	No	No	DHF	No
92	AIIMS	473975	Shital Kumar	7	F	2	24-Sep-96		No	No	No	DHF	No
93	AIIMS	473915	Felix Thomas	26	F	Ansari Nagar 9	24-Sep-96		No	No	No	DHF	No
94	AIIMS	473967	Waqi Khan	19	M	Bara Tuti 2	24-Sep-96		No	No	No	DHF	No
95	AIIMS	473866	Jyoti	15	F	Chirag Delhi 10	24-Sep-96		No	No	No	DHF	No
96	AIIMS	471231	Jose	1	M	Sadiq Nagar 9	30-Aug-96		No	No	No	DHF	No
97	AIIMS		Saumya	4	F	Pahar Ganj 2	11-Sep-96		No	No	No	DHF	No
98	AIIMS	121170	Subha	4	F	Laxmi Nagar 12	18-Sep-96		No	No	No	DHF	No
99	AIIMS	473312	Sapna	6	F	Nehru Place 10	18-Sep-96		No	No	No	DHF	No
100	DDU	31303	Harish	25	M	Bankanar Vill. 15	23-Sep-96		No	No	No	DHF	No
101	AIIMS	473308	Umesh Kumar	40	M	Subji Mandi 9	18-Sep-96		No	No	No	DHf	No
102	AIIMS	473219	Roop Kishore	24	M	Ghaziabad 15	18-Sep-96		No	No	No	DHF	No
103	AIIMS	473214	Poonam	11	F	Greater Kailash 9	18-Sep-96		No	No	No	DHF	No
104	AIIMS	473232	Dr. Nisha	27	F	Masjid Moth 9	18-Sep-96		No	No	No	DHF	No
105	GANGA	29838	Bushra	8	M	Bara Hindu Rao 2	13-Sep-96		No	No	No	DHF	No
106	GANGA	30019	Kapiko A. Mahr	12	M	Gole Mkt. 13	15-Sep-96		No	No	No	DHF	No
107	GANGA	29834	Jai Veer Khura	5	M	Greater Kailash 9	13-Sep-96		No	No	No	DHF	No
108	GANGA	29532	Sarika Bhagat	12	F	Rani Bagh 7	11-Sep-96		No	No	No	DHF	No
109	GANGA	29801	Master Arora	5	M	Gujaranwala To 5	13-Sep-96		No	No	No	DHF	No
110	AIIMS	473660	Ranjan Jena	26	M	2	21-Sep-96		No	No	No	DHF	No
111	AIIMS	473610	Rajesh	6	M	Hari Nagar 4	21-Sep-96		No	No	No	DHF	No
112	AIIMS	473605	Sachdeva	4	M	A.V. Nagar 9	21-Sep-96		No	No	No	DHF	No
113	AIIMS	SA-121455	Harima	6	F	Jangpura 10	21-Sep-96		No	No	No	DHF	No
114	AIIMS	SA_121449	Khalid	7	M	2	21-Sep-96		No	No	No	DHF	No

Pat_ID	Hosp_ID	Hosp_N	Name	Age	Sex	Locality	Onset	AdmitDa	Tour	Plat_co	MP_	Type	Died
115	AIIMS	SA-121444	Tanika Gupta	3	F	Safdarjang Encl. 9		21-Sep-96	No	No	No	DHF	No
116	AIIMS	SA-121441	Lalit	12	F	Gole Mkt. 13		21-Sep-96	No	No	No	DHF	No
117	AIIMS	SA-121427	Rajani	7	F	Tagore Garden 4		21-Sep-96	No	No	No	DHF	No
118	AIIMS	473654	Rajesh Luthra	25	M	Sriniwas puri 9		21-Sep-96	No	No	No	DHF	No
119	AIIMS	473613	Sumit	1	M	Kotla Mubarakp 10		21-Sep-96	No	No	No	DHF	No
122	AIIMS	473711	Jasdeep Singh	10	M	A.V. Nagar 9		22-Sep-96	No	No	No	DHF	No
124	AIIMS	473702	Roni	5	F	Ansari Nagar 9		22-Sep-96	No	No	No	DHF	Yes
126	AIIMS	121475	Jenin	7	M	Masjid Moth 9		22-Sep-96	No	No	No	DHF	No
127	AIIMS	121472	Gaurav	1	M	Ansari Nagar 9		22-Sep-96	No	No	No	DHF	No
128	AIIMS	121470	Puneet Sharma	9	M	A.V. Nagar 9		22-Sep-96	No	No	No	DHF	No
129	AIIMS	121461	Deepak	8	M	A..V.Nagar 9		22-Sep-96	No	No	No	DHF	No
130	AIIMS	121459	Anuj	5	M	Moti Bagh 9		22-Sep-96	No	No	No	DHF	No
131	AIIMS	121457	Konika	7	F	H.M. Pur 15		22-Sep-96	No	No	No	DHF	No
132	AIIMS	121456	Preeti	6	F	Trilokpuri 12		22-Sep-96	No	No	No	DHF	No
133	AIIMS	121453	Taranjeet	5	F	Jangpura 10		22-Sep-96	No	No	No	DHF	No
134	AIIMS	473548	Deepak	36	M	Jaipur 15		23-Sep-96	No	No	No	DHF	Yes
135	SJH	61968	Girish	60	M	Noida 15		18-Sep-96	No	No	No	DHF	No
136	SJH		Panchsheel	14	M	Baba Colony		16-Sep-96	No	No	No	DHF	No
137	SJH	C/148075	R.N. Singh	50	M	Nanak Pura 9		18-Sep-96	No	No	No	DHF	No
138	SJH	C/95378	Om Parkash	25	M	Dakshinpuri 9		16-Sep-96	No	No	No	DHF	No
139	SJH	C/95319	Ajay	25	M	Soami Nagar 9		15-Sep-96	No	No	No	DHF	No
140	SJH	148236	Harish	27	M	Lajpat Nagar 10		19-Sep-96	No	No	No	DHF	No
141	SJH	146991	Govinda	25	M	Vasant Vihar 9		20-Sep-96	No	No	No	DHF	No
142	SJH	62053	Jeet Bahadur	30	M	Vasant Vihar 9		20-Sep-96	No	No	No	DHF	No
143	SJH	62077	Rajeev	20	M	Indira Park 4		20-Sep-96	No	No	No	DHF	No
144	SJH	C/148752	Ramurisina	20	M	Badarpur 10		16-Sep-96	No	No	No	DHF	No
145	SJH	C/148414	Subhash	21	M	Faridabad 15		18-Sep-96	No	No	No	DHF	No
146	SJH	C/148376	Sunita	13	F			18-Sep-96	No	No	No	DHF	No
147	SJH	62017	Snam Akina	9	F	Hauzrani 9		22-Sep-96	No	No	No	DHF	No
148	SJH	61977	Ajahid	7	M	Hauzrani 9		20-Sep-96	No	No	No	DHF	No
149	SJH	62159	A.B. Singh	7	M	Kotla Mubarakp 10		22-Sep-96	No	No	No	DHF	No
150	SJH	62171	A.K.Shan	8	M	Lodhi Raod 10		19-Sep-96	No	No	No	DHF	No
151	AIIMS	473862	Jenam	7	M	Masjid Moth 9		23-Sep-96	No	No	No	DHF	No
152	AIIMS	4773860	Dr. Rajesh Vas	27	M	Ansari Nagar 9		23-Sep-96	No	No	No	DHF	No
154	AIIMS	473854	Rupal	6	F	S.J.Enclave 9		23-Sep-96	No	No	No	DHF	No
155	AIIMS	473854	Dr.Janya Devis	20	F	Chattarpur 9		23-Sep-96	No	No	No	DHF	No
157	AIIMS	473891	Dr.Sridhar	30	M	Masjid Moth 9		23-Sep-96	No	No	No	DHF	No
158	AIIMs	473759	Girish	8	M	Khanapur 9		23-Sep-96	No	No	No	DHF	No
159	AIIMS	473724	Sanjay	20	M	Trilokpuri 12		23-Sep-96	No	No	No	DHF	No
160	AIIMS	473718	Mani Nath Jha	23	M	Chandnichowk 1		23-Sep-96	No	No	No	DHF	No
161	AIIMS	121358	Anuj Pukhral	11	M	Pushp Vihar 9		23-Sep-96	No	No	No	DHF	No
162	AIIMS	472639	G. Jyoti	1	F	New Delhi 10		13-Sep-96	No	No	No	DSS	Yes
163	AIIMS	472674	Garima	11	F	Alaknanda 9		13-Sep-96	No	No	No	DHF	No
164	AIIMS	120918	Simran	1	F	Malviya Nagar 9		14-Sep-96	No	No	No	DHF	No
165	AIIMS	120915	Ravi	1	M	2		14-Sep-96	No	No	No	DHF	No
166	AIIMS	120914	Vijender	12	M	Ansari Nagar 9		14-Sep-96	No	No	No	DHF	No
167	AIIMS	120913	Paul Mathew	1	M	A.V. Nagar 9		14-Sep-96	No	No	No	DHF	No
168	AIIMS	120907	Sonu	7	M	Safdarjang Hos 9		14-Sep-96	No	No	No	DHF	No
169	AIIMS	120905	Annu	3	F	Uttam Nagar 8		14-Sep-96	No	No	No	DHF	No
170	AIIMS	472861	Teena	9	F	A.V. Nagar 9		14-Sep-96	No	No	No	DHF	No
171	AIIMS	120929	Akash	1	M	Madangir 9		15-Sep-96	No	No	No	DHF	No
172	AIIMS	120922	Shreeni	7	F	A.V. Nagar 9		15-Sep-96	No	No	No	DHF	No
173	AIIMS	120921	Dhurv Suri	11	M	New Friends Col 10		15-Sep-96	No	No	No	DHF	No
174	AIIMS	472898	B.Rachel	1	F	Lajwanti Garden 4		15-Sep-96	No	No	No	DHF	No
175	AIIMS	472859	Ajay	11	M	Dabri Extn. 8		14-Sep-96	No	No	No	DHF	Yes
176	AIIMS	473049	Dr.J.Singh	26	M	Masjid Moth 9		19-Sep-96	No	No	No	DHF	No
177	AIIMS	473049	Mona	4	F	Saurav Vihar 10		16-Sep-96	No	No	No	DHF	No

Pat_ID	Hosp_ID	Hosp_N	Name	Age	Sex	Locality	Onset	AdmitDa	Tour	Plat_co	MP_	Type	Died
178	AIIMS	473037	Bhola	3	M	Kotla Mubarakp	10	16-Sep-96	No	No	No	DHF	No
179	AIIMS	473017	Shristhi	1	F	A.V. Nagar	9	16-Sep-96	No	No	No	DHF	No
180	AIIMS	472994	Sonia	6	F	V.K.Dutt Colony	—	16-Sep-96	No	No	No	DHF	No
181	AIIMS	472968	Bijender	12	F	Ansari Nagar	9	16-Sep-96	No	No	No	DHF	No
182	AIIMS	472961	Akash	1	M	Ambedkar Naga	9	16-Sep-96	No	No	No	DHF	No
183	AIIMS	472945	Mohd. Asim	7	M	Ambedkar Naga	9	16-Sep-96	No	No	No	DHF	No
184	AIIMS	120991	Vikram	7	M	Gautam Nagar	9	16-Sep-96	No	No	No	DHF	No
186	AIIMS	121067	Rajni	7	F	Tagore garden	4	17-Sep-96	No	No	No	DHF	No
187	AIIMS	121056	Monica	10	F	Masjid Moth	9	17-Sep-96	No	No	No	DHF	No
188	AIIMS	121051	Deepak	5	M	Dabri Extn.	8	17-Sep-96	No	No	No	DHF	No
189	AIIMS	120992	Sunil Kumar	11	M	Lodhi Road	10	17-Sep-96	No	No	No	DHF	No
190	AIIMS	473163	Indu	11	F	Rewari, Haryan	15	17-Sep-96	No	No	No	DSS	Yes
191	AIIMS	473092	Neeraj	4	M	Ansari Nagar	9	17-Sep-96	No	No	No	DHF	No
192	AIIMS	473023	Phillip	9	F	A.V. Nagar	9	17-Sep-96	No	No	No	DHF	No
193	AIIMS	473070	Simmi	10	F	Mayur Vihar	12	17-Sep-96	No	No	No	DHF	No
194	AIIMS	473063	Master Arun	7	M	Sangam Vihar	10	17-Sep-96	No	No	No	DHF	No
195	AIIMS	473056	Ravinder	25	M	Ghaziabad	15	17-Sep-96	No	No	No	DHF	No
196	RML		Amir Lohar	0	M	Pandav Nagar	3	26-Sep-96	No	No	No	DHF	No
197	RML		Harpreet Kaur	0	F	Tilak Nagar	4	26-Sep-96	No	No	No	DHF	No
198	RML		AmitaKumar	13	F	Shakarpur	12	26-Sep-96	No	No	No	DHF	No
199	RML		Y.P Kumar	0	M	Lawrence Road	7	26-Sep-96	No	No	No	DHF	No
200	RML		Sunil		M	Sultanpuri	8	26-Sep-96	No	No	No	DHF	No
201	RML		S. Dey	0	M	Kalibari, New D	13	26-Sep-96	No	No	No	DHF	No
202	RML		Bhupesh	8	M	Uttam Nagar	8	26-Sep-96	No	No	No	DHF	No
203	AIIMS	C-2/ICU-I	Dr.Sakat Agga	24	M	Ansari Nagar	9	23-Sep-96	No	No	No	DHf	No
204	AIIMS	C-2/ICU	Dr.Rajan Jena	26	M	Masjid Moth	9	23-Sep-96	No	No	No	DHF	No
205	AIIMS	C-2/17	Jagdeep Singh	16	M	A.V. Nagar	9	23-Sep-96	No	No	No	DHF	No
206	AIIMS	D-2/9	Manju	21	F	Ansari Nagar	9	23-Sep-96	No	No	No	DHF	No
207	AIIMS	D-2/11	Dinesh Mehra	14	M	Faridabad	15	23-Sep-96	No	No	No	DHF	No
212	AIIMS	C-5/24	Taranjit	5	F	Jangpura	10	23-Sep-96	No	No	No	DHF	No
213	AIIMS	D-5/ICU	Jariam Jhon	7	M	Masjid Moth	9	23-Sep-96	No	No	No	DHF	No
214	AIIMS	D-5/ICU	Poonam	11	F	Greater Kailash	9	23-Sep-96	No	No	No	DHF	No
217	AIIMS	D-5/37	Master Deepak	6	M	Dabri Extn	8	23-Sep-96	No	No	No	DHF	No
220	AIIMS	D-5/40	Geatash	9	M	A.V. Nagar	9	23-Sep-96	No	No	No	DHF	No
221	AIIMS	D-5/41	Master Bhola	3	M	2		23-Sep-96	No	No	No	DHF	No
225	AIIMS	AB-6/22	Chitra Sharma	55	F	A.V. Nagar	9	23-Sep-96	No	No	No	DHF	No
227	AIIMS	AB-6/29	Roop Kishore	24	M	Mayur Vihar	12	23-Sep-96	No	No	No	DHF	No
228	AIIMS	D-6/28	Amit	10	M	Madangiri	9	23-Sep-96	No	No	No	DHF	No
229	AIIMS	Pvt-108	Dr. B.N. Sharm	30	M	A.V. Nagar	9	23-Sep-96	No	No	No	DHF	No
230	AIIMS	Pvt-503A	Jenny Andrew	12	F	A.V. Nagar	9	23-Sep-96	No	No	No	DHF	No
231	AIIMS	Pvt.506	Nisha	27	F	Masjid Moth	9	23-Sep-96	No	No	No	DHF	No
232	AIIMS	473710	Neelam	7	F	Hauz Khas	9	22-Sep-96	No	No	No	DSS	Yes
233	AIIMS	473689	Jagdish	19	M	Saidul Ajaib Villl	15	22-Sep-96	No	No	No	DHF	Yes
234	GANGA	301775	Ankita Verma	11	F	Old Rajinder Na	3	21-Sep-96	No	No	No	DHF	No
235	SJH	62253	Manoj	11	M	Savitri Nagar	9	24-Sep-96	No	No	No	DHF	No
236	SJH	62278	Lalu	1	M	Sangam Vihar	10	24-Sep-96	No	No	No	DHF	No
237	SJH	62286	Dinesh	5	M	S.J. Enclave	9	24-Sep-96	No	No	No	DHF	No
238	SJH	62297	Nitin	12	M	Sadiq Nagar	9	24-Sep-96	No	No	No	DHF	No
239	SJH	62342	Sandeep	9	M	Bhagwan Nagar	11	24-Sep-96	No	No	No	DHF	No
240	SJH	62364	Pratish	7	M	Nanak Pura	9	24-Sep-96	No	No	No	DHF	No
241	SJH	62368	Parul	9	M	Sarojini Nagar	13	24-Sep-96	No	No	No	DSS	No
242	SJH	62385	Farkan	7	M	Hauz rani	9	24-Sep-96	No	No	No	DHF	No
243	SJH	62409	Anju	9	F	Masjid Moth	9	24-Sep-96	No	No	No	DSS	No
244	SJH	62421	Hukam Singh	8	M	IIT-Campus	9	24-Sep-96	No	No	No	DSS	No
245	SJH	62429	Bhupender	5	M	Kotla Mubarakp	9	24-Sep-96	No	No	No	DSS	No
246	SJH	62493	Sagar	5	M	Nehru Stadium	10	24-Sep-96	No	No	No	DHF	No
247	SJH	62200	Rekha	25	F	Madanpur	9	24-Sep-96	No	No	No	DHF	Yes

Pat_ID	Hosp_ID	Hosp_N	Name	Age	Sex	Locality	Onset	AdmitDa	Tour	Plat_co	MP_	Type	Died
248	SJH		Rajesh	12	M	Kamal Cinema 3	24-Sep-96		No	No	No	DHF	No
249	SJH	62229	Parminder	18	M	Laxmi Bai Naga 9	24-Sep-96		No	No	No	DHF	No
250	SJH	149124	Guphan	15	M	Janakpuri 8	24-Sep-96		No	No	No	DHF	No
251	SJH	148749	Vicky	25	M	Sangam Vihar 10	24-Sep-96		No	No	No	DHF	No
252	SJH	149129	B.P.Tyagi	30	M	Basai Dara Pur 4	24-Sep-96		No	No	No	DHF	No
253	SJH	149172	Yudhishtar	21	M	Katwaria Sarai 9	24-Sep-96		No	No	No	DHF	No
254	SJH	62499	Pintoo	12	M	Basant Vihar 9	24-Sep-96		No	No	No	DHF	No
255	SJH	149567	Akal	18	M	Lajpat Nagar 10	25-Sep-96		No	No	No	DHF	No
256	SJH	149261	Gablu	20	M	Faridabad 15	25-Sep-96		No	No	No	DHF	No
257	DDU	81630	Vineeta	11	F	Raghuvir Nagar 4	26-Sep-96		No	No	No	DHF	No
258	KWS	12076	Megha	8	F	Tilak Nagar 4	19-Sep-96		No	Yes	No	DSS	No
259	SJH	96240	Dhishankon	21	M	Kotla Mubarakp 10	25-Sep-96		No	No	No	DHF	No
260	SJH	96263	Rajinder	27	M	Aliganj 10	25-Sep-96		No	No	No	DHF	No
261	SJH	148690	Anita	21	F	R.K.Puram 9	25-Sep-96		No	No	No	DHF	No
262	SJH	149621	Shiv Kishan	36	M	Gautam Nagar 9	25-Sep-96		No	No	No	DHF	No
263	SJH	149691	Vijay	32	M	Satyam Vihar	25-Sep-96		No	No	No	DHF	No
264	SJH	149755	Madhav	20	M	Madakani Encla	25-Sep-96		No	No	No	DHF	No
265	SJH	149521	Vinod Kumar	28	M	Haryana 15	25-Sep-96		No	No	No	DHF	No
266	SJH	149665	Vinod Kumar	17	M	Ashram 10	25-Sep-96		No	No	No	DHF	No
267	SJH	1492864	A.K.Singh	27	M	Lodhi Road 10	25-Sep-96		No	No	No	DHF	No
268	SJH	149837	Shiv Prasad	29	M		25-Sep-96		No	No	No	DHF	No
269	SJH	62610	Laxmi	19	F	Badarpur 10	25-Sep-96		No	No	No	DHf	No
270	SJH	149885	Reena Malhotr	14	F	Old Govindpuri 10	25-Sep-96		No	No	No	DHF	No
271	SJH	148985	Mangeram	16	M	Zamrudpur 9	25-Sep-96		No	No	No	DHF	No
272	STH	223554	Parvej	6	M	Industrial Area	11-Sep-96		No	No	No	DHF	No
273	STH	1222798	Konika	9	F	Loni,UP 15	07-Sep-96		No	No	No	DHF	No
274	SJH	61542	Paramjeet	11	F	Dakshinpuri 9.	21-Sep-96		No	No	No	DHF	No
275	SJH	61491	Deepak	3	M	Sadiq Nagar 9	21-Sep-96		No	No	No	DHF	No
276	SJH	61489	Puneet	12	M	Laxmi Bai Naga 9	21-Sep-96		No	No	No	DHF	No
277	SJH	61451	Geeta	8	F	Sangam Vihar 10	21-Sep-96		No	No	No	DHF	No
278	SJH	61458	Shivam	6	M	Sarojini Nagar 10	21-Sep-96		No	No	No	DHF	No
279	STH		Sanny	8	M	Sadar Bazar 2	26-Sep-96		No	No	No	DSS	Yes
280	LNJP	964815	Pinky	3	F	Ashok Nagar 4	26-Sep-96		No	No	No	DHF	No
281	LNJP	964748	Vivek	2	M	Sidhartha Basti,	26-Sep-96		No	No	No	DHF	No
282	LNJP	964798	Rahul	10	M	Gandhi nagar,D 12	26-Sep-96		No	No	No	DHF	No
283	LNJP	964809	Anu	6	F	Tagore Road 4	26-Sep-96		No	No	No	DHF	No
284	LNJP	964864	Deepak	4	M	Delhi Gate 1	26-Sep-96		No	No	No	DHF	No
285	LNJP	964942	Annu	2	F	LKR park	27-Sep-96		No	No	No	DHF	No
286	LNJP	964919	Kapil	10	F	Delhi Gate 1	27-Sep-96		No	No	No	DHF	No
287	LNJP	964252	Manish	6	M	Narayana Gaon 3	26-Sep-96		No	No	No	DHF	No
288	LNJP	963872	Rakhee	8	F	Vinod Nagar 12	23-Sep-96		No	No	No	DHF	Yes
290	SUNDER	5325	Rohit	9	M	Dabri village 8	11-Sep-96		No	No	No	DHF	No
291	SUNDER	5478	Nihar	5	M	Ashok Vihar 7	18-Sep-96		No	No	No	DHF	No
292	SUNDER	5514	Nishant Gujral	12	M	Madhipur, Ronla 7	20-Sep-96		No	No	No	DHF	No
293	SUNDER	5542	Baby Asha	6	F	Ashok Vihar 7	21-Sep-96		No	No	No	DHF	No
294	SUNDER	5554	Baby Gurjeet	7	F	Majlis Park 5	21-Sep-96		No	No	No	DHF	No
295	SUNDER	5636	Neeraj	8	M	Ashok Vihar 7	25-Sep-96		No	No	No	DHF	No
296	AIIMS	474355	Kanchan	10	F	Sewa Nagar 10	27-Sep-96		No	No	No	DSS	No
297	AIIMS	474346	Manju	12	F	Seelam Pur 11	27-Sep-96		No	No	No	DHF	No
298	AIIMS	474339	Pragya	6	F	Saket 9	27-Sep-96		No	No	No	DHF	No
299	AIIMS	474315	Vasim	12	M	Seelampur 11	27-Sep-96		No	No	No	DHF	No
300	AIIMS	474311	Vidhisha	9	F	Vivek Vihar 12	27-Sep-96		No	No	No	DHF	No
301	AIIMS	474308	Gopal Sharma	21	M	Gaziabad, U.P 15	27-Sep-96		No	No	No	DHF	No
302	AIIMS	474286	Neeraj	9	M	N.Delhi 10	27-Sep-96		No	No	No	DHF	No
303	AIIMS	474284	Manju	11	F	Tuglakabad 10	27-Sep-96		No	No	No	DHF	No
304	AIIMS	474262	B.Pooja	5	F	Sarojini Nagar 9	27-Sep-96		No	No	No	DHF	No
305	AIIMS	474253	ijay Kumar	30	M	Shakarpur 12	27-Sep-96		No	No	No	DHF	No

Dr. Meera D Menon DIS: 14-91
Bangalore.

Model Surveillance Districts
Action Plan

Objectives:

1. Develop and strengthen surveillance activities to promote early detection of outbreaks and appropriate field action for the prevention and control of outbreaks.
2. Strengthen laboratory support for confirmation of diagnosis from clinical specimens and for monitoring of environmental samples.
3. Improve clinical management of reportable and epidemic prone diseases.
4. Strengthen basic entomological support services as relevent to surveillance and detecting early warning signals
5. Establish data base of reportable diseases and previous outbreaks.
6. Institute an exclusive network of electronic communications for rapid transmission of information
7. Ensure community participation in prevention and control of outbreaks.

Methodology:

Strengthen and utilise existing manpower, laboratory and health infrastructural facilities through:

1. Training
2. Modernisation of office and laboratory equipment
3. Strengthening of linkages for disease surveillance from peripheral to central levels
4. Networking with state/regional and national institutions through electronic means of communication.
5. IEC activities.

Training:

1. Categories to be trained:-

   a) Rapid response team at state level (state level officer designated by state govt as nodal officer for disease surveillance, one epidemiologist/public health specialist, one microbiologist, one entomologist, one clinician and one statistician)
   b) District rapid response team (same at (a) at district level). In the absence of entomologist at District level, either epidemiologist or microbiologist may be trained for same.
   c) PHC medical officers (app. 25)
   d) MPWs (male and female) (app. 150)
   e) Lab personnel (app. 40)
   f) Clinicians at state level - 25

2. Except for training of the Rapid Response teams at NICD, other training will be at state/district level. State rapid response team will act as trainers for training other medical & paramedical personnel at state/district level. NICD faculty will participate in the training programmes at state/district levels.

Training Material:

(a) Modules to be used:

   ⇒ principles of outbreak prevention and control
   ⇒ case definitions and differential diagnosis of reportable and epidemic prone diseases
   ⇒ clinical management, guidelines for referral
   ⇒ laboratory tests at PHC & district levels, collection, storage & transportation of samples to state/regional/national lab availability of media and reagents
   ⇒ guidelines for entomological surveillance
   ⇒ data collection, analysis, channels for reporting, line listing, nil reporting, computer software
   ⇒ practical drills for outbreak investigations & control

(b) Training material will be drafted and printed by NICD

Laboratory strengthening

1. Checklist for documentation of available facilities and identification of gaps at PHC and district levels
2. Visit to facilities at district level/selected CHCs & PHCs by NICD faculty/state officers
3. Training in laboratory techniques by NICD faculty at district level
4. Supply of essential equipment and supplies by NICD
5. Issue of guidelines for collection and transportation of samples for which testing facilities are not available at district level.
6. Networking with state level and NICD through fax & E-mail by district level

Clinical Management

1. Issue guidelines for case definition, differential diagnosis and clinical management
2. Train selected medical officers
3. Networking with state level/NICD through fax & E-mail by district level

Data base

1. Establish computer facilities with appropriate software and fax
2. Use relevant formats for feedback and reports
3. Prepare annual reports including investigation and follow up of early warning signs of outbreaks.

Community participation

1. Use monthly visits to each village by FHWs for immunization sessions to convey appropriate seasonal messages on outbreak prevention and to collect information of unusual events
2. Hold *open house* meeting on fixed day & time every month at PHC/CHC/district level which private practitioners/members of the public could attend. Topic for discussion could be seasonally appropriate
3. Rapid response and investigation of untoward/unusual events reported by the public
4. Involvement of school teachers and students by organising meetings and preparation of relevant health education material
5. Involvement of ICDS as above
6. Involvement of NGOs

Other Government Department

1. Inter-department committee at state/district level at senior level. Meetings to be held regularly at pre-determined frequency of 2/3 months under the chairmanship of Director of Health Services and Chief Medical Officer respectively.
2. Feedback on important events/potential outbreaks to be provided by state/district health authorities as appropriate to members of the above committee.

Supply of essential equipment, supplies & reagents

1. Computer facilities at state and district levels
2. Fax at state & district levels
3. Telephone facilities at CHC and PHC levels by State Govts
4. Lab equipment as appropriate
5. Contingencies for procurement of supplies and reagents as appropriate
6. Contingency funds for sample collection/transportation etc.

Monitoring & Evaluation

Overall monitoring will be the responsibility of NICD and it will be done in cooperation with State health authorities. Year ending evaluation shall be performed by an independent group which will be coordinated by NICD. A monthly newsletter shall be published by NICD to give feedback to various units regarding activities of model districts.

DIS 14.12

ISSUES RELATED TO THE LAUNCHING OF A DISEASE SURVEILLANCE PROGRAMME

- **TECHNICAL**
- **ADMINISTRATIVE**
- **FINANCIAL**

TECHNICAL ISSUES:

1. Prioritisation of diseases to be put under surveillance
2. Surveillance type for each disease
3. Case definitions
4. Format for collection of information/report
5. Flow of information
6. Frequency of reporting
7. Analysis & interpretation of data and action
8. Monitoring for completeness & regularity

9. Laboratory support

- Identification of laboratories
- Supply of materials for sample collection
- Formats for sending samples
- Sample transportation
- Laboratory supplies
- Laboratory reporting

10. Rapid Response Team

- Constituent
- Job responsibilities
- Mobility & other supports
- Mechanism of activities

11. Feedback mechanism

12. Contingency plan for outbreak control

13. Periodic publication of surveillance data/reports

14. Periodic evaluation mechanism

15. Trained and motivated manpower development

ANALYSIS & INTERPRETATION OF DATA AND ACTIONS AT DIFFERENT LEVELS

Analysis (of variables):

* Number of cases & deaths
* Sex ratio
* Time of occurrence
* Place of occurrence
* Incidence
* Attack rate (age specific)
* Seasonal variation
* Case fatality rate
* % of samples found +ve
* % of sensitivity to antibiotics
* % of serogroups/types

Interpretation:

* Endemicity (same/less/more)
* Clustering of cases/deaths
* Increased mortality
* Impending outbreak situation

Action:

* Regular reporting sending
* Giving feedback
* Alerting for outbreak
* Outbreak investigation

29 September, 1997

RRT Training Course:

1000-1115	Role of lab. Infrastructure required, tests to be performed, networking etc:
	Dr Rajesh Bhatia
1130-1300	Diagnosis of cholera, LA, HbsAg, & meningitis, biosafety
	Dr Shashi Khare
1400-1530	Water bacteriology
	Dr KV Chandersekharan
1600-1730	Microscopic examination plague, TCoCon- TB
	Dr Sunil Gupta

NATIONAL DISEASE SURVEILLANCE PROGRAMME

TRAINING OF STATE LEVEL OFFICERS
(22.9.97-3.10.97)

Group-II. NICD Seminar Room

Facilitator: 1. Dr A C Dhariwal
2. Dr B K Sainanee
3. Dr Jagvir Singh
4. Dr D Bora

Participants: 1. Dr Mahesh Bora, SNO, Rajasthan
2. Dr Santhamma Joseph, SNO, Kerala
3. Dr Kamal Chand Singhal, Physician
4. Dr Niti Talsania, P & SM
5. Dr Meera D. Meundi, Micro – Bangalore
6. Dr S.K. Ghosh, Entomology
7. Shri Inder Singh, Statistician
8. Dr Sanjay Mallik, Medical Spl.