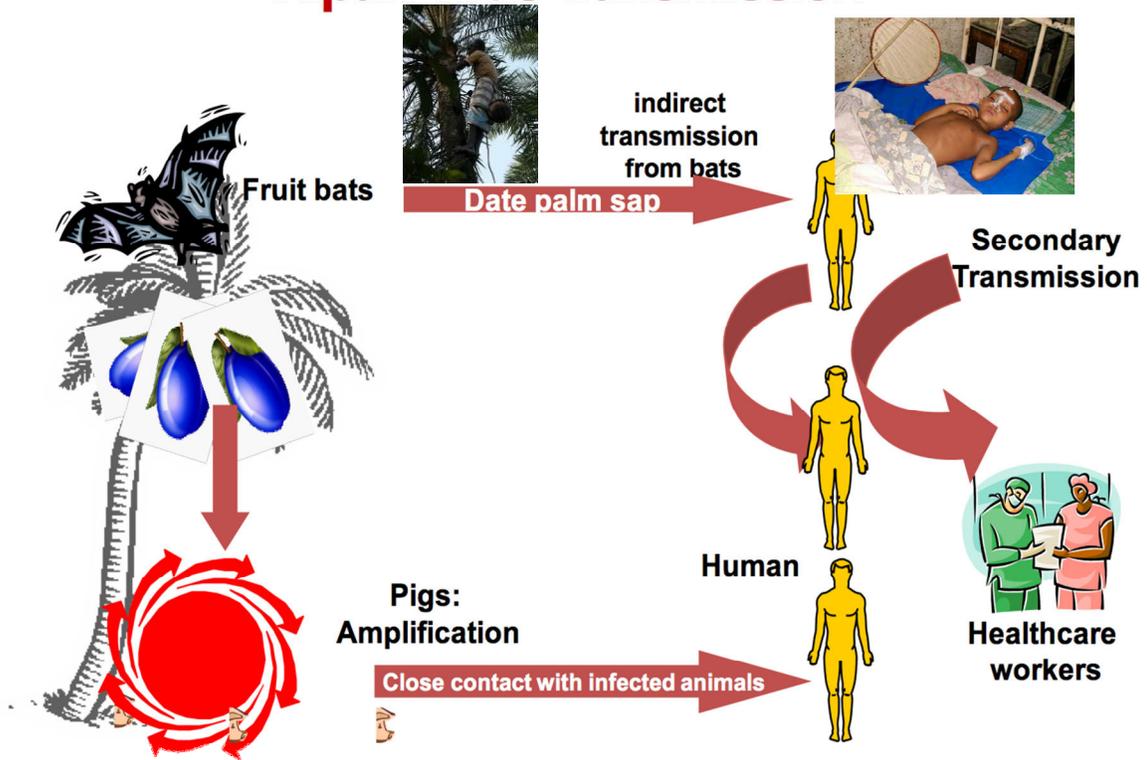


Surveillance, Prevention and Control of Nipah Virus Infection: A Practical Handbook

Nipah virus transmission



Acronyms and abbreviation

AES	acute encephalitis syndrome
ALRI	acute lower respiratory infection
BSL	biosafety level
CSF	cerebrospinal fluid
ELISA	enzyme-linked immunosorbent assay
HCW	health-care worker
IATA	International Air Transport Association
ICU	Intensive Care Unit
IgG	immunoglobulin G
IgM	immunoglobulin M
IHR	International Health Regulations
JE	Japanese encephalitis
NiV	Nipah virus
PCR	polymerase chain reaction
PPE	personal protective equipment
RNA	ribonucleic acid
RRT	Rapid Response Team

RT-PCR Reverse transcription polymerase chain reaction

SOP standard operating procedure

WHO World Health Organization

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PREFACE

Nipah virus encephalitis is an emerging zoonotic disease of public health significance that is caused by a highly pathogenic paramyxovirus. Nipah virus infection is an example of an emerging disease that appeared recently and recurrent outbreaks have been reported in some countries. In addition to being a serious public health problem, past Nipah virus outbreaks have shown that the disease can have strong detrimental economic effects. This is dependent upon the involvement of animal reservoirs, mode of transmission and human behaviour in the affected countries.

Although human cases have been reported from only a few countries (Bangladesh, India, Malaysia and Singapore), fruit bats, the main reservoir of Nipah virus, are present in many other countries of the South-East Asia Region. Nosocomial transmission, disruption of the health-care system and community disorder are well-known features of these outbreaks in affected countries. Acute public health events cannot be easily prevented due to various factors such as increased human-animal interaction, sociocultural behaviour and food habits. However, the negative public health impact of Nipah virus can be mitigated through appropriate public health interventions.

Although it is an emerging disease, recurrent outbreaks are still limited to some countries, and the status of other countries is unknown. There is neither a specific treatment nor a vaccine for the prevention of Nipah virus infection. It has therefore become imperative to develop guidelines based on the limited experience in

outbreak investigation, laboratory diagnosis and case management of patients in health facilities. Experts engaged in outbreak investigation, laboratory diagnosis and case management of Nipah virus infection in the Region were brought together by the WHO Regional Office for South-East Asia to develop and finalize a practical handbook on surveillance, prevention and control of Nipah virus infection. This publication is the end result of that exercise, and is intended to assist field epidemiologists, clinicians, public health professionals and laboratory professionals in early detection and isolation of human cases and prevention of further human-to-human transmission. Establishing appropriate surveillance systems and strengthening existing systems will be necessary in both affected and non-affected countries.

The publication of these guidelines is relevant, as emerging infectious diseases such as Ebola virus disease and Middle-East respiratory syndrome coronavirus are known to be of bat origin, and surveillance, prevention and control measures are similar. I hope that these guidelines will be helpful to Member States to establish and strengthen appropriate surveillance systems so that Nipah virus outbreaks can be detected quickly and appropriate control measures can be initiated.

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The draft of the Practical Handbook on Surveillance, Prevention and Control of Nipah Virus Infection was developed by a team lead by Dr Gyanendra Nath Gongal, Scientist, International Health and Regulations unit under WHO Regional Office for South-East Asia in consultation with lead writers for each chapter. The lead writers were chosen because of their expertise in the field and their willingness to undertake the work. Literature searches were conducted by the writing teams and lists of references were added. All the chapters were discussed in detail at an informal expert consultation in Bangkok in June 2011.

This handbook would not have been possible without the support of the following experts who have practical experience of many years of working in outbreak investigation, surveillance, laboratory diagnosis, case management and control of Nipah virus infection.

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It was decided to revise the handbook in the context of emerging infectious diseases such as Crimean-Congo haemorrhagic fever and the potential threat of Middle East respiratory syndrome and Ebola virus disease so that it would be useful for epidemic-prone diseases. The document was peer-reviewed by Dr Sirenda Vong and Dr Anuj Sharma.

PURPOSE OF HANDBOOK

The purpose of this handbook is to help health personnel and health authorities at all levels to:

- update current knowledge about Nipah virus infection;
- provide a standard case definition for surveillance and outbreak investigation of Nipah virus infection;
- provide comprehensive information on the epidemiology and techniques for diagnosis, case management, prevention and control of Nipah virus infection;
- provide guidance for outbreak investigation of Nipah virus infection based on past experiences; and
- strengthen the capacity of emergency response to outbreaks.

This handbook is intended as a technical support to field epidemiologists, clinicians, public health professionals and laboratory professionals. Nipah virus epidemiology and risk factors may differ from country to country, which will influence the nature of an outbreak investigation. These factors must be taken into consideration. These guidelines may be modified appropriately and utilized for the development of manuals for targeted groups by national health authorities. They should have the approval of appropriate authorities, conform to national policies and meet local needs.

Animal health concerns exist but these are not covered in this handbook. Health authorities should liaise with animal health and wildlife authorities to carry out Nipah virus surveillance in bats and domestic animals and develop national contingency plans accordingly.

1. INTRODUCTION

1.1. Background

Nipah virus (NiV) infection is a zoonosis that was first recognized in a large outbreak of 265 suspected cases and 105 deaths (40% case fatality) in peninsular Malaysia from September 1998 through April 1999 (1). Patients were diagnosed primarily with acute encephalitis after contact with sick pigs infected with NiV. The outbreak was initially thought to be due to Japanese encephalitis, but the causative agent was later identified as NiV. This outbreak caused widespread panic and fear in Malaysia leading to considerable social disruption and tremendous economic loss due to the mass culling of over one million pigs. In addition, 11 abattoir workers in Singapore developed NiV infection during March 1999 following close contact with pigs imported from Malaysia (2). However, no new outbreaks have been reported from these countries since May 1999.

Bangladesh and India have experienced outbreaks of NiV infection since 2001. Although the outbreaks in Bangladesh have been smaller, the case-fatality rates have been repeatedly higher (~75%) than those from the initial outbreak in Malaysia and Singapore (40%). However, the clinical case definition used in Bangladesh differs from that used during the Malaysia outbreak and focuses on severe neurologic signs and symptoms (1,3,4).

Consumption of raw date palm sap contaminated by flying bats was the primary source of human NiV infection in the Bangladesh outbreaks (3,4). Strong evidence indicative of human-to-human transmission of NiV was found in Siliguri, India, in 2001 and in Bangladesh in 2004 (3,4). The role of pigs or other domestic animals in disease transmission in Bangladesh is unknown and subject to further investigation.

1.2. Infectious agent

NiV is named after the Malaysian village where it was first discovered (5). The virus is a highly pathogenic paramyxovirus and one of two virus species in the genus *Henipavirus*, the other being Hendra virus (6).

Sequence analysis of virus isolated from clinical samples obtained from persons affected by the outbreaks in Bangladesh and India were closely related compared with that in Malaysia, while indicating large nucleotide heterogeneity (3,7,8).

2. EPIDEMIOLOGY

2.1. Introduction

NiV is highly pathogenic in humans, with bats acting as a reservoir. In Malaysia, the virus was believed to be introduced into pig farms by fruit bats and spread easily among pigs; it was transmitted to humans who came in close contact with infected

animals (9). NiV antibodies were detected in dogs, cats, goats and horses; however, only in those that were exposed to infected pigs (10).

Outbreaks of NiV infection in South Asia have a strong seasonal pattern and a limited geographical range. All the outbreaks occurred during the months of winter and spring (December–May). This could be associated with several factors such as breeding season of the bats, increased shedding of virus by bats and the date palm sap harvesting season.

The evidence of seasonal preference of transmission in *Pteropus lylei* bats was recently demonstrated in a study in Thailand (11). The period April–June (peaking in May) was the peak time when viral ribonucleic acid (RNA) could be detected in bat urine. This was associated with a fluctuation of population numbers that was observed only in May and correlated with young bats leaving the nest to fly.

The NiV cases tend to occur in a cluster or as an outbreak, although 18% of the cases in Bangladesh were isolated cases (4).

There is strong evidence that the emergence of bat-related viral infections communicable to humans and animals is associated with the loss of natural habitat of bats (12).

2.2. Reservoir

Fruit bats (or also known as flying foxes) of the genus *Pteropus*, family Pteropidae, have been identified as a natural reservoir host of NiV(13). A seroprevalence study in Malaysia implicated four fruit bat species: *Pteropus hypomelanus*, *P. vampyrus*, *Cynopterus brachyotis* and *Eonycteris spelaea* (13). Subsequently, NiV was isolated from the urine of a free-living colony of *P. hyomelanus* in Malaysia (14).

The world distribution of *Pteropus* fruit bats extends from the west Indian Ocean islands of Mauritius, Madagascar and Comoros, through Pakistan, South and South-East Asia, to the Philippines, Indonesia, the Pacific Islands and Australia (15). These bats are migratory and known to travel over considerable distances within the Asia-Pacific Region. Evidence of NiV and/or their markers of infection has been reported in fruit bats in Bangladesh (16), India (17), Cambodia (18), China (56), Thailand (19), Indonesia and Timor-Leste (20, 55). The status of NiV infection in bats in other countries of the South-East Asia Region is not known.

2.3. Mode of transmission and incubation period

Infected bats shed the virus in their urine and potentially from other excretions and secretions such as faeces, saliva, urine and birthing fluids, but as host reservoir, they are asymptomatic carriers. NiV is highly contagious among pigs and is spread by infected droplets. Pigs acquire NiV and act as an intermediate and possibly amplifying host after contact with infected bats or their secretions. The mode of transmission of NiV infection can be direct as

well as indirect. In Malaysia, it is thought that a rapid spread through intensively reared pigs and between farms may be due to fomites, that is, carrying the virus on clothing, equipment, boots, vehicles, etc. (48).

2.3.1. Direct transmission

2.3.1.1. Pig-to-human transmission

Direct human contact with infected pigs was identified as the predominant mode of transmission in humans when it was first recognized in Malaysia (21). In the 1998–1999 outbreaks, 93% of infected people were pig farmers or abattoir workers.

2.3.1.2. Human-to-human transmission

There is circumstantial evidence of human-to-human transmission in India in 2001. During the outbreak in Siliguri, 33 health workers and hospital visitors became ill after exposure to patients hospitalized with NiV illness, suggesting nosocomial infection (3). Subsequent person-to-person transmission occurred from close physical contact, especially with body fluids. Strong evidence indicative of human-to-human transmission of NiV was also found in Bangladesh in 2004 (4).

The virus is readily identified in the saliva, urine of infected patients and in cerebrospinal fluid (CSF) of encephalitic patients (1,8). Respiratory secretions appear to be particularly important for person-to-person transmission of NiV

(52). This underlines the importance of infection control precautions in a hospital setting and among family caregivers.

2.3.1.3. Bat-to-human transmission

During the Bangladesh outbreak, the virus is suggested to have been transmitted either directly or indirectly from infected bats to humans.

2.3.2. Indirect transmission

Drinking of fresh date palm sap, possibly contaminated by fruit bats (*P. giganteus*), may have been responsible for the transmission of NiV to humans in Bangladesh (22). The consumption of date palm sap is popular in a number of countries in South-East Asia including Bangladesh, India, Indonesia and Thailand as well as in Malaysia and Philippines. Fruit bats also consume date palm sap and can contaminate it with saliva, urine and faeces. This is the means by which NiV is thought to have been transmitted from infected fruit bats to humans (23).

2.3.3. Incubation period

The incubation period is from 4 to 18 days. In Bangladesh, the median incubation period was 8 days (24).

The period and duration of infectiousness of NiV infection are not known.

2.4. NiV in the South-East Asia Region

Bangladesh and India have reported human cases of NiV infection. Indonesia, Thailand and Timor-Leste have identified antibodies against NiV in the bat population and the source of the virus has been identified (13,19, 55).

The first identification of NiV virus as a cause of an outbreak of encephalitis in the South-East Asia Region was reported in 2001 in the Meherpur district of Bangladesh (25). Since then, outbreaks of NiV encephalitis have been reported almost every year in Bangladesh, mostly from the western and north-western regions.

India reported outbreaks of NiV encephalitis in West Bengal State, in 2001 and 2007 (3), with 71 cases and 54 deaths. During January and February 2001, an outbreak of febrile illness associated with neurological features was observed in Siliguri, West Bengal (26). A second outbreak was reported in 2007 in the Nadia district of West Bengal. Thirty cases of fever with acute respiratory distress and/or neurological symptoms were reported, and five cases were fatal. The geographical distribution of NiV outbreaks in Bangladesh and India between 2001 and 2014 is shown in Fig. 1.

Fig.1. Geographical distribution of outbreaks of NiV infection in Bangladesh and India, 2001–2014



3. Epidemiological (disease) surveillance

Epidemiological surveillance is the ongoing systematic collection, recording, analysis, interpretation and dissemination of data reflecting the current status of the community or population so that action may be taken to prevent or control a disease. In case of a rare

disease with an epidemic potential (like NiV infection), surveillance can use both case-based (hospital-based) and event-based (community-based) surveillance systems.

3.1. Objectives

The main objective of NiV disease surveillance is to rapidly detect and potentially forecast epidemic activity. The objectives of surveillance that are most applicable to NiV infection include:

- detection of outbreaks quickly for control interventions;
- monitoring trends in the distribution and spread of the disease, over time and geographically;
- facilitating planning and resource allocation on the basis of lessons learnt from interventions and their impact;
- evaluation of the effectiveness of prevention and control measures.

3.2. Setting up a surveillance system

Ideally, surveillance of NiV infection in humans should be performed in countries where there has been an outbreak or evidence of infection in bats. Most importantly, the system must ensure that surveillance is linked to further investigation and response and the ability to control or mitigate the epidemics. The major attributes for such surveillance include sensitivity of detection and timeliness of reporting.

Effective surveillance must include laboratory support. Suspected patients should be tested using both serological and molecular techniques, i.e., enzyme-linked

immunosorbent assay (ELISA) and/or reverse transcription polymerase chain reaction (RT-PCR). Quality control for laboratory diagnostics should be provided by national or international reference laboratories. At a minimum, diagnostic laboratories should be able to perform immunoglobulin (Ig) M antibody ELISA as a frontline screening test.

Such laboratory testing for NiV should be done as soon as possible. If laboratory testing for NiV is not available at country level, appropriate arrangements should be made for transportation of samples to a facility capable of conducting the testing abroad.

3.2.1. Event-based surveillance

Cases of NiV infection commonly occur in clusters. Therefore, event-based surveillance is crucial to identify clusters of acute encephalitis syndrome (AES) or acute lower respiratory infection (ALRI) cases of unknown aetiology, which may help in early detection of NiV outbreaks. The definition of acute AES and pneumonia cases and a cluster are given in Appendices 1 and 2, respectively.

Clusters of cases can be identified in the community or in a health-care facility.

3.2.2. Case-based (hospital-based) surveillance

The key component of the AES surveillance system is the tertiary and district/provincial hospitals with laboratory capacity. Clinicians in selected hospitals should be trained in case detection, cluster identification and reporting. One to two

medical officers should be specifically designated for surveillance in each hospital depending on the number of hospital beds.

3.2.3. Surveillance in bats

Surveillance of NiV in bat reservoirs by ELISA and/or RT-PCR in countries where no outbreak has been reported can provide important information about virus circulation and facilitate further strategic planning for control and prevention of NiV outbreaks in humans.

3.3. Standard case definitions

The cases are defined for clinical case identification or for surveillance activities. The suggested standard case definitions for Nipah cases are as follows:

1. Suspected (clinical) Nipah case: Patient with an epidemiological link or from a community affected by an outbreak who has:

- i) fever with acute onset of altered mental status or seizure and/or
- ii) fever with headache and/or
- iii) fever with acute onset of cough with shortness of breath.

2. Probable Nipah case: Suspect cases, and/or who died before complete diagnostic specimens could be collected, including a serum antibody test 14 days after onset of illness.

3. Confirmed Nipah case: Suspected/probable case having, in addition, laboratory confirmation of NiV infection either by:

- i) IgM antibody against NiV identified in serum or CSF, or
- ii) NiV RNA identified by RT-PCR from respiratory secretions, urine or cerebrospinal fluid, or
- iii) isolation of NiV from respiratory secretions, urine or cerebrospinal fluid or other tissue specimens.

4. OUTBREAK OR EMERGENCY PREPAREDNESS AND RESPONSE FOR NiV

4.1. Preparedness

Preparedness in terms of technical and logistical management of a Nipah outbreak is essential in countries with recurrent outbreaks. The best response to a Nipah outbreak is being able to detect cases as early as possible and prevent further infections.

4.1.1. Enhancing surveillance during the NiV transmission season

Surveillance should be intensified during the Nipah season from January through May, when most Nipah outbreaks have been identified. This will increase the possibility of identifying NiV infection and understanding the characteristics of the

virus. Blood, CSF, urine and throat swabs are collected from suspected patients and sent to the reference laboratories.

4.1.2. Awareness building in hospitals and raising community awareness

- Encourage and train health-care workers to maintain standard infection control precautions, e.g., personal hygiene, use of personal protective equipment (PPE), and manage encephalitis or neurological patients appropriately.
- Disseminate information to communities through multimedia, leaflets, posters and meetings (group, community and market) encouraging people:
 - to stop consumption of raw date palm sap;
 - not to eat fruit partially eaten by bats;
 - cover the mouth and nose while caring for unconscious patients;
 - wash hands with soap and water before and after feeding and taking care of patients.

4.1.3. Infection control in health-care settings should be in place

- Implement standard infection control precautions.
- Acquire and maintain PPE stock and other equipment needed in epidemiological investigations and outbreak response.

4.1.4. Planning for outbreak response: some major components

4.1.4.1. Formation of a multisectoral team

Since NiV infection is a zoonosis and outbreaks may be associated with multiple factors such as animal reservoirs, sociocultural practices, food habits and possible human-to-human transmission, a multidisciplinary team is needed, and preparation should be done for pre-outbreak, outbreak and post-outbreak phases.

A multisectoral team should be built up at national and local levels for the monitoring, evaluation and response to unusual acute public health events and outbreak response, including Nipah outbreaks. The team should have a holistic, multidisciplinary approach consisting of public health personnel, clinicians and laboratory personnel. The multisectoral team may consist of the following professionals (depending on the evolving and country-specific situation) who would bring relevant expertise in outbreak investigation and response:

- epidemiologist
- microbiologist
- anthropologist and/or social scientist
- veterinarian
- ecologist.

National or subnational level – Rapid Response Team (NRRT): The NRRT should be assigned from institutes at the national/provincial level and partner institutes.

District/provincial level – District Rapid Response Team (DRRT): The DRRT consists of the head of health services at the district/provincial level and clinical and laboratory expertise, and other expertise from the public health department.

4.1.4.2. Evaluate and ensure the supplies for sample collection, storage and shipment of samples:

- Assess PPE in stock;
- Assess sample collection instruments;
- Assess sample storage capacity in the laboratory;
- Evaluate laboratory capacity for NiV testing (e.g., biosafety, quality, skills, human resources and consumables for NiV virus testing);
- Evaluate hospital capacities for isolation facilities and ability to treat Nipah patients in Nipah-prone areas.

4.2. Alert and outbreak investigation

The outbreak investigation should lead to formulation of an appropriate public health intervention as soon as the source and mode of transmission are known. In the meantime, control measures mitigating known risk factors should be implemented as soon as NiV transmission is suspected.

4.2.1. Investigation of a suspected case or cluster of suspect cases:

4.2.1.1. Standard Operating Procedures (SOPs) for sample collection and transportation in place:

- Surveillance physician will take verbal consent from patient or patient's family member;
- Collect 5 ml venous blood;
- If possible, collect 3 ml extra-CSF when appropriate;
- Aliquot 1 ml serum and 1 ml CSF samples in 1.8 ml cryovial tube. Try to aliquot serum and CSF samples in three cryovial tubes;
- Label the cryovial tube with: type of samples (serum/CSF), patient name and identification number, and date of sample collection;
- Store the serum and CSF samples in liquid nitrogen if possible, or -20°C freezer for short-term storage if liquid nitrogen is not available;
- Ship samples in liquid nitrogen tank or ice pack to assigned centre for laboratory diagnosis;
- Store samples in -70°C freezer for longer-term storage;
- A list of potential national or international reference laboratories should be pre-established. There can be several for different purposes: a frontline laboratory would be the WHO Collaborating Centre for laboratory diagnosis of viral diseases with BSL 3 or BSL 4 facilities (see list of WHO Collaborating Centres and other institutions for laboratory diagnosis, surveillance and response in Appendix 4).

4.2.1.2. Templates of data collection instruments pre-developed and in place for quick use

These templates should include the following:

- line listing of all cases;
- case reporting form;
- questionnaire for case-control studies or other relevant studies;
- forms for sample collection.

4.2.1.3. SOP for activating and conducting outbreak investigation teams

This SOP is commonly country-specific as the process relies on the administrative structures and capacity or resources of a given country. Therefore a country-based manual or protocol for outbreak investigations should be in place in at-risk countries for Nipah outbreaks. A more generalized national SOP manual for all emerging or re-emerging infectious diseases of international concern could be developed focusing on a mechanism of response and roles and responsibilities of different parties.

The following are some of the key components to prepare a team for outbreak investigation:

1) National or Subnational Rapid Response Team (RRT)

Should an outbreak of NiV virus disease be suspected and/or reported, the National RRT should be activated and should meet together to:

(1) Plan and conduct the investigation;

(2) Request further technical support if needed (e.g., further analysis and interpretation, risk communication, initiate control).

2) Administrative SOP for field work in place: administrative clearance, organize supplies, travel arrangements:

- approval/permission from competent authority;
- arrangement for accommodation;
- arrangement for security, if needed;
- arrange vehicle;
- supplies:
 - medicines
 - sample collection instruments
 - PPE
 - disinfectants, hand sanitizer
 - basic medical and investigation equipment, e.g., stethoscope, thermometer, GPS instrument, etc.

3) SOP for rapid mobilization of additional or experts teams

If the NiV outbreak is confirmed, an experienced Nipah outbreak investigation team comprising an epidemiologist, clinician, veterinarian and anthropologist or social scientist can move to the field within 24 hours of outbreak reporting.

4.2. 1.4. Nipah outbreak investigation

The overall objective of investigating Nipah outbreaks is to control the outbreak and prevent future outbreaks. Any Nipah (or suspicion of) outbreaks should be investigated as the disease is of public health concern with potentially devastating consequences.

The specific objectives include the following:

- to determine the extent of the outbreak;
- to characterize the populations at greatest risk and to identify specific risk factors;
- to provide practical recommendations to strengthen control and prevention measures.

Key steps when conducting Nipah outbreak investigation

Step 1: Activate preparation plan for outbreak investigation (details above).

Step 2: Confirm the outbreak.

One of the first tasks of the initial investigation team is to verify that a suspected cluster of cases is indeed a real outbreak with common cause. Some will be unrelated cases of the same disease, and others will turn out to be real cases of AES or ALRI but of unrelated diseases. This step consists of confirming the diagnosis through visiting the outbreak affected areas to (1) examine the patients and/or review the medical charts to describe and understand the clinical presentation; (2) collect blood, CSF and throat

swab samples at the time of admission/ first contact, and follow-up serum samples 2 weeks after the onset of illness for testing.

A Nipah outbreak is defined as the identification of at least one laboratory-confirmed case.

Step 3: Define and identify cases.

The investigators should develop or adapt standardized case definitions appropriate to the outbreak context (see details in standard case definitions). Testing for NiV infection should be performed when there are: (i) clusters of AES due to an unknown agent or (ii) patients with AES due to an unknown agent living in or near NiV zones.

Patients with AES should also be tested for NiV infection when they are exposed to a cluster of unexplained neurological/pulmonary illness in animals, such as horses and pigs.

Step 4: Case-finding

In many outbreaks, including Nipah outbreaks, the first cases that are recognized are usually a small proportion of the total number. Retrospective and prospective case-findings are crucial to determine the true magnitude and geographical extent of the outbreak.

Active case-finding should be conducted:

Among close contacts:

- A close contact is defined as “a patient or the person who came in contact with a Nipah case (confirmed or probable cases) AND stayed in the room or veranda or vehicle for at least 15 minutes”.
- Record contacts for potential follow-up if need be. They are to be followed up in case of occurrence of illness (up to 18 days). Serum specimens should be collected in case of symptom onset
 - in high-risk groups or in groups exposed to the source
 - through enhancing surveillance in the outbreak area and the at-risk areas for case-finding in the community

Step 5: Evaluate the outbreak in relation to ‘time, place and person’

- establish a line-list of current and previous cases;
- draw an epidemic curve;
- analyse and interpret the data to identify potential sources of transmission.

Step 6: Develop and evaluate hypotheses

Once step 5 has been done, investigators should have some hypotheses regarding the source and/or mode of transmission and the exposures that caused the disease. These hypotheses should be compared with established facts.

Step 7: Refine hypotheses and carry out additional studies

If step 6 is not conclusive, these hypotheses can be refined to look for new modes or vehicles of transmission and be evaluated through conducting case–control studies.

Step 8: Implement control and prevention measures (see response section below)

Step 9: Communicate findings and information about risks (i.e., outbreak report)

- Develop an outbreak report and disseminate to concerned authorities.
- Learning from the outbreak includes detailing:
 - new findings
 - major limitations during outbreak investigation
- Resume the activities of pre-outbreak phase.

4.3. Additional considerations with respect to Nipah outbreaks

When the Nipah outbreak is confirmed, the investigation team needs to:

- Immediately inform the local, regional and national authorities.
- Inform the partners/stakeholders (notably those involved at local level): treating hospitals, patients' relatives.
- Declare the Nipah outbreak to WHO under the International Health Regulation 2005 (IHR) via **National IHR focal points** (see detail below in the response section).

Notification and assessment of Nipah outbreak and/or cases to WHO should be based on the following four criteria described in Annex II of IHR 2005. A "yes" to any of the four criteria would lead to notifying WHO under Article 6 of the IHR.

- Is the public health impact of the Nipah outbreak and/or cases serious?
- Are the Nipah outbreak and/or cases unusual or unexpected?
- Is there a significant risk of international spread?
- Is there a significant risk of international travel or trade restrictions?

4.3.1. Conduct rapid risk assessment

Some of the major risk assessment questions should include the following:

- What is the risk of occurrence of further cases from the detected outbreak?
- What is the risk of spread of the infection?
- What is the risk of major impact of the current outbreak on the health-care system?

4.3.2. Evaluate the impact of control measures

Each outbreak should be thoroughly investigated, and lessons learnt from each outbreak should be evaluated and documented so that control measures can be reviewed and modified as required.

4.3. 3. Develop further research with the objective of identifying determinants of infection or severity and determining modes and dynamics of infection

The populations to be investigated would be those exposed to NiV:

4.3.3.1. Health-care workers (HCWs)

There is evidence of nosocomial transmission in India and Bangladesh, and one nurse was positive to Nipah IgM antibody in Malaysia (3, 4). HCWs are to be trained for infection control and prevention (see below). Surveillance should be in place to detect

any suspected cases among HCWs. In addition, a study should be conducted to identify asymptomatic cases among HCWs who provided service to Nipah patients. Among these, positive cases should be subsequently compared with negative ones to determine risk factors for infection and understand the dynamics of transmission. Some components of the study could include:

- Make a list of HCWs who provided care to Nipah patients.
- Take consent from HCWs.
- Interview at-risk HCWs using an exposure questionnaire, about 3 weeks after the last exposure to NiV-infected patients.
- Collect 5 ml of blood for serology testing about 3 weeks after the last exposure to NiV-infected patients.

4.3.3.2. Communities potentially exposed to NiV

The investigation should encourage involvement of multidisciplinary and multisectoral team using a one-health approach. For instance, investigators should have the support of microbiologists and their laboratories to conduct community-based seroprevalence surveys (detection of recent antibody response) to determine the extent of the outbreak via detecting subclinical and/or asymptomatic cases. Asymptomatic cases could be further compared with controls to identify risk factors for infection.

Anthropologists or other social scientists with extensive community-based experience could help propose additional behaviour risk factors to be tested in a case–control study. Anthropologists should work with communication/health promotion specialists to

develop communication messages combining both local explanatory models and biomedical models using local terms and languages, and deliver the message in such a way that it is meaningful to the community.

Veterinarians and eco-health specialists should join the investigation to conduct studies collecting specimens from animals and the environment in the outbreak settings. Zoonotic and environmental investigations during an NiV outbreak primarily aim to determine the primary reservoir, likely source of the virus, route of transmission and the extent of the spread of the virus in animals. Georeferenced positive specimens could be analysed with positive human cases to better understand the dynamics of transmission.

4.4. Response

As soon as a Nipah outbreak is confirmed, national authorities should implement control measures based on known risk factors. The interventions should be based on a multisectoral approach and include/understand the following strategic objectives:

1. Establishment of a coordination committee for outbreak prevention, and control activities and resources mobilization; the role of this committee is to ensure the general coordination of operations. It must clearly define the responsibilities of the various teams and the route of information during outbreak response operations.

2. Setting up partnerships with the media to ensure media monitoring and better risk communication.
3. Formation of a referral system with the principal objective of easing transfer of cases to the appropriate case-management health-care settings.
 - a. Active detection for new Nipah cases and their transfer to the case-management ward.
 - b. Follow up all contacts during 18 days after their last unprotected exposure to Nipah patient(s) or infected animal or tissue (e.g., laboratory) and their transfer to the case-management ward if they fall sick.
4. Set up a social mobilization and medical education programme whose principal role is to inform the public and promote practices that decrease community transmission of the disease.
5. At the foci zone, the medical team should ensure safe case management of Nipah patients by complying with the following guidelines:
 - a. Respect patients and their families' dignity and rights, in particular their right for information on disease and treatment,
 - b. Set up a specific Nipah case-management ward that ensures biosafety of in-patient care,
 - c. Set up infection prevention and control measures for safe patient care,
 - d. Organize the safe transport of patients from their residence to the ward,
 - e. The express consent of patients is necessary for any hospitalization. In the event of patient's refusal to be hospitalized, the medical team should organize, temporarily, a patient's care at home with his/her family support.

- f. Organize safe burials while respecting the funeral ceremony,
 - g. Set up psychosocial support (patients, family, HCWs).
6. Outside the foci zone, to prevent secondary foci, the medical team should reinforce standard infection prevention and control measures in health care in all health centres of the affected district and all hospitals catering to the outbreak zone.
7. Establishment of links with the animal health sector to:
- a. Continue monitoring the cause of disease and death in domestic animals and wildlife.
 - b. Test samples and alert public health authorities as needed.
 - c. Control slaughtering/butchering activities of domestic animals and wildlife, at home, and in markets and slaughterhouses.
8. Media and communication
- a. Designate a spokesperson in the outbreak team.
 - b. Designate a spokesperson at the national level who communicates with national media.
 - c. Regularly update reports to be sent to assigned authority.
 - d. Conduct regular meetings with press and community.
 - e. Distribute information, education and communication material.

4.5. In the aftermath of the outbreak (evaluation)

4.5.1. Declare the end of the outbreak

The health ministry declares the end of the outbreak. The date of outbreak end is equal to twice the mean incubation period for Nipah counted from the last infectious contact with a confirmed or probable case.

The national authorities should use the announcement of the end of the outbreak to acknowledge national and international field teams as well as the media. They should also formally present their solidarity and their empathy to the victims, their families and the affected populations.

4.5.2. Writing a final report of the outbreak control activities

The report objective is to describe the activities undertaken during the epidemic as well as constraints and difficulties encountered. It should include technical aspects (final epidemiological analysis, clinical investigations, etc.), as well as administrative and financial aspects. The report should be published to achieve wider dissemination of findings and lessons learnt.

4.5.3. Archive outbreak documents and files

- Gather all the reports, files, photographs, videos and other documents related to the outbreak management.
- Store all the documents in a place accessible for their later use.

4.5.4. Evaluate the management of the outbreak

The evaluation of the management of the outbreak response will review the performance of the various components of the strategy: coordination, relationship to the media, surveillance system, social mobilization programme, clinical management and logistics.

The aim of the evaluation is to determine lessons learnt to improve the future management of epidemics. This evaluation should be led by a team comprising national and technical partners.

4.5.5. To resume activities of the pre-outbreak period

See Section 3: Epidemiological surveillance.

5. CLINICAL MANIFESTATIONS

5.1. Clinical signs and symptoms

NiV infection has been found to present in the following forms:

1. Asymptomatic and subclinical infection
2. Classical form of acute Nipah encephalitis
3. Relapsed encephalitis
4. Late-onset encephalitis

5.1.1. Asymptomatic and subclinical infections

Asymptomatic infection was reported in 8% of patients with laboratory-confirmed cases in Malaysia (28); however, there was no evidence of asymptomatic NiV infection in Bangladesh and India during outbreaks, although cases of mild and nonspecific features in the illness were identified (28). Subclinical and mild diseases were identified in Bangladesh and India; these mild symptoms were nonspecific, indistinguishable from flu-like symptoms and included slightly raised temperature, malaise and body aches (9, 21). However, information is limited to indicate the actual proportion of infection that falls in this category.

5.1.2. Classical presentation of NiV infection:

The classical form is an acute and rapidly progressive encephalitis with or without respiratory involvement in all age groups. The Nipah encephalitis presents with 3–14 days of fever and headache, followed by drowsiness, disorientation and mental confusion. The acute encephalitis progresses then to coma within 24–48 hours, with high mortality rate.

The respiratory involvement consists of non-productive cough during the early part of the disease, which can evolve later to severe acute lower respiratory disease, i.e., from breathing difficulties to acute respiratory distress syndrome (ARDS) with chest radiographs of some patients showing diffuse bilateral opacities covering the majority of lung fields, consistent with ARDS. These neurological and respiratory features suggest involvement of the brain stem (21).

The clinical presentation of NiV infection in Bangladesh and India differed from that in Malaysia and Singapore. In Bangladesh, severe respiratory disease was more common, with 62% of cases having cough and 69% developing severe respiratory symptoms. In the outbreak in Siliguri, India, respiratory symptoms were reported in 51% of patients. By contrast, in Malaysia, 14% of patients had a non-productive cough on presentation; only 6% of chest radiographs were abnormal and these abnormalities were mild and focal. However, in the Malaysia–Singapore outbreak, 2 out of 11 patients among abattoir workers in Singapore presented with pneumonia without encephalitis (21).

The other important difference in clinical manifestation is that the dramatic, persistent, segmental myoclonus seen in 32–54% of patients in the Malaysian outbreak was not observed in the patients from Bangladesh and India, even though generalized hyporeflexia, common in Malaysian patients, was also noted in the Bangladesh outbreaks (53).

The case fatality rate was higher in Bangladesh at 73% compared with 39% from Malaysia, but this difference could be explained by more sophisticated clinical care provided in Malaysia. Authors suggest that variation in viral genome and the relative lack of implementation of infection control practices may explain the predominance of respiratory involvement, and the importance of human-to-human spread in the Bangladesh and Indian outbreaks compared with that of Malaysia and Singapore (52).

Post-encephalitis sequelae have been commonly observed in NiV infection. One third of Nipah survivors in Bangladesh have moderate to severe objective neurological dysfunction 7–30 months after infection (52).

5.1.3. Relapsed encephalitis: A case is considered to be relapsed encephalitis if the neurological symptoms recur after recovery from encephalitis.

Relapsed encephalitis with acute onset of fever, headache, seizures and focal neurological signs occurring months to years after recovery from the initial acute encephalitis is another characteristic feature of Nipah encephalitis seen in about 10% of the Malaysian patients during follow-up (53). Although there was report of delayed onset neurological abnormalities seen in 4 out of 22 patients in a follow-up study in Bangladesh, manifesting in oculomotor palsy and cervical dystonia, none reported fever, seizures or headache during onset of the new neurological deficit as was seen in the Malaysian patients (53).

5.1.4. Late-onset encephalitis: If the neurological signs and symptoms of encephalitis develop after more than 10 weeks of the initial exposure, it is known as late-onset encephalitis.

5.2. Differential diagnoses

Measles, Japanese encephalitis (JE), cerebral malaria, bacterial meningitis and herpes simplex encephalitis and other viral encephalitis (e.g., EV71-associated encephalitis) should be taken into consideration for differential diagnoses of NiV encephalitis. Initial confusion of NiV encephalitis with JE was probably due to the first noticeable outbreak of disease occurring in a pig population in Malaysia. JE is endemic in countries of the South-East Asia Region and pigs act as a reservoir of JE virus. Laboratory diagnosis may include investigations for JE virus since JE is one of the common causes of AES. NiV infection was confused with measles when nosocomial infection was reported among health workers in Siliguri in 2001. It was identified as Nipah-like virus and later confirmed as NiV virus encephalitis after one year.

Nipah cases tend to occur in clusters in outbreaks. This helps to distinguish between NiV and JE. Only one in every 200 persons infected with JE virus develops clinical illness (30). Therefore, it would be extremely unusual to have multiple cases of JE in the same family or in the same village within a few days of each other. Even if infection is widespread, the clinical attack rate from JE is simply too low. A comparison of the characteristics of NiV, JE and herpes simplex encephalitis is presented in Appendix 3.

5.3. Pathogenesis

Data on pathological changes in NiV infection in humans have been limited and so far solely based on Malaysian studies. Brain pathology of Nipah encephalitis cases showed

evidence of necrotizing vasculitis. The main pathology appeared to be widespread ischaemia and infarction caused by vasculitis-induced thrombosis across multiple organs; however, direct neuronal invasion may also play a major role in the pathogenesis of the encephalitis.

Alveolar haemorrhage, pulmonary oedema and aspiration pneumonia were often encountered in the lungs (29). These may lead to pneumonia and ARDS ultimately.

6. CASE MANAGEMENT

There is no effective specific treatment for NiV infection. Treatment is symptomatic and supportive. Severely ill individuals need to be hospitalized and may require Intensive Care Unit (ICU) support.

Because NiV encephalitis can be transmitted person-to-person, standard infection control practices and proper barrier nursing techniques are important in preventing nosocomial infections.

6.1. General management

1. Symptomatic and supportive treatment should be started immediately in all clinically suspected cases.
2. Ensure patient isolation (preferably in a separate ward/room).

3. Institute barrier nursing, e.g., personal protection using masks, gloves, gowns, shoe covers and hand-washing with soap and water before and after handling/visiting patients.
4. Provide symptomatic and supportive treatment:
 - a. Fluid maintenance and electrolyte balance.
 - b. Nutrition, e.g., nasogastric tube feeding/total parenteral nutrition if necessary.
 - c. Anticonvulsant, e.g., intravenous diazepam, phenytoin if necessary.
 - d. Oropharyngeal suction in closed circuit.
 - e. Oxygen inhalation using disposable cannulae.
 - f. Bronchodilators through large spacer devices.
 - g. Admission criteria for ICU care to be decided by national authority.
 - h. Referral criteria to be decided by national authority.

6.2. Specific management

There is no confirmed effective specific treatment for NiV infection in humans to date. However, ribavirin was used in an open-label trial in Malaysia (54). During the Malaysian Nipah outbreak, 140 patients treated with ribavirin were retrospectively compared with 52 control Nipah patients who did not receive ribavirin (54). As a result, fewer treated patients died (32% versus 54%, $P < 0.05$). However, as treatment allocation was not randomized, it is possible that treated

patients had better clinical outcomes because they received better general medical care than the untreated patients.

As NiV is closely related to Hendra virus, experiences acquired for medical care management of Hendra virus cases may be useful for NiV cases.

In May 2010, in Queensland, Australia, an experimental drug (monoclonal antibodies neutralizing both Nipah and Hendra viruses) for Hendra virus was offered as a trial to a mother and a daughter who were exposed to the virus from their infected horse (31). This was the first time that the monoclonal antibody therapy was used in humans in post-exposure treatment. The mother and her daughter did not develop Hendra virus infection, although it is still not known whether treatment was effective or whether the patients were never infected. Monoclonal antibodies against Hendra and Nipah have been shown since 2009 to be highly effective for post-exposure protection of experimentally infected primates and ferrets (32, 33).

It is possible that availability of post-exposure treatment could be highly beneficial and feasible during responses to Nipah and Hendra outbreaks in the future, especially for local health workers who are mobilized for case management of infected patients. From an ethical perspective, it is legitimate to ask that efforts are made to make the post-exposure vaccine affordable, available and accessible for needy people in all affected countries.

7. INFECTION PREVENTION AND CONTROL

7.1. General considerations

All hospitals should adhere to standard infection control precautions for all patient-care activities and aerosol-generating procedures. Standard precautions assume that every person is potentially infected or colonized with a pathogen that could be transmitted in the health-care setting. These precautions have been developed by WHO in the form of guidelines.

There are 10 key elements that consist of guidance on hand hygiene, injection safety, facial protection, glove and gown wearing, waste disposal, linen cleaning, environmental cleaning, patient care equipment and respiratory hygiene.

In case of NiV infection in health-care settings, additional measures, such as droplet, contact and airborne precautions should be applied. Droplet precautions rely on isolation (one-patient isolation rooms or cohorting [i.e., grouping patients infected with the same infectious agents together to confine their care to one area and prevent contact with susceptible patients]) and keeping the patient with an existing roommate.

A patient that meets the criteria for a suspect Nipah case should immediately be isolated and infection control precautions instituted. In general, hospitals in at-risk areas need to be prepared for the management of Nipah cases via hospital screening,

admission procedures and triage, and the management of visitor access and movement should be in place to minimize potential exposures.

Health-care administrators should ensure that all health-care personnel (doctors, nurses, technologists, ward helpers, cleaners/sweepers and others), receive job- or task-specific education and training on infection control and prevention. In particular, attention should be given to settings where caregivers in hospitals also include family members. Training for infection control should also involve these caregivers.

Who should be trained for standard precaution

- health personnel (doctors, nurses, technologists, ward helpers, cleaners/sweepers and others);
- patients;
- family caregivers.

When to apply standard precautions

- handling patients (suspected or confirmed) during:
 - o assessment of the patient,
 - o transportation of the patient,
 - o management of the patient,
 - o handling contaminated equipment linen and waste,
 - o taking care of patients following discharge, and

- o handling the deceased.
- handling specimens: collection, processing, transportation, testing of specimen;
- cleaning floors, toilets, furniture, instruments, equipment;
- washing used linen, utensils;
- cleaning and decontamination of isolation unit/facilities;
- waste disposal.

How and where

Infection control in hospital settings

- Raise awareness of health-care workers about:
 - o use of personal protective equipment,
 - o nosocomial infection, isolation and treatment of Nipah patients.
- Administrative control:
 - triaging before admission,
 - o maintaining respiratory hygiene and cough etiquette,
 - o arrange isolation facilities,
 - o waste decontamination,
 - o waste disposal/incineration,
 - o environmental cleaning (e.g., hospital bed, premises, washroom) and
 - o adequate ventilation.

- PPE
 - o Hand hygiene: Handwashing with soap and water or alcohol-based handrub before and after patient contact,
 - o Wearing of PPE when performing an aerosol generating procedure or a patient examination.

7.2. Specific infection control precautions for NiV

7.2.1. Infection control during management of Nipah encephalitis in isolation unit/facilities

7.2.1.1. Infection control during entry into isolation unit

PPE is an important component of infection prevention in isolation facilities. The use of full barrier PPE is ideal before entering into an isolation unit. However, if full barrier PPE is not available, individuals must at least use a mask, gloves and gown/apron for their protection.

Caregiver or attendants must:

- follow instructions made in the local language/dialect;
- practice full barrier precaution;

- apply standard and droplet/contact precaution;
- ensure respiratory hygiene/cough etiquette for patients and others;
- check /activate negative pressure ventilation; if available;
- practice minimum and essential handling of patients and equipment;
- restrict all patient-care equipment to a single patient (if not possible; these must be cleaned and properly disinfected before reuse);
- clean and disinfect used patient-care equipment properly; and
- practice hand sanitation.

(Note: Instruct patients to avoid unnecessary touching of objects within the facility.)

Patients must:

- put on appropriate PPEs;
- clean secretions and visible soiling with wet swabs frequently and discard in designated covered waste bins and sanitize hand;
- be educated on hygienic use of toilets and other facilities;
- instructed to avoid unnecessary touching of objects within the facility;
- decontaminate used clothes; utensils and other belongings according to protocol;
and
- restrict movement within and outside the facility (if it is necessary for a patient to move outside of the isolation facility a mask must be worn).

Measures to be followed by health-care personnel and visitors (family members and friends) during exit from an isolation unit:

- enter into anteroom/changing room;
- sterilize and decontaminate gloves with soap, water and 70% alcohol, spirit or other disinfectants before removing;
- remove PPEs carefully in order and avoid any aerosol generation;
- place used PPEs in appropriate container/biohazard bags for proper decontamination and disposal;
- wash hands and sanitize.

Measures to be followed by health personnel in general

Before patient discharge:

- provide instructions and materials to patient and caregivers on infection control;
- record patient's address and telephone number for follow-up.

After patient discharge:

- dispose of or clean and disinfect patient equipment as per protocol;
- change and launder linen without shaking;
- clean surfaces as per protocol;
- dispose waste after decontamination.

Measures to be followed by patients during discharge/referral to other facility

- enter into anteroom or changing room;
- remove PPE;
- put on mask and clean clothing;
- sanitize hands;
- issue instructions for infection control measures to be followed at home when necessary.

7.2.2. Measures to be taken by health administrator for hospital management

- Post a biohazard sign in front of isolation unit.
- Limit numbers of HCWs/family members/visitors.
- If necessary, additional beds should be placed at least one metre (three feet) apart from each other.
- Ensure continuous and adequate supply of PPE, hand sanitizers, disinfectants, detergents, equipment and other logistics.
- Clean and disinfect the patient's room at least once daily. Frequently touched surfaces (e.g. doors, table top, bed rails, etc.) should be cleaned more often.
- Designate an infection control official to be in charge of the isolation unit.
- Follow standard precautions for washing dishes/eating utensils and linen.
- Contaminated wastes must be decontaminated properly before disposal.

- Monitor exposed HCWs for signs and symptoms (mainly in case of fever).
- Advise the transporting HCWs and the receiving facility staff about the necessary infection control precautions in case of transfer of patients.
- Clean and disinfect environmental surfaces in the patient examination room or other areas where the patient was located.
- Training and education for health-care personnel on infection control should be performed locally and refresher training is recommended.
- Conduct post-exposure prophylaxis, if necessary.
- Record patient address and telephone number for follow up.

Health-care facility managerial activities include:

- education and awareness-building programmes;
- orientation and training;
- adequate staffing;
- adequate logistics, reagents and supplies.

7.2.3. Cleaning, decontamination, waste disposal for isolation unit facilities

Cleaning and decontamination of environmental surfaces:

- Wipe bed rails, furniture, walls and other contaminated items, and floor with a clean cloth soaked with disinfectant cleaning solution.

7.2.4. Measures for decontamination of waste of isolation unit

Waste: Keep in leak-proof bag within designated covered bin. This includes:

- blood/blood products, body fluids, used saline bag, used bandages and dressings,
- human tissue, body parts, placenta, etc.

7.2.5. Infection control in the mortuary

Secretions and excretions from a deceased person due to infectious diseases like NiV infection are considered to be equally infectious as those from a living infected person. The following standard precautionary measures have to be taken during handling a body during transportation, washing and burial or cremation:

- Handwashing with soap and water should be done immediately after handling the corpse.
- During transport, deceased persons should be carried in an air-sealed bag; if not possible, by covering with clothes.
- During transport of a deceased body from hospital to home, avoid close contact with deceased's face, especially respiratory secretions.
- Avoid close contact with the deceased's face, especially respiratory secretions, during grieving situations.
- Mortuary staff should wear PPE (disposable surgical mask, gloves and gown) while handling the corpse.
- Cover face with a piece of cloth during washing/ritual bath of the deceased body.

- Wash hands with soap; if possible take bath with soap, immediately after performing ritual washing.
- Wash reusable items (cloths, utensils, etc.) with soap/detergent and clean mattress, quilt/comforter, pillow, etc., and dry under sunlight.

7.2.6. Infection control in the community

- At national level: conduct mass media campaign and telecast of short documentary film on NiV transmission and prevention.
- Disseminate health messages at the community level (person-to-person, courtyard, group meeting, local market, local schools).
- Present Nipah transmission and prevention messages through the multimedia at the community level:
 - Try to avoid coming into close contact with the patient,
 - Cover nose and mouth when going near the patient,
 - Wash hands with soap and water after handling the patient,
 - Wash hands before and after feeding the patient.

8. LABORATORY DIAGNOSIS AND BIOSAFETY

NiV is a highly pathogenic organism and it is classified as a biosafety level (BSL) 4 agent. Therefore, it is important that samples from suspect patients (and animals) are handled carefully according to biosafety regulations. However, BSL 2 laboratory

facilities are sufficient for routine diagnosis if the virus is inactivated during specimen collection (4).

Most countries in the South-East Asia Region do not have adequate laboratory facilities for diagnosing infections caused by NiV. However, Bangladesh, India and Thailand have developed their laboratory capacity for diagnostic and research purposes.

Laboratory diagnosis of a patient with a clinical history of NiV can be made during the acute and convalescent phases of the disease by using a combination of tests.

During the early stage of illness – virus isolation and RT-PCR from throat and nasal swabs, CSF, urine, and blood is recommended.

During the convalescent phase, antibody detection by ELISA (IgG and IgM) from serum or CSF may be used.

Initial screening tests recommended are serology (ELISA) and immunohistochemistry, neither of which amplify infectious virus, and so are safer tests to carry out in the laboratory. Especially in fatal cases, immunohistochemistry on tissues collected during autopsy may sometimes be the only way to confirm a diagnosis.

8.1. Collection, storage and transportation of samples

The samples that may be collected include blood (serum), CSF, tissues from various organs, urine and throat/nasal swabs (28). Serum should be separated from the clotted blood samples as soon as possible (but within 24 hours, to avoid haemolysis) and prior to freezing and storage at -70°C .

Specimens for virus isolation and molecular detection of virus should be fresh tissues (from brain, lung, kidney, spleen and heart), CSF, urine or throat/nasal swabs. Samples for viral isolation should be collected in viral transport medium. Specimens for RT-PCR can be collected in guanidine thiocyanate buffer, which inactivates the virus.

Clinical samples should be submitted to designated laboratories in specially designed containers, with packing and shipping as per the International Air Transport Association (IATA), Dangerous Goods Regulations for shipping specimens from a suspected zoonotic disease. The clinical samples should be packed for air transport to the laboratory by a trained person in accordance with the IATA Packing Instructions 602 and 650. The recipient country will require a valid import permit, hence prior consultation with the reference laboratory is imperative.

Samples should be transported at 4°C if they can arrive at the laboratory within 48 hours; if shipping time will be over 48 hours, the samples should be sent frozen on dry ice or liquid nitrogen. Samples should not be held at -20°C for long periods.

NiV surveillance in bats can be performed on pooled bat urine samples for isolation or RT-PCR testing. These are collected from beneath trees where bats roost. This method should replace techniques of mist-netting or shooting of bats.

8.2. Laboratory diagnosis of NiV infections

The following laboratory techniques may be used (51):

virus isolation

histopathology and immunohistochemistry

serology

serum neutralization test

RT-PCR

electron microscopy

8.2.1. Virus isolation

Ideally, virus isolation should be attempted to confirm any new NiV outbreak. However, NiV being a BSL-4 level agent, biosafety considerations require a BSL-4 laboratory facility (52).

NiV can be grown in a range of cultured cells in the laboratory. It grows well in Vero cells, with development of characteristic syncytia with the nuclei arranged around the periphery of the multinucleated cell. Identification of virus isolates may be done by immune staining of fixed, infected cells, neutralization with specific antisera, RT-PCR of

culture supernatants and electron microscopy. Isolation of NiV from the CSF has been strongly associated with mortality (35).

8.2.2. Histopathology and immunohistochemistry

Immunohistochemistry is highly recommended for initial NiV virus diagnosis. It is one of the safest tests as it is performed on formalin-fixed tissues. Since the primary pathology occurs in the vascular endothelium, viral antigen can be detected in a range of tissues. The sensitivity and specificity is not very high, but sensitivity can be improved by sampling an adequate number of animals at necropsy, perhaps over a period of a few days if the disease is progressing on a farm. Also, an adequate range of tissues should be sampled (35, 43, 49).

8.2.3. Serology

Indirect ELISA may be used for detecting IgG and capture ELISA for IgM antibodies against NiV, and can be conducted from inactivated serum in BSL-2 facilities. The IgG ELISA test is used for serosurveillance in humans, swine, bats and peridomestic and domestic animals. Since NiV is closely related to Hendra virus, ELISAs using Hendra virus or NiV antigen may cross-react and could be used detect antibodies to both viruses (14, 36, 37, 38).

8.2.4. Serum neutralization test

The serum neutralization test is the accepted reference serological test, but because NiV is a BSL-4 level agent, biosafety considerations require that this work be carried out in a BSL-4 facility (35, 42).

8.2.5. RT-PCR

There is a range of PCR techniques described to detect RNA from NiV including conventional RT-PCR, nested conventional RT-PCR, and real-time RT-PCR. Detection of viral RNA and its quantitation using real-time RT-PCR can be done in serum, CSF, throat swabs, urine and viral cultures from the CSF, throat swabs or tissues (49, 52).

RT-PCR is the most practical test in countries where either BSL-4 laboratory or ELISA reagents are not available. BSL-2 facilities are sufficient for conducting molecular tests if the samples can be first inactivated during specimen collection. RT-PCRs can be used for detection of viral sequences in fixed or fresh tissue or CSF specimens (36, 39, 40, 41).

8.2.6. Electron microscopy

Negative contrast electron microscopy and immuno-electron microscopy of culture media provides information on structure and antigenic activity of viruses in cell culture (49, 50, 52).

8.2.7. Other clinical laboratory tests

Common haematologic abnormalities in NiV infection include thrombocytopenia (30%) and leucopenia (11%). Elevated liver enzymes have been seen in 40% of patients, and hyponatraemia is sometimes found. Haemoglobin, renal indices and electrolytes other than sodium are usually normal. CSF white count and proteins are elevated in about 75% of cases, although normal CSF white counts were reported in all cases in one outbreak (3). However, CSF glucose remains within normal limits.

8.3. Safety in the laboratory

In laboratories doing serological testing, particularly in outbreak situations, several strategies have been adopted to reduce the risk of exposure of laboratory personnel to Hendra virus and NiV. Sera may be gamma-irradiated (6 kiloGreys) or heat-inactivated at 56 °C for 30 minutes (49).

Since NiV is a highly infectious and dangerous pathogen, it is safer to use ELISA and RT-PCR than serum neutralization or virus isolation.

Propagation of viruses should only be done under BSL-4 conditions. Virus isolation for diagnostic purpose from an unknown suspected NiV infected case could be conducted under BSL-3 conditions. However, when a paramyxovirus-like cytopathic effect is observed, the culture has to be transferred to a BSL-4 condition or inactivated immediately prior to other laboratory assays (35).

It is important to strictly adhere to standard precautions, including contact precautions while handling laboratory samples. Aerosol and droplet precautions may be required for some procedures.

PPE such as protective clothes/apron, gloves, mask, cap, etc., should also be used depending on the risk of the test procedure.

Laboratory waste management should also be in place with segregation of waste at source. Hand hygiene through handwashing and hand rubs would also be important precautions to avoid transmission of the infection in the laboratory.

9. PREVENTION

The epidemiology of NiV infection may differ from country to country. Preventive action should be based on identified risk factors, mode of transmission and sociocultural behaviour facilitating the transmission of NiV.

9.1. Vaccine development

A vaccine for animals is under development. A recombinant subunit vaccine formulation protects against lethal NiV challenge in cats (44). ALVAC canary pox vectored Nipah F and G vaccine appears to be a promising vaccine for swine and has potential as a vaccine for humans (45).

9.2. Information, education and communication

The preventive measures are designed to prevent transmission of the virus from reservoir animals (bats, pigs) to humans and stop transmission of NiV from human to human. To prevent animal-to-human transmission, the exposure of the public to NiV through bat-contaminated fresh date palm sap (as in Bangladesh) or contact with infected pigs (as in Malaysia) must be minimized. Human-to-human transmission is prevented by reducing the exposure of care workers to the secretions of seriously ill person keeping in mind the social and health-care context in the country.

Health promotion activities should be planned and implemented on the basis of established modes of transmission, sociocultural background and available resources.

9.2.1. Target audiences for health promotion are:

1) Opinion leaders (administration, traditional society, community, women groups, religious communities, sports bodies)

- to identify at the national, regional and local level;
- to mobilize;
- to use for social interventions and mediation.

2) The community in general

- to provide information on disease and prevention measures;
- to assess their understanding of the disease and mode of transmission;

- to inform at-risk populations (palm date juice collector, traditional healers, etc.) on the specific risks;
- to inform community networks (women);
- to prepare the community to accept control measures;
- to encourage behaviour changes that decrease NiV transmission;
- to encourage the community to report quickly new suspect cases to surveillance teams.

3) Patient care providers (family, friends and others)

- to participate in public awareness campaigns for Nipah prevention and control before the outbreak season (if it is known);
- to share practical experience in safe handling and care of patients at the community level.

It is important to understand the anthropology of the disease to know the sociocultural background of the community.

9.2.2. Media and risk communication

- Develop a joint communication plan with all ministries involved (ministries of health, agriculture, environment), which has to be trust-based, transparent, quick and respectful of the population/community's legitimate doubts and fears.
- Identify and target the most effective media and press vehicles.

- Communicate with journalists on a regular basis and sensitize them on the key issues.

Box 1. Key elements the prevention of NiV transmission

- Early case detection based on rapidly developed case definitions and prompt alert of local health personnel.
- Early management in isolation and care in the hospital or in the home.
- Adequate and precise infection control measures:
 - o in the community
 - o in the hospital
 - o in isolation facilities
 - o among family caregivers
- Accurate reporting
- Successful awareness programmes

The following action could be taken at different levels:

9.2.3. At community level:

Stop entry of virus from reservoir to host:

- Motivate people to stop drinking raw date palm juice.
- The date palm cultivators (known in Bangladesh as *gachis*) may be requested to use some barriers such as *bana*, a locally produced jute-sheet widely used in rural Bangladesh, to prevent bats from drinking the date juice from the plants. Training programmes for the *gachis* and others to use *bana* and not to sell or drink raw date juice must be held.
- In case of a Nipah outbreak where the source is infected pig or other animals, pig farmers and local livestock officials should be alerted about the possibility of infection and precautions to be taken (personal hygiene and good farming practices).

9.2.4. For patients:

- Report sickness or fever to local health personnel after consuming raw date palm juice.
- Practice frequent handwashing with soap.
- Cover mouth and nose with a napkin, piece of cloth or mask (if possible) while talking.
- Hygienic use of toilet and other facilities.
- Avoid unnecessary touching of objects.

- Decontaminate used clothes, utensils and other belongings with soap and water.
- Limit social contact of visitors and family members.

9.2.5. For caregivers:

- Home isolation: keep the patient in a separate room. If not possible, they should be maintained in a partitioned area.
- Do not eat leftover food from the patient.
- Sleep in a separate bed or in the opposite direction, maintaining more than hand distance (ideally at least two hands) from patient's face while giving care face-to-face.
- Practice frequent handwashing with soap and water when exposed to a patient or contaminated objects.
- Cover mouth and nose with a napkin, piece of cloth or mask (if possible) while talking.
- Practice hygienic use of toilet and other facilities.
- Avoid unnecessary touching of objects.
- Decontaminate used clothes, utensils and other belongings with soap and water.

9.2.6. Activities for local health authorities to change behaviour of drinking raw juice:

- Conduct awareness raising programme at the community level.
- Create mass public awareness to change behaviour by health messages such as leaflets, posters, banners, campaigns, loud-hailers, local focal group discussions, street dramas, local folklore, video film shows, etc.
- Deliver health messages before the outbreak season, if there is an established season.
- Conduct advocacy meetings with local leaders, religious leaders (*imams*, temple heads, bishops, etc.), school teachers and popular figures on early detection of suspected cases, the severity of NiV infection, and the measures for prevention and control.
- At the subdistrict and district levels during the Nipah season, strategies to combat NiV may be discussed during monthly meetings held at the subdistrict and district administration offices and in the presence of representatives of major political parties and members of the local councils (municipalities, unions, etc.).

9.3. Health education and community participation

Past experience shows that NiV encephalitis has a sociocultural and economic impact on the community. Since there is no specific treatment or vaccine available for the prevention of human infection, HCWs are often victims of NiV infection. Possible outbreaks create havoc among family members and health workers. Any health communication message should be socially acceptable and it should be focused on the following issues:

1. How to recognize the disease and whom to contact if there is a suspected case.
2. How to prevent bat-to-food-to-human and bat-to-domestic animal-to-human disease transmission.
3. How to prevent human-to-human transmission.

Simple key health messages used in Bangladesh

- Wash hands with soap before eating and after cleaning and feeding patients.
- Do NOT eat patient's leftover food.
- Sleep in separate bed, or in opposite direction to patient.
- Maintain more than one hand distance (ideally one metre) from patient's face while giving care face-to-face.

In the absence of a vaccine and effective treatment, the only way to reduce infection in people is by raising awareness of the risk factors and educating people about the measures they can take to reduce exposure to the virus.

For each epidemic, the main mode of transmission must be identified by the investigation teams, and key messages must be selected by health promotion experts according to the local context and priority interventions.

Public health educational messages should focus on the following:

- **Reducing the risk of bat-to-human transmission**

Efforts to prevent transmission should first focus on decreasing bat access to date palm sap. Freshly collected date palm juice should also be boiled and fruits should be thoroughly washed and peeled before consumption.

- **Reducing the risk of human-to-human transmission**

Close physical contact with NiV-infected people should be avoided. Gloves and protective equipment should be worn when taking care of ill people. Regular handwashing should be carried out after caring for or visiting sick people.

- **Reducing the risk of domestic-animal-to-human transmission**

Gloves and other protective clothing should be worn while handling sick animals or their tissues, and during slaughtering and culling procedures.

Key messages for prevention of corpse-to-person transmission:

Avoid contact with corpse's face, especially respiratory secretion, during transportation of corpse from hospital to home.

9.4. Intersectoral coordination

NiV encephalitis is a zoonotic disease that also affects domestic animals, primarily pigs. Intersectoral coordination is very important in the control and prevention of NiV outbreaks as the disease is related to agriculture, farming and sociobehavioural practices at the community level. The involvement of agriculture, animal husbandry and food ministries, and the local administration is crucial for decision-making and the implementation of appropriate public health interventions. Coordinated epidemiological and field investigations and cross-checking of samples at both human and animal laboratories may be considered.

Journalists, electronic media personnel, social activists, community leaders and nongovernment organizations all play an active role in early case reporting, social mobilization and raising public awareness, which is essential in the rapid control of any outbreak. Discussions with media people during the preoutbreak time will help develop a common understanding with and constructive role for the media during outbreaks.

The district and subdistrict administration should be involved in discussions on Nipah virus. Such discussions should occur not only during outbreaks, but also prior to the season to highlight the possibility of an outbreak. Possible prevention measures can be discussed at routine intersectoral monthly meetings chaired by district or subdistrict administrators and involving elected people's representatives. These would be crucial for better coordination and cooperation at the community level.

Local community leaders such as members of the local councils, village heads, religious leaders, school teachers, doctors, pharmacists and primary health-care providers can all act in their own capacity, and it is therefore important that they are kept up-to-date with planned awareness-building processes.

10. LESSONS LEARNT FROM PREVIOUS NIPAH VIRUS OUTBREAKS

10.1. NiV outbreaks in Malaysia and Singapore

The NiV outbreak in Malaysia and its subsequent spread to Singapore caught both countries relatively unprepared, and the main lessons that were learnt from it are as follows:

Think out of the box!

A new disease may resemble and thus be mistaken for, a familiar one. The danger of preconceived ideas, and of fitting a disease into an existing mould, was well illustrated by the early phase of NiV outbreak.

Openness and partnership is essential

Free sharing of information and cooperation among scientists and medical doctors, both locally and internationally, was one of the most positive aspects of the outbreak.

Proper diagnosis is key

The discovery of the novel NiV was a significant turning point in the control of the outbreak. The initial supposition that the JE virus was the cause of disease was incorrect, and much time and effort were wasted in the control of vectors associated with and vaccination against JE virus (46). It is noteworthy that the outbreak, which had raged for 6 months, was controlled 2 months after the discovery of the virus.

Compensation is crucial for better surveillance and disease control

The proactive decision by the Government of Malaysia to compensate pig farmers for the loss of pigs was crucial in stopping the surreptitious smuggling of pigs out of outbreak areas, and in bringing about an acceptance of massive pig-culling in all affected communities.

The Malaysian NiV outbreak emphasized the need for sharing information on any unusual illnesses in animals and humans, an open-minded approach, and intense collaboration and coordination between the medical profession, veterinarians and wildlife specialists in the investigation of such illnesses.

Environmental mismanagement (such as deforestation and forest firing or burning) has far-reaching effects, including the encroachment of wildlife into human habitats and the subsequent introduction of zoonotic infections into domestic animals and humans (47).

10.2. NiV outbreaks in India

The first outbreak of NiV in Siliguri, eastern India, triggered a big scare among the public because of the clustering of deaths in space and time. Cases and deaths among doctors and health-care providers, and rumours of it being pneumonic plague or some unknown disease with no clue to diagnosis and treatment resulted in a panic situation with scores of people fleeing the town.

Person-to-person transmission, including nosocomial transmission, which was not reported in earlier NiV outbreaks in Malaysia and Singapore, was apparent in the Siliguri outbreak. For this reason, NiV was not considered in the differential diagnosis.

The lesson learnt is that in an outbreak of unknown aetiology, we need to be careful before we exclude newer pathogens as possible aetiological agents. Also, laboratory confirmation may not be forthcoming early in such outbreaks. Therefore, there is an urgent need to strengthen epidemiological investigation capabilities so that possible mode(s) of transmission and the reservoir of infection can be identified and appropriate control measures instituted in a timely manner.

The Siliguri outbreak highlighted the importance and urgency of establishing a strong surveillance system supported by a network of state-of-the-art laboratories.

10.3. NiV outbreaks in Bangladesh

NiV infection is an emerging epidemic-prone disease with potentially severe consequences related to high case-fatality rates. Knowledge about the disease is limited. The international public health community is still learning from the two distinct types of outbreaks that have emerged to date: pig-related outbreaks in Singapore and Malaysia and date palm sap-related outbreaks that were also propagated through human-to-human transmission in Bangladesh and India. The

lessons learnt from the outbreak investigation in Bangladesh can be summarized as follows:

Multiple pathways of transmission

The primary lessons learnt in the investigation of Nipah outbreaks concern the epidemiology of human NiV infection, which has been detailed in an earlier section. Some of these particular characteristics of Nipah epidemiology impact how an effective outbreak investigation is conducted. There are two potential phases in the transmission of NiV to humans. This multistage transmission pathway complicates the outbreak investigation because individuals are infected by NiV at different times and by different pathways.

Since these are not always point source outbreaks with a single exposure, there may be reduced statistical power to identify the multiple pathways of transmission. To address this characteristic of Nipah transmission, investigators should explore potential multiple pathways of transmission for each case. In addition, early efforts at prevention should focus on interrupting transmission at multiple points. In Bangladesh, for example, outbreaks that began as point common source outbreaks from exposure to date palm sap later became outbreaks sustained by person-to-person transmission.

Person-to-person transmission of Nipah does not occur in every outbreak, but there is always some risk of this, and some of the most important steps to save lives involve encouraging family caregivers and health-care personnel to take prudent

steps to prevent transmission. In Bangladesh, only 7% of persons infected with NiV transmitted the virus to other people (24). These Nipah transmitters all died and it appears that persons who came in contact with them during the final day or two of their lives were at highest risk of contracting Nipah infection.

Fear and panic during Nipah outbreak

Nipah outbreaks are community crises. In a typical outbreak, several previously healthy people became sick and died within a matter of a few days or weeks. There was considerable fear and anxiety and often panic in the affected communities. Outbreaks are also crisis periods for the health-care system. Early in the outbreak, families brought sick patients to hospital, but most of these people, who were previously healthy, died. Communities then often lost faith in hospitals, and even accused hospital staff of neglecting their sick family members or administering incorrect medication that hastened their death.

During such outbreaks health-care providers are also under stress and may be reluctant to treat patients. They see themselves at risk of contracting infection, and often feel they can do little to help their patients. They often do not have the infection control training, facilities or supplies required to protect themselves and other patients.

Strong leadership by the health system is crucial for effective response to the panic generated by these outbreaks. Rapid diagnosis of the cause of the outbreak and providing accurate essential information to the local health and political authorities as well as the communities themselves can both reduce panic and spur the

adoption of appropriate behaviour to prevent ongoing transmission. Anthropologists or others with expertise in understanding community concerns and communicating in a crisis are key participants in an outbreak response team, both to reduce community anxiety and to deliver prevention messages.

Multidisciplinary investigations

We learn the most from outbreaks and manage them best with a multidisciplinary team. Epidemiologists can efficiently organize and oversee an outbreak investigation. They are in the best position to identify risk factors and assess the extent of the outbreak. Physicians can provide clinical information to local physicians on the diagnosis and management of Nipah patients and appropriate strategies to implement infection control. Indeed, it is important to select physicians who will have credibility among health-care providers in the affected community and who have a good understanding of how to apply infection control practices within the resource constraints that prevail in an outbreak setting.

Microbiologists are essential to confirm the diagnosis of Nipah infection. Veterinarians assess if domestic animals are affected and if they may be involved in the transmission of NiV to people. Anthropologists or other social scientists with extensive community-based experience are able to listen to the community's concerns and communicate these to the broader investigation team. They also help to frame and deliver prevention messages in terms that are meaningful to the community.

Nipah clusters

Nipah outbreaks tend to occur in clusters. Sporadic cases do occur, but unlike most other causes of encephalitis, multiple people within the same geographical area tend to become infected with Nipah during the same time-frame. Thus, if HCWs recognize one or two Nipah cases, it is prudent to look for more. Sick neighbours may have used a different hospital, or not visited a health centre at all, or sought care from a traditional healer. Rapidly investigating smaller clusters enables earlier recognition of larger outbreaks, and earlier opportunities to stem panic and implement prevention.

This tendency for Nipah cases to cluster is a characteristic that can also be exploited to both recognize outbreaks and focus surveillance. In Bangladesh, less than 1% of patients who present with symptoms of meningoencephalitis have NiV infection. However, among patients who are part of a cluster of two or more persons meeting the case definition and who live within a 30-minute walk from each other and develop symptoms within three weeks of each other, 32% can develop Nipah infection. Thus a surveillance system that focuses on scarce diagnostic resources on clusters of cases is much more likely to recognize Nipah than a surveillance approach that attempts to assess every patient or a random subset of patients.

Major highlights: Lessons learnt from Nipah outbreak in Bangladesh

1. NiV infection has a high mortality rate of up to 80% in settings without ICU support, which can be reduced to 40% when appropriate ICU services can be administered in a timely fashion. However, the fatality rate is still very high and so prevention rather than cure is more appropriate and cost-effective.

2. The key to controlling the outbreak and reducing mortalities is early detection of the outbreak and installing preventive measures as soon as possible.
3. Proper and prompt field investigations with risk assessments were found to be very useful in the early detection of the outbreaks. Facility-based infection control measures were employed, including standard case management, and increasing levels of intersectoral coordination raised community awareness.
4. Preventive measures work very well not only for outbreak control but also when routinely used prior to the proven Nipah season.
5. The principles of outbreak detection, response and control used in Nipah outbreaks could be successfully used in similar settings in case of other outbreaks with known or unknown agents.
6. Intersectoral coordination in dealing with any disease outbreaks or public health events of concern, as shown in Nipah outbreaks, can play a major role for the rapid control of outbreaks or unwanted impacts, including preventing morbidities and mortalities.
7. The media can play a very positive and proactive role in outbreak control when they are kept well informed and trusted as partners.

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12. READING MATERIALS

Manual on the Diagnosis of Nipah Virus Infection in Animals is available at: www.fao.org/docrep/005/ac449e/ac449e05.htm#bm05.1

International requirements for transport of infectious materials is available at: www.iata.org

Detailed information on surveillance of NiV in animals can be found in following links:

www.fao.org/docrep/010/ah690e/ah690e00.htm

www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/

Guidance to set-up event-based surveillance can be found at:

www.wpro.who.int/emerging_diseases/documents/docs/eventbasedsurv.pdf

A WHO guideline on the rapid risk assessment process for public health events can be found at:

http://whqlibdoc.who.int/hq/2012/WHO_HSE_GAR_ARO_2012.1_eng.pdf

Guidance for contact and air-borne precautions can be found at:

www.who.int/csr/resources/publications/EPR_AM3_E3.pdf?ua=1

Epidemic- and pandemic-prone acute respiratory diseases – Infection prevention and control in healthcare. Aide memoire WHO, 2008.

Guidance for Standard Precautions in Health Care can be found at:

www.who.int/csr/resources/publications/standardprecautions/en/index.html

More information on International Health Regulations (2005) can be found at: www.who.int/csr/ihr/en/.

Appendix 1

Suspected acute meningoencephalitis cases

- Acute meningoencephalitis syndrome case definition
 - History of fever or documented fever (axillary temperature $>38.5^{\circ}\text{C}$ [101.3°F]) with any of the following brain pathology:
 - o altered mental status or
 - o new onset seizures* (excluding simple febrile seizure*)
 - o new neurological deficit either diffuse or localized** to the brain).

OR

- Pulmonary presentation
 - Illness < 7 days duration **and**
 - Fever (axillary temperature $>38.5^{\circ}\text{C}$) **and**
 - Severe shortness of breath (i.e. dyspnoea prevents patient from walking unassisted for 10 steps) **and**
 - Chest radiograph consistent with diffuse acute respiratory distress syndrome.

*Child 6 months to 6 years old whose only finding is a fever and single generalized seizure lasting <15 minutes with recovery of consciousness within 60 minutes.

**Focal neurological deficit: aphasia, ataxia, hemiparesis, cranial nerve deficits, dysphagia, unilateral sensory or motor dysfunction, partial/focal/general seizures.

Appendix 2

Cluster definition and identification

- **Cluster definition**

- A cluster is defined as two or more patients meeting the case definition who occurring within 21 days of each other or who live within walking distance of 30 minutes of each other or have had contact with another patient with similar illness.

- **Cluster identification**

- With each new case that is added to the list, the surveillance physician will check the address of the line-listed cases in the previous three weeks. If they are from the same village or ward, they will probably fit the cluster definition.

- The surveillance physician will also ask the case whether anyone they know has had a recent onset of a similar illness in their family, relatives, village or ward who were either hospitalized or did not visit a hospital. If the answer is yes, they will be likely form a cluster.

- If they are from the same subdistrict but different wards or villages, the latest line-listed case can be asked about the address of the previous case and distance from it. Be sure to keep other information from the line listing confidential. If they are within 2 km or 30 minutes from each other, they likely form a cluster.

Appendix 3

Differential diagnosis of Nipah virus, Japanese encephalitis (JE) and herpes simplex (HS) encephalitis

Characteristics	Nipah virus	Japanese encephalitis	Herpes simplex encephalitis
Agent	Nipah virus (Paramyxovirus family)	JBE virus (RNA, Flavivirus)	HS virus
Incubation period	Median 10 days (range: 2–21 days)	5–14 days	Mean 4 days (range: 2–12 days)
Transmission	Drinking raw date palm juice, human-to-human (close physical contact with Nipah case), animal (pig) to man	<i>Culex</i> mosquito (vector); human-to-human not reported	Human-to-human, respiratory, droplet
Site of involvement	Corticosubcortical areas of cerebrum/cerebellum, brain stem	Thalamus, cortex, cerebellum, anterior horn cells (AHC)	Frontotemporal area

Clinical features	Fever, headache, altered sensorium but specially associated with segmental myoclonus and respiratory involvement	Fever, headache, altered sensorium (100%) followed by convulsions and meningeal sign, abnormal movements	Same as JE but typically associated with a constellation of frontotemporal features with aphasia or mutism, personality change, and focal or generalized seizures
Serology / PCR	IgM /IgG (ELISA), PCR	Antigen or antibody in blood /CSF	CSF PCR for HSV DNA is diagnostic
CSF	Pleocytosis (10–60 cells/mm ³), ↑,protein (30–60 mg/L), normal glucose	Pleocytosis (10–9800cy ⁶ /L),L)prot ein (900 mg/L), normal glucose	Lymphocytic pleiocytosis (typically 10typiccells/mm ³), normal glucose, and increased protein (0.6 to 6 g/l). Red blood cells and xanthochromia may be present
EEG ^a	The main EEG findings are continuous diffuse slowing with focal predominance and discharges mainly at temporal and frontal regions	Diffuse slow waves (theta/delta), burst suppression	High voltage periodic lateralizing epileptiform discharges

Computed tomography	Extensive low density areas in both cerebral hemispheres	Non-enhancing, low density lesions in thalamus, basal ganglia, brain stem	Hypodense lesion in one or both frontal or temporal lobes and sometimes areas of hyperintensity representing small haemorrhages
Magnetic resonance imaging	Confluent areas of signal changes in corticosubcortical region of both cerebrum and cerebellum	T2-weighted images, extensive hyperintense lesions in thalamus, cerebrum, cerebellum	T1 hypointense and T2/FLAIR/BWI image showing hyperintensity lesions in frontotemporal lobe ^b
Prognosis	40% CFR with ICU care, 75% CFR without adequate care ^c	Mortality 8.5–20%	High mortality (20–30% with acyclovir)

^a EEG, electroencephalography.

^b FLAIR/BWI, fluid attenuated inversion recovery/bonded water index.

^c CFR, case fatality rate.

Appendix 4

List of WHO Collaborating Centres and other institutions having facilities for laboratory confirmation of Nipah outbreaks and surveillance in the WHO South-East Asia Region

Laboratory diagnosis

WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research

National Institute of Virology

(Indian Council of Medical Research)

20/ A, Dr Ambedkar Road.

Post Box No. 11,

Pune 411001, India

Tel. No. : 91-020-26127301 / 91-020-26006290

Fax No. : 91-020-26122669/ 91-020-26126399

Email: director@niv.co.in

Website: <http://www.niv.co.in>

WHO Collaborating Centre for Research and Training on Viral Zoonoses

Department of Medicine (Neurology) and Neuroscience Centre for Research and Development

Chulalongkorn University Hospital

Bangkok, Thailand

Tel: 662-2564000 Fax: 662-6523122

Email: fmedthm@gmail.com

Website: <http://www.cueid.org/>

Surveillance and response

Institute of Epidemiology, Disease Control and Research (IEDCR)

Mohakhali, Dhaka-1212

Dhaka

Bangladesh

Phone: +880-2-9898796, 9898691

Fax: +880-2-9880440

Website: <http://www.iedcr.gov.bd>

Email: director@iedcr.gov.bd

International Centre for Diarrhoeal Disease Research, Bangladesh

GPO Box 128

Dhaka 1000

Bangladesh

Phone: (+88 02) 9827001–10

Fax: (+88 02) 9827075, 9827077

Email: nfo@icddr.org

Blurb

Nipah virus encephalitis is a highly pathogenic emerging zoonotic disease of public health significance in Asia. Although Nipah virus was first identified in Malaysia in 1998 with a mortality rate of 40%, it has appeared in Bangladesh and India with even greater lethality. Although human cases have been reported from only a few countries, fruit bats, the main reservoir of Nipah virus, are present in many other countries of the South-East Asia Region. There is no specific treatment, nor vaccine, for the prevention of Nipah virus infection. It has, therefore, become imperative to develop guidelines based on the present limited experience in outbreak investigation, laboratory diagnosis and case management of patients in health facilities.

Considering the above facts, WHO Regional Office for South-East Asia came up with a practical handbook on surveillance, prevention and control of Nipah virus infection so that human cases can be detected promptly and further human-to-human transmission can be prevented. This handbook is intended as a technical support to field epidemiologists, public health and laboratory professionals.