NIPAH VIRUS INFECTION IN ANIMALS
AND CONTROL MEASURES IMPLEMENTED IN PENINSULAR MALAYSIA

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Summary: Between October 1998 and May 1999 the spread of a new pig disease characterized by a pronounced respiratory and neurological syndrome, sometimes with sudden death of sows and boars, was noticed on pig farms in Peninsular Malaysia. The disease appeared to be closely associated with a viral encephalitis epidemic in the pig farm workers. A previously unrecognized paramyxovirus, related to but distinct from the Australian Hendra virus, was later identified in this outbreak. The new virus is named 'Nipah' and was confirmed by molecular characterization to be the same agent responsible for the human and pig diseases. This paper attempts to describe the new disease and some of the epidemiological findings among other animals as well as the control programmes which were instituted to contain and eliminate the virus in the national swine herd.

Since October 1998 to May 1999, a new pig disease with zoonotic implications, characterized by a pronounced respiratory and neurologic syndrome and sometimes with sudden death of sows and boars, was discovered to be spreading among certain pig farms in Peninsular Malaysia. The pig disease was identified to be associated with the viral encephalitis epidemic in pig farm workers resulting in 265 human cases and 105 deaths. A new virus, belonging to the paramyxoviridae family, was discovered and named 'Nipah' after the village 'Sungai Nipah' where the virus was first isolated from a human case. Nipah virus was confirmed by molecular characterization to be the same agent responsible for both the human and pig disease (1). The Nipah virus is also related to the Hendra virus which has killed two people and 16 horses in Australia since 1994.

1. EPIDEMIOLOGICAL BACKGROUND

1.1. Host range of the virus

Pigs, dogs and humans were infected with the virus in the outbreaks in Peninsular Malaysia. Other animals such as cats and horses could be infected, but only in situations where they were exposed to infected pigs. So far only two dogs, one cat and one horse exposed to infected pigs were confirmed as being immunohistochemically infected.

Pigs appeared to be the source of the virus infection in humans and other animals. A case control study of risk factors for human infection by the Nipah virus during an outbreak of severe human encephalitis in Malaysia concluded that direct, close contact with pigs, especially sick pigs, was the primary source of human infection. Other animals may be the source of some infection but the fact that the outbreak stopped after the culling of pigs suggests that infected pigs are ultimately required to sustain transmission.

The origin and reservoir of Nipah virus are still not clear, though preliminary wildlife surveillance has shown fruit bats of the genus Pteropus to have neutralizing antibodies and has identified them as a species requiring further study (2). Currently, virus isolation and polymerase chain reaction (PCR) work for bats are being carried out in the Centers for Disease Control (CDC), in Atlanta.
1.2. Disease occurrence in Peninsular Malaysia

The new pig disease was presented as an outbreak in the Tambun, Ulu Piah, and Ampang (TUA) areas in the vicinity of Ipoh city, in the State of Perak; in the Sikamat, Sungai Nipah, Kg, Sawah and Bukit Pelanduk areas in the State of Negeri Sembilan; and in Sepang and Sg. Buloh in the State of Selangor. National swine testing and a surveillance programme, based on antibody determination and carried out from April to July 1999, had identified previous infection in another 50 farms which were located outside the earlier outbreak areas in the states of Perak, Malacca, Penang, Selangor and Johore. There were no new cases reported in August and September 1999. The last recorded cases, either in humans or in pigs, were in May 1999.

1.3. Clinical signs

Based on observations of the natural infection of pigs in the States of Perak, Negeri Sembilan, and Selangor, clinical manifestations were observed to vary with the age of the pigs. Sows were noted to show primarily with the neurologic syndrome while in porkers the respiratory syndrome predominate. Clinical disease in pigs, however, can also be very subtle. A large proportion of pigs on farms can appear to be infected asymptomatically as farmers have reported that farm workers develop the disease after the pigs have recovered. The incubation period in pigs is estimated to be from 7 to 14 days (2). Based on human cases currently under treatment, the incubation period ranges from 4 to 18 days with the first symptom being a severe headache. Severe cases result in coma and death.

1.4. Pathology

The lung and the brain were the key organs affected. The majority of the cases showed mild to severe lung lesions with varying degrees of consolidation, emphysema and petechial to ecchymotic haemorrhages. Histologically, the main lesion is a moderate to severe interstitial pneumonia with widespread haemorrhages and syncytial cell formations in the endothelial cells of the blood vessels of the lung. Generalized vasculitis with fibrinoid necrosis, haemorrhages, and infiltration of mononuclear cell sometimes associated with thrombosis were observed notably in the lung, kidney, and brain tissues. Non-suppurative meningitis and gliosis are the other significant findings in the brain. Immunohistology showed a high concentration of the viral antigens in the endothelium of the blood vessels, particularly in the lung. Evidence of viral antigens in the cellular debris in the lumen of the upper respiratory tract suggests the possibility of virus transmission through expired air. In human, similar lesions were observed but were more severe in the brain.

1.5. Origin and spreading of the disease

The Nipah virus epidemic is believed to have started in the state of Perak and moved down south to the States of Negeri Sembilan and Selangor. It is established that the mode of viral transmission among pig farms within and between the States of Peninsular Malaysia was movement of pigs. This was especially true, during the epidemic of the disease in the State of Perak, when there was a ‘fire sale’ which dispersed pigs across the country to the states of Negeri Sembilan, Selangor, Penang, Malacca and Johore. Active trading of pigs between states is a normal practice in Peninsular Malaysia, which had a standing pig population (SPP) of 2.4 million. During trading, it is believed that supposedly infected but asymptomatic pigs were moved from farm to farm within and between the States. Evidence has shown that farms that did not receive supposedly infected animals remained uninfected during the swine testing and surveillance programme, even though they are located immediately adjacent to an infected farm. Observations from the swine testing and surveillance programme showed no positive antibody results on farms that took prompt action to cull populations of grower pigs for fattening that that they had received from sources supposedly infected by or exposed to Nipah virus infection. This could be thanks to the current practices of housing growers and breeders in different barns, which have helped to reduce the exposure to and the transmission of virus between these animals. Furthermore, grower pigs normally leave the farm for slaughter by the age of six-month which further reduces the period of virus shedding. On the other hand, it has been observed that farms that have received replacement breeders from supposedly infected farms were found to be antibody positive during the surveillance programme.

The mode of transmission between farms in farming communities has been attributed to several possible factors, such as sharing boar semen and possibly dogs and cats. It is suspected that lorries carrying infected pigs with contaminated urine and excreta picked up dogs and cats which then might have introduce the virus onto other farms.

The disease was observed to spread rapidly among pigs on infected farms. The mode of transmission between pigs within a farm was possibly through direct contact of infected pigs’ excretory and secretary fluids such as
urine, saliva, pharyngeal and bronchial secretions. This is especially likely when pigs are kept in close confinement. Mechanical transfer by dogs and cats and the use of the same needles or equipment for health intervention and artificial inseminations or sharing of boars’ semen within the farm are also possible reasons for rapid spread. Transmission studies in pigs in the Australian Animal Health Laboratory (AAHL), Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia have established that pigs could be infected by the oral route and parental inoculation and have demonstrated the excretion of virus via oral-nasal routes. It has also observed that infection spreads quickly to the in-contact pigs and neutralizing antibodies have been detected on day 14 (2).

The early epidemiology of the disease in Ipoh, in the State of Perak, and how the pig was first infected with the disease remains undetermined. Epidemiology of other animals in the transmission of the disease is also unknown.

2. STAMPING OUT POLICY

A stamping out policy to cull all pigs in the outbreak area was adopted as an early reaction to the infection in pigs.

2.1. Legislation

The Department of Veterinary Services in Malaysia is empowered under the provisions of the Animals Ordinance, 1953, to control and prevent the spread of a disease by gazetting the new disease and identifying areas as infected areas, disease control areas and/or disease eradication areas. On 20 March 1999, viral encephalitis was declared by the Minister of Agriculture as a disease falling within the scope of the Ordinance and a policy was immediately developed for eradication of the disease by mass culling of diseased and in-contact pigs. On some farms where human fatalities or pigs dying with clinical signs of Nipah virus infection were reported, farmers surrendered their pigs and the pigs were culled. Farms that were abandoned by farmers were also taken over by the department and all the pigs were culled. From the information available at that time, culling included all farms from the TUA areas in Perak, all farms in Negeri Sembilan and Sepang in Selangor. A total of 901 228 pigs from 896 farms were destroyed in the infected areas between 28 February to 26 April 1999. The army, police, volunteers from non governmental organizations (NGO), Department of Veterinary Services and other government agencies were involved in the culling operation. Culling was carried out by shooting the pigs and burying them in deep pits. The culling of pigs in these areas successfully controlled the human epidemic in the States of Negeri Sembilan, Perak and Selangor. During the mass culling period, farmers and residents of the villages were evacuated from the infected areas. All interstate movement of pigs was also banned during this period. The evidence of infection in dogs in an outbreak area in Sepang, led to the decision to shoot all stray dogs in infected areas at that time.

At the same time, peri-domestic animals and dog studies were conducted together with the CDC to evaluate possible transmission of the virus through these animals.

2.2. Financial arrangements

Two funds, namely the humanitarian fund to relieve hardship caused by the loss of family members and the trust fund to relieve financial losses due to pig culling were set up by the public sector to assist farmers.

In terms of movement control, interstate movement and farm to farm movements were stopped. All live pig exports to Singapore were also stopped.

The mass culling programme for pigs was discontinued when all the known infected areas had been covered and an enzyme-linked immunosorbent assay (ELISA) test was made available to identify infected farms.

3. SURVEILLANCE PROGRAMME

After the mass culling of pigs in outbreak areas, it was considered essential to institute a national testing and surveillance programme to determine the status of the disease in Peninsular Malaysia.

3.1. Programme objectives

- to protect public health in Malaysia.
- to restore public confidence in the pig industry and in pork and pork products for human consumption by ensuring that pigs entering abattoirs are uninfected.
- to eradicate Nipah virus infections from the Malaysian national swine herd and population and to rid Malaysia of Nipah virus infection.

3.2. Surveillance programme

3.2.1. Surveillance programme for all pig farms: a statistically significant number of sows from all the pig farms will be bled and tested twice, at least three weeks apart. An ELISA test developed by experts from CDC and CSIRO, in the AAHL, has been implemented in the Veterinary Research Institute in Malaysia to test the animals. All farms outside the infected areas showing no confirmed human cases are declared as having ‘Provisionally Approval’ for slaughter only. These farms will be allowed 90 days to comply with Federal requirements to be declared ‘Approved’. At the end of the 90 day period beginning at the start of the programme, farms that did not participate in the testing programme will have their pigs culled.

3.2.2. A surveillance programme for abattoirs consisting of random blood sampling provides assurance that pigs entering the market are uninfected. The established farm code that is unique to each pig farm is to be used. Pigs entering abattoirs must have farm code tattooed on the back and animals without the farm code tattoo will not allow to be slaughtered. Trace back on animals tested positive using farm code as farm site identification will subject affected farm to be quarantine immediately and classified as High Risk Farm.

3.2.3. Testing protocol for high risk farm: farms with a history of human disease or animal disease, and positive test results in the abattoir, are classified as High Risk Farms. High Risk Farms will be subjected to quarantine and given priority for testing. Quarantine is lifted only after the farm has received the Certificate of Test from the Director General, Department of Veterinary Services (DG-DVS), after obtaining negative results on two blood tests made at least three weeks apart. Farms testing positive on follow up will be culled accordingly.

3.3. Programme preparation

3.3.1. Establishment of a laboratory facility at the Veterinary Research Institute (VRI) in Ipoh for the safe implementation of a serological testing programme.

3.3.2. Development of standard operating procedures for the laboratory and field personnel to enable the safe handling of blood specimens for serological testing.

3.3.3. Establishment of a serological testing capability at VRI to reliably detect antibodies to Nipah virus in swine. The cross-reactivity between Nipah and Hendra viruses has facilitated the early application of indirect ELISA for screening. Rapid screening using an ELISA for Hendra during the early outbreak period has shown that on infected farms most of the adult pig population, particularly the sows, had been exposed to the infection. An indirect IgG ELISA using Nipah antigen has been developed in Veterinary Research Institute (VRI) in Peninsular Malaysia with the help of scientists from CDC, Atlanta and AAHL, Australia. Initial studies have indicated that screening sow blood showed the most success in detecting an infected farm. This observation, together with the availability of ELISA for testing Nipah infection, formed the basis of the second phase of the national swine testing and surveillance programme, which was launched on 21 April 1999. Since the programme had to be completed in the shortest time possible in order to prevent further virus spread, attempt to limit the potential threat to humans and to relieve the financial hardship to individual farmers. To achieve this objective, a test was needed which could process many samples in one day (up to 1000) and provide results on the same day.

The tests available in overseas laboratories were the serum neutralisation test (SNT) and the indirect enzyme-linked immunosorbent assay (ELISA). The SNT is one of the most sensitive assays available for virus-specific antibody detection. However, it was not appropriate for this surveillance programme for the following reasons:

- the test requires the use of active virus, and so must be carried out under biosecurity level 4 (BSL4) conditions, which are available in only a few laboratories internationally.
- working under BSL4 conditions, only a few hundred samples can be tested per day.
- the SNT test takes three days to complete.
- because the test relies on cell culture, technical difficulties may result in the test not being available on a daily basis.

Since it had been calculated that approximately 20,000 pig sera would need to be screened twice and that a period of three months was agreed to be an acceptable length of time for the programme, a rapid test was needed. The ELISA, using 96-well plates to allow many samples to be run, was considered the only possible way to achieve the goals of the programme. This type of assay is internationally recognised as the most appropriate herd screening method in such surveillance situations.

3.4. National sampling strategy to be developed

For the national swine herd and population the strategy would be expected to detect all Nipah infected farms and reassure consumers that Nipah exposed pigs are not entering the food chain.

It was worked out that each farm was to be sampled twice with a minimum interval of three weeks between sampling to detect IgG. Based on the current testing information, a statistically significant number of sows was tested on each farm. The minimum number of sows was calculated to be 15 on each farm. If a farm has sows housed in physically separate barns, each barn must have at least six sows sampled and tested.

3.5. Management strategies to be developed

For the implementation of the programme, including collection and submission of specimens, confidential communication of results to the Director General, Veterinary Services, and monitoring of programme progress. All blood test results are entered electronically at VRI and transmitted daily to the headquarters. All positive test results from VRI are released only through DG-DVS or his assigned Deputy Director Generals to the State Veterinary (SVO) on the same day that the result is released. If test results are positive, a letter of notification will then be issued by DG-DVS to the SVO. The same letter will be copied and delivered to the farm owner upon sealing of the farm for quarantine or for culling purposes. The two types of notification letter are:

- letter of notification of a High Risk Farm that will be issued to farms with positive testing results from abattoir, and to farms with human or animal disease;

- letter of notification of a Positive Farm that will be issued to farms with either first or second positive blood testing. The affected farm is declared infected when positive results are obtained from either the first or the second blood testing.

4. FIRST RESULTS OF THE SURVEILLANCE PROGRAMME

Hereafter is a summary of the national surveillance programmes showing the total number of pig farms tested positive and negative classified by States of Peninsular Malaysia:

<table>
<thead>
<tr>
<th>States</th>
<th>Farms</th>
<th>First bleed</th>
<th>Second bleed</th>
<th>Total Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Closed</td>
<td>Positive</td>
</tr>
<tr>
<td>Johore</td>
<td>77</td>
<td>73</td>
<td>4</td>
<td>66</td>
<td>7</td>
</tr>
<tr>
<td>Kedah</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Kelantan</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Melaka</td>
<td>98</td>
<td>93</td>
<td>5</td>
<td>89</td>
<td>4</td>
</tr>
<tr>
<td>N. Sembilan</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pahang</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Penang</td>
<td>335</td>
<td>333</td>
<td>2</td>
<td>318</td>
<td>12</td>
</tr>
<tr>
<td>Perak</td>
<td>197</td>
<td>191</td>
<td>6</td>
<td>190</td>
<td>1</td>
</tr>
<tr>
<td>Perlis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Selangor</td>
<td>154</td>
<td>146</td>
<td>8</td>
<td>143</td>
<td>1</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>864</strong></td>
<td><strong>25</strong></td>
<td><strong>829</strong></td>
<td><strong>25</strong></td>
<td><strong>10</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>889</strong></td>
<td><strong>889</strong></td>
<td><strong>854</strong></td>
<td><strong>50</strong></td>
<td><strong>5.62</strong></td>
</tr>
</tbody>
</table>

Details of the protocols of the national programme are given in Appendix 1.
5. OTHER MEASURES

5.1. Control zone around the contaminated farm

Where one farm in a district is declared infected, a control zone of 0.5 km will be declared around the farm, effective from the day the result is reported. All farms in the control zone will be declared High Risk Farms and given first priority for testing. Pigs from the control zone will not be permitted to move to slaughter or for any other purpose until such time as the farms show two blood draw negative tests and are awarded the Certificate of Test from the DG-DVS.

5.2. Certificate of test

Certificate of test will be awarded to farms having attained two negative blood test results. This certificate is also required, due to high risk factors, for a farm under quarantine in order to obtain a slaughter permit for movement to the abattoir.

A task force was formed and met weekly to monitor the progress of the programme. A total of 889 farms were tested nationwide from 21 April to 20 July 1999. Among these, 50 farms were found positive. Positive farms were considered infected and a total of 172,750 pigs were destroyed on these farms by the end of July 1999. On the average, 5.6% of all the pig farms examined in Peninsular Malaysia were found to be positive for Nipah virus.

5.3. Abattoir monitoring and testing programme

Currently a monitoring programme is being developed to provide continued monitoring of Nipah virus in pigs entering the abattoir for slaughter. The programme will introduce an ear notching system to identify pigs from all the coded farms and allow them to be traced back to their farms of origin if tests reveal infected pigs. The abattoir sampling will be directed at porkers that are between 6-8 months, and not culled sows, and therefore any infection found will be relatively recent.

5.4. Training

A continued educational programme for farmers focusing on the danger of the new pig disease will also be undertaken. Farmers will be educated on the detection of clinical signs and basic personal safety practices on the farms. They are to report immediately to the veterinary department any incidence of abnormal morbidity or mortality in pigs or other animals on the farms. They are advised to avoid direct contact with sick or infected pigs or other animals and wear appropriate protective attires, which include boots and gloves, while handling pigs and excreta. They need to exercise good personal hygiene by washing hands with soap or detergent after handling pigs.

6. SURVEY OF THE INFECTION IN DIFFERENT SPECIES

6.1. Dogs and cats

A preliminary report on a survey of dogs for possible Nipah virus infection in Bukit Pelanduk and Sepang gave the following results:

A serology study conducted in March 1999 in an actively infected area in Bukit Pelanduk showed a 55% (36/66) prevalence in 66 dogs tested and in April 1999 in Sepang, 6 (23%) of 26 dogs were found to have antibodies reactive to Nipah virus. A study was conducted from 11 to 13 May 1999, about three months after the initial incidence of the Nipah virus case in Bukit Pelanduk and Sepang, to determine whether Nipah virus was actively circulating within the dog population.

Two hundred and forty-nine blood samples, taken using filter strips from adult dogs around Bukit Pelanduk and Sepang, were collected and examined for the presence of Nipah virus antibodies using the IgG Elisa method, after the culling operation of pigs in April 1999. The dogs were sampled along two line sections between known Nipah virus infected areas. The first section began at Bukit Pelanduk and continued south-east in intervals of 0-8 km, 8-15 km and 15-20 km to Lukut and Port Dickson towns. The second section was established at a similar distance from Sepang extending north-east to Salak Tinggi. The sampling strategy was chosen to determine whether Nipah virus-infected dogs were limited to the areas where swine infection with
Nipah virus had occurred and also whether secondary transmission among the dog population had taken place. During the period of study, questionnaires were completed by veterinary personnel for stray and pet dogs in and around households selected along these sections from the localities chosen. Blood samples and tissue specimens, chilled on ice and in formalin, were collected from 88 strays while filter strips were used for blood sampling from 161 pet dogs. Four out of a total of 249 samples analysed (2%) were found to have antibodies reactive with the Hendra virus. The positive dogs were from the following locations: in Bukit Pelanduk, one pet dog in the 1-8 km range and one stray dog within the 8-15 km range; and in the Sepang section, the two positive pet dogs were from the 1 to 8 km range. The absence of evidence in the dog population at a distance greater than 15 km from the actively infected areas and the very low prevalence in dog populations nearer to the actively infected areas strongly suggests that the virus was not spreading among the dog population and that the transmission of Nipah virus to dogs occurred only in areas where active pig infection was known to have occur.

Number of stray and pet dogs tested positive (with specific antibodies) in Bt. Pelanduk and Sepang

<table>
<thead>
<tr>
<th>Area</th>
<th>Distance</th>
<th>Stray</th>
<th>Pet</th>
<th>No. Positive</th>
<th>No. Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bt. Pelanduk</td>
<td>0-8 km</td>
<td>1</td>
<td></td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>8-15 km</td>
<td></td>
<td>1</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15-20 km</td>
<td></td>
<td></td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Sepang</td>
<td>0-8 km</td>
<td>2</td>
<td></td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>8-15 km</td>
<td></td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15-20 km</td>
<td></td>
<td></td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>No. Tested</td>
<td></td>
<td>88</td>
<td>161</td>
<td>4</td>
<td>249</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>1.1%</td>
<td>1.9%</td>
<td>2%</td>
<td></td>
</tr>
</tbody>
</table>

The results of SNT from AAHL on blood samples collected in dogs and cats after the active phase in Perak State were negative in 396 dogs and in 114 cats.

6.2. Horses

More than 3,200 serum samples were collected from polo, equestrian and race horses and dispatched to AAHL in March and April 1999 for screening using ELISA and SNT. The results showed that only two horses out of 47 horses in a polo club tested positive by SNT, giving a prevalence of 4.3%. Examination of these two horses did not reveal clinical disease and the animals were apparently healthy. The horses were euthanised and necropsy did not reveal any gross lesions. Both specimen sent to AAHL were negative for immunohistochemistry and PCR. There was no isolation of virus from the tissues and blood samples. Epidemiological investigations revealed that the affected horses were at one time stationed at pig farms and could have possibly contracted the infection there.

6.3. Other species

The results from CDC Atlanta on blood samples collected in Bukit Pelanduk and Sepang during the active phase in March 1999 indicated that the following animals had antibodies reactive to Nipah virus: goats: 1.5% (1/65), cats: 6.4% (1/24), birds: 6.4% (7/109) and rodents: 0.4% (1/278). Currently, virus isolation and PCR work are in progress at CDC.

REFERENCES

NATIONAL TESTING AND SURVEILLANCE PROGRAMME FOR NIPAH VIRUS IN PIGS

1. Objective: To identify any infected farms outside the current areas of containment

2. Priority areas for testing:
   a) High risk farms: Farms that have had a confirmed human case or a report of Nipah disease in swine. These will be given first priority for testing to rapidly identify possible new foci of infection. Pigs from high risk farms will not be moved to abattoirs.
   b) Abattoirs: A random sampling programme will monitor pigs entering the food chain to ensure that these are uninfected. Positive antibody tests will require farm identification and follow-up testing.
   c) Farms outside the identified containment areas: The pig population will be sampled within 90 days, with sampling to commence on 21 April 1999.

3. Testing protocol for high risk farms:
   a) A history of human and animal disease will be established, noting previous animal movements.
   b) The premises will be inspected for sick pigs.
   c) Necropsies will be conducted on representative clinically affected animals, including collection of a serum sample and tissues from kidney, lung (three different areas), bronchi, trachea and brain. (NB Brain is still under consideration and may be left out as a safety measure.)
   d) Fifteen randomly selected sows and 15 pigs of the same age group(s) as the sick animals, especially animals from the same and adjacent pens, will be bled for serum.
   e) Sera will be tested to detect IgG.
   f) Formalin fixed tissues will be examined histologically and by immunohistochemistry.

4. Testing protocol for abattoirs:
   a) Random sampling of pigs slaughtered at abattoirs will be implemented, according to a programme to be approved by the Director General of Veterinary Services.
   b) Pigs at Government abattoirs will be regularly sampled and those in private slaughterhouses will be sampled on an occasional basis.

5. Testing protocol for farms:
   a) To increase consumer confidence that the national pig population has been adequately sampled, it has been agreed that all farms be tested, using the Farm Code as the site-specific identifier.
   b) All farms outside the containment area showing no confirmed human cases should be declared as 'Provisionally Approved' for slaughter only. These farms will be allowed 90 days to comply with Federal requirements to be declared 'Approved'. After a period of 90 days from the start of the programme, farms that did not participate in the testing programme will have their pigs culled with no compensation.
   c) Based on present testing information, a statistically significant number of sows will be tested on each farm. The minimum number of sows that will be tested from any one farm is 15. If farms have sows housed in physically separated buildings, each building must have at least six animals sampled but the total number of tested animals must be at least 15.
   d) Two separate blood collections will be required from each farm no less than three weeks apart and not from the same animals. All blood samples collected must be submitted to the nearest Regional Veterinary Laboratories for processing and consignment to the Veterinary Research Institute (VRI).
   e) Serum samples will be tested in VRI using ELISA test approved by the Director General, Department of Veterinary Services (DG-DVS).
   f) It is the responsibility of the farmer to pay for the bleeding and the government will pay for the test.

6. Response to Positive Test Results
   All results from VRI will be reported to DG-DVS, who will direct the following actions:
   1) Upon completion of two blood draws and in the presence of negative test results, a farm will be issued a Certification of Test from the DG-DVS.
   2) Farms testing positive will be considered infected, and subject to immediate quarantine and movement restrictions.
3) Where a farm has been identified as infected, the DG-DVS will advise the Director of Veterinary Services of the State to take immediate action.
4) Where one farm in a district has been declared infected, a control zone of 500 metres will be declared around that farm, effective from the time the results are reported.
5) All pigs on infected farms will be culled. Infected farms are those tested serologically positive.
6) All farms in the control zone will be declared High-Risk Farms and testing will be given first priority.
7) Pigs from control zones will not be permitted to be moved for slaughter or for any other purpose until such time as the farms have been tested and cleared.

7. General Guidelines:

7.1. Isolation of Pig Farms
   a) During the 90-day testing period there will not be any movement of pigs between pig farms, including for artificial insemination.
   b) To ensure separation between pig farms, vehicles transporting pigs to the abattoir will carry pigs from only one farm at a time and will not enter a second farm en route to the abattoir.
   c) Vehicles transporting pigs will be thoroughly cleaned and disinfected at the abattoirs after each delivery.

7.2. Safety of Personnel

   The published clothing and equipment recommendations of the Task Force for personal safety will be followed:
   a) Inspecting farms and bleeding of apparently healthy pigs:
      • Protective eye goggles or face shield
      • HEPA mask
      • Surgical gloves
      • Coveralls, long sleeve
      • Knee-high protective boots (gum boots)
   b) Necropsy of pigs and bleeding of suspect Nipah-infected pigs:
      • Protective eye goggles or face shield
      • PAPR (positive air pressure respirator) mask
      • Surgical gloves (double glove)
      • Water impervious apron
      • Coveralls, long sleeve
      • Knee-high protective boots (gum boots)
   c) Specimen handling in the laboratory:
      • Protective eye goggles or face shield
      • HEPA mask
      • Surgical gloves
      • Laboratory gown (lab coat)
   d) Report all needle sticks or exposures to the Task Force HQ and seek immediate medical treatment.

7.3. Disinfection after operations on farms:

   a) Ensure that necropsied animals are buried.
   b) Use viricidal disinfectants such as sodium-hyperchlorite, Betadine, Dettol, lysol, Vircon or Savlon.
   c) Before leaving the farm, clean blood from equipment and boots, then disinfect with an appropriate disinfectant.
   d) All specimens should be labelled appropriately, and the containers be disinfected by spraying and double-bagged.
   e) Change into clean clothes before leaving the premises.
   f) Wash all clothing at least daily and do not use the same clothing between farms (waterproof disposable overalls may be disinfected by spraying with disinfectant between farms). Wash vehicles, including tires and wheels, with detergent before leaving the farm.