Nipah virus infection of pigs in peninsular Malaysia

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Summary
Between late 1998 and 1999, the spread of a new disease of pigs, characterised by a pronounced respiratory and neurological syndrome, sometimes accompanied by the sudden death of sows and boars, was recorded in pig farms in peninsular Malaysia. The disease appeared to have a close association with an epidemic of viral encephalitis among workers on pig farms. A previously unrecognised paramyxovirus was later identified from this outbreak; this virus was related to, but distinct from, the Hendra virus discovered in Australia in 1994. The new virus was named 'Nipah' and was confirmed by molecular characterisation to be the agent responsible for the disease in both humans and pigs. The name proposed for the new pig disease was ‘porcine respiratory and neurological syndrome’ (also known as ‘porcine respiratory and encephalitis syndrome’), or, in peninsular Malaysia, ‘barking pig syndrome’. The authors describe the new disease and provide the epidemiological findings recorded among infected pigs. In addition, the control programmes which were instituted to contain the virus in the national swine herd are outlined.

Keywords

Introduction
A number of significant pig diseases are normally present in peninsular Malaysia. These include classical swine fever, Aujeszky's disease and porcine reproductive and respiratory syndrome. However, between late 1998 and 1999, the spread of a new disease of pigs, characterised by a pronounced respiratory and neurological syndrome, sometimes accompanied by the sudden death of sows and boars, was recorded in pig farms in peninsular Malaysia. Initially, the new syndrome was not identified because the pig morbidity and mortality rates were not excessive, and the clinical signs were not markedly different from those of other diseases of pigs in peninsular Malaysia. However, the disease appeared to be closely related to an epidemic of viral encephalitis among pig farm workers. Attention was again focused on the mysterious pig disease as numerous and stringent measures used to control Japanese encephalitis failed to contain the incidence of the viral encephalitis epidemic in the pig farm workers. A new virus named 'Nipah', belonging to the Paramyxoviridae family, was discovered and later confirmed to be the agent responsible for disease in both humans and pigs (1). Between October 1998 and May 1999, 265 cases of viral encephalitis were recorded in humans involved in pig farming activities; 105 of these cases resulted in death.

An early and accurate diagnosis is essential to ensure the prompt control of any new, emerging or exotic disease of animals. The authors therefore attempt to provide some essential information concerning this new pig disease, including epidemiological findings and information regarding the control programmes which were instituted to contain the outbreak.

Proposed name of the pig disease
The names 'porcine respiratory and neurological syndrome' and 'porcine respiratory and encephalitis syndrome' (PRES) have been proposed as technical names on account of the pronounced respiratory and neurological syndromes associated with the disease. The unusual loud barking cough
is another characteristic feature of the disease that differs from the other respiratory diseases of pigs recorded in peninsular Malaysia, and thus 'barking pig syndrome' (BPS) has been suggested as the common name for the disease.

**Aetiology**
The disease is caused by a new virus, Nipah, named after the village Sungai Nipah in the State of Negeri Sembilan from which the virus was first isolated from a human case. Nipah is an enveloped ribonucleic acid (RNA) virus, belonging to the family *Paramyxoviridae* and is closely related to, but distinct from, the Hendra virus isolated in 1994 in Australia. The virus is relatively unstable in vitro and can be readily disinfected with common detergents. The virus grows very well in cell lines such as African green monkey kidney (Vero), baby hamster kidney (BHK) and porcine spleen (PS) (Fig. 1).

**Pathogenesis**
The pathogenesis of the disease is still undetermined.

**Natural hosts**
Pigs, dogs and humans were infected with the virus during the outbreaks in peninsular Malaysia. Other animals such as cats, horses and goats were infected, but only if exposed to infected pigs.

The origin and reservoir of Nipah virus is still not clear. Preliminary wildlife surveillance has demonstrated neutralising antibodies in fruit bats of the genus *Pteropus*. The role of this species in transmission of the disease requires further study (H. Field, J. Yob, A. Rashdi & C. Morrissy, unpublished findings).

**Occurrence of disease in peninsular Malaysia**
The new pig disease presented as an outbreak in several locations, as follows:
- the areas Ulu Piah, Tambun and Ampang, near the town of Ipoh, in the State of Perak
- Sikamat, Sungai Nipah, Kampong Sawah and Bukit Pelanduk areas in the State of Negeri Sembilan
- Sepang and Sungai Buloh in the State of Selangor.

A national swine testing and surveillance programme based on antibody determination was undertaken between April and July 1999. The programme identified previous infection in a further fifty farms which were located in the States of Perak, Malacca, Penang, Selangor and Johore, which are all situated outside the initial outbreak areas.

**Epidemiological findings**
The Nipah virus epidemic is believed to have originated in the State of Perak and moved south to the States of Negeri Sembilan and Selangor. The mode of transmission of the virus among pig farms within and between States of peninsular Malaysia, was the movement of pigs. At the time of the epidemic in the State of Perak, significant movement of pigs occurred due to a 'fire sale' which dispersed pigs across the country. Pigs were moved to the States of Negeri Sembilan, Selangor, Penang, Malacca and Johore. Active pig trade was normal practice in peninsular Malaysia which had a large standing pig population (SPP) (2.4 million) at the time of the outbreak. As a result of trade, supposedly infected pigs (perhaps with no signs of the disease) were moved from farm to farm, both within and between States. Evidence has shown that farms that did not receive animals suspected of infection remained free from infection during the testing and surveillance programme, although such farms were located immediately adjacent to an infected farm. Observations obtained from the programme indicated that infection was not detected on farms which received grower pigs for fattening if the farm took prompt action in culling all animals suspected of infection. This could be due to the current practice of housing growers and breeders in different barns, which helps to reduce exposure or virus transmission between these animals. Furthermore, grower pigs usually leave the farm for slaughter before the age of six months, thereby further reducing the period of virus shedding. In contrast, farms that received replacement breeders from suspected infected farms gave positive results in the surveillance programme.

Transmission between farms in farming communities was attributed to several possible means, for example, sharing boar semen and transmission by dogs and cats. It is suspected that dogs and cats were infected with urine and excreta from lories carrying affected pigs, and subsequently introduced the virus to uninfected farms.

The disease was observed to spread rapidly among pigs on infected farms. Transmission between pigs on the same farm is possibly through direct contact with excretory and secretory fluids such as urine, saliva, pharyngeal and bronchial secretions. This mode of transmission could be significant as
pigs are kept in close confinement. Mechanical transfer by dogs and cats, the use of unsterilised needles or equipment for health intervention and artificial insemination and sharing of boar semen within the farm are also implicated. Transmission studies in pigs at the Australian Animal Health Laboratory (AAHL) of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, have established that pigs could be infected by either the oral route or by parenteral inoculation and have demonstrated the excretion of virus via oronasal routes. Infection was observed to spread quickly to the in-contact pigs and neutralising antibodies were detected on day 14 (P. Daniels, unpublished findings).

The origin of the initial outbreak of the disease in Ipoh, State of Perak, and the method of initial transmission to pigs is still undetermined. The role of other animals in the transmission of the disease is also unknown.

Clinical signs

Based on observations of the natural infection of pigs in the States of Perak, Negeri Sembilan and Selangor, the clinical patterns were observed to vary according to the age of the pigs. Sows were noted to present primarily the neurological syndrome, while in porkers, the respiratory syndrome predominated. However, clinical disease in pigs can also be very subtle. A large proportion of pigs on a farm can appear to be infected asymptomatically as farmers have reported that farm workers develop disease after the pigs have recovered. The incubation period in pigs is estimated to be seven to fourteen days (P. Daniels, unpublished findings).

Weaners and porkers

Pigs from four weeks to six months of age usually presented acute febrile illness (≥ 39.9°C) with respiratory signs ranging from rapid and laboured respiration to a harsh non-productive cough (loud barking cough). Severe cases may present haemoptysis and less severe cases, open mouth breathing. One or more of the following neurological signs may accompany the respiratory signs:

- trembling and neurological twitches
- muscle spasms and myoclonus
- rear leg weakness and varying degrees of spastic paresis or lameness
- unco-ordinated gait when driven and hurried
- generalised pain, especially in the hind quarters.

Mortality is low, from less than 1% to 5%, but the infection rate approaches 100%. Disease manifestation can be asymptomatic, mild or fulminant. When stress is reduced, severe cases may recover or show less severe signs.

Boars and sows

Boars and sows present similar symptoms and infection may be accompanied by sudden death or acute febrile illness (≥ 39.9°C), with laboured breathing, increased salivation (drooling or frothy), nasal discharge (serous or mucopurulent or bloody), and possible early abortion for sows (first three months). Some or all of the following neurological signs can also be present:

- agitation and head pressing
- tetanus-like spasm and seizures
- nystagmus
- champing of the mouth
- apparent pharyngeal muscle paralysis, which may explain an inability to swallow, frothy salivation and the tongue hanging out of the mouth.

Suckling pigs (piglets)

Disease has been identified in suckling pigs with a mortality rate of approximately 40%. However, it is unclear whether mortality is due to disease in piglets or results from the inability of the sow to nurse the piglets (Fig. 2). Apparently healthy but confirmed seropositive sows were observed to nurse apparently healthy piglets. The majority of the infected piglets showed the following signs:

- open mouth breathing
- leg weakness with muscle tremors
- neurological twitches.

Pathology

The majority of the cases demonstrated mild to severe lung lesions with varying degrees of consolidation, emphysema and petechial to ecchymotic haemorrhages (Fig. 3). The cut surface of the lung showed distension of the interlobular septa. The bronchi and trachea may be filled with a frothy fluid (with or without blood). Brain tissue may show...
Severe lung lesions with varying degrees of consolidation, emphysema, petechial and ecchymotic haemorrhages.

Fig. 3
Courtesy of Dr Aziz Jamaluddin, Veterinary Research Institute, Malaysia.

Generalised congestion and oedema. Kidney tissue may show congestion, both on the surface and the cortex, but appeared normal in many cases. Other visceral organs also appeared to be normal.

Histologically, the principal 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. Generalised vasculitis with fibrinoid necrosis, haemorrhages, and infiltration of mononuclear cells sometimes associated with thrombosis, were observed notably in the lung, kidney and brain tissues. Non-suppurative meningitis with gliosis was the other significant finding 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 suggested the possibility of virus transmission through expired air (Fig. 4).

Fig. 4
Safety attire worn during post-mortem examination and specimen collection from pig carcasses.
Courtesy of Dr Aziz Jamaluddin, Veterinary Research Institute, Malaysia.

Laboratory tests
Serological testing for Nipah virus, using an enzyme-linked immunosorbent assay (ELISA), is currently available in peninsular Malaysia. The Nipah antigen is inactivated by gamma-irradiation. The test sera are inactivated by adding both sodium dodecyl sulphate and tetra-ocdythe-noxypoly-ethoxyethanol and by heating at 56°C for 1 hour. Laboratory diagnosis using the serum neutralisation test (SNT), polymerase chain reaction (PCR) and virus isolation should be performed in a biosafety level 4 laboratory.

Treatment
Treatment of infected pigs is not recommended since the disease is transmissible to humans. Treatment using hyperimmune serum has not yet been attempted in humans.

Control and eradication
With the discovery of the aetiology of the pig disease, an immediate ‘stamping-out’ policy was instigated to cull all pigs in the outbreak areas in the first phase. A total of 901,228 pigs from 896 farms were destroyed in the infected areas from 28 February to 26 April 1999. The culling of pigs in these areas successfully controlled the human epidemic in the States of Negeri Sembilan, Perak and Selangor. The culling programme ceased when an ELISA was made available to identify infected farms in a national swine testing and surveillance programme.

The cross-reactivity between Nipah and Hendra viruses facilitated the early application of indirect ELISA for screening. Rapid screening using an ELISA for Hendra had shown that on infected farms most of the adult pig population, particularly the sows, had been exposed to infection. An indirect immunoglobulin G (IgG) ELISA, using Nipah antigen, was developed in peninsular Malaysia and initial studies indicated that screening of sow blood gave the highest confidence of 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 that was launched on 21 April 1999. The programme stipulated that each farm was to be sampled twice, with a minimum interval of three weeks between sampling. 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 fifteen on each farm. If sows were housed in physically separate barns, at least six sows from each barn were sampled and tested. A total of 889 farms were tested nation-wide between 21 April and 20 July 1999. Of these, 50 farms were found positive. Farms recorded as positive were considered infected and a total of 172,750 pigs from these farms were destroyed at the end of July 1999. On average, 5.6% of all pig farms examined in peninsular Malaysia were found to be positive for Nipah virus.
Currently, a control programme is being developed to provide continued monitoring of all pigs entering an abattoir. The programme will introduce an ear notching system to identify pigs from all the coded farms and allow these pigs to be traced back to farms of origin if tests reveal pigs are infected. A continued educational programme for farmers, highlighting 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 in the farms. Any incidence of abnormal morbidity or mortality in pigs or other animals on the farm must be reported immediately to the veterinary department. Farmers are advised to avoid direct contact with sick or infected pigs or other animals, and wear appropriate protective attire, which includes boots and gloves, while handling pigs and excreta. Good personal hygiene should be exercised by washing hands with soap or detergent after handling pigs.

Disinfectants such as sodium hypochlorite are recommended for use in the pig farms of peninsular Malaysia.

Conclusion

The Nipah virus outbreak has had a significant impact on the pig industry in peninsular Malaysia, with a marked reduction in the SPP and the total number of farms. By 31 July 1999, the total SPP was reduced from 2.4 million to 1.32 million, and the number of farms was reduced from 1,885 to 829. The episode has also caused a dramatic change in the direction of the future of the pig farming industry. In Negeri Sembilan, for example, pig rearing in previously infected areas will be prohibited. Pig farming will only be allowed in identified pig farming areas (PFA), as designated by the Ministry of Agriculture, Malaysia. Restocking of farms where animals were culled will also be subject to State decision. The policy and new conditions for restocking infected farms require further study. As an alternative, farmers are being encouraged to undertake other agricultural and livestock activities.

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Infection due au virus Nipah chez les porcs en Malaysia péninsulaire

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Résumé

Une nouvelle maladie porcine, caractérisée par un syndrome respiratoire et neurologique prononcé, avec parfois mort subite des truies et des verrat, est apparue fin 1998 et s’est propagée en 1999 parmi les élevages de porcs en Malaysia péninsulaire. La maladie semblait étroitement liée à une épidémie d’encéphalite virale qui affectait le personnel employé dans les élevages. Le paramyxovirus causal, non reconnu auparavant, a par la suite été identifié ; il s’agissait d’un virus apparenté au virus Hendra décrit en Australie en 1994, quoique distinct de celui-ci. On lui a donné le nom de virus « Nipah » ; le type moléculaire a confirmé que cet agent était responsable de la maladie chez l’homme comme chez l’animal. L’appellation proposée pour cette nouvelle maladie porcine est « syndrome respiratoire et nerveux du porc », également connue sous le nom de « syndrome respiratoire et d’encéphalite du porc » ; en Malaysia péninsulaire, on l’appelle « syndrome de la toux rauque du porc ». Les auteurs décrivent la nouvelle maladie et résument les observations d’ordre épidémiologique réalisées sur les porcs infectés. Ils présentent également les
Enfermedad por cerdos por virus Nipah en la Malasia peninsular
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Resumen
Desde finales de 1998 y durante 1999, se observó en las explotaciones de cerdos de la Malasia peninsular la extensión de una nueva enfermedad porcina, caracterizada por un fuerte síndrome respiratorio y neurológico que conducía a veces a la muerte repentina de puercas y verracos. La enfermedad parecía presentarse estrechamente asociada a una epidemia de encefalitis de origen vírico que afectaba a trabajadores de granjas porcinas. Ulteriormente se detectó la presencia en ese brote de un paramixovirus hasta entonces desconocido, afín pero no idéntico al virus Hendra descubierto en 1994 en Australia. Por caracterización molecular se confirmó que el nuevo virus, al que se dio el nombre de virus “Nipah”, era el agente causal tanto de la enfermedad humana como de la porcina. El nombre propuesto para esa nueva patología porcina es “síndrome respiratorio y neurológico porcino”, conocida también como “síndrome respiratorio y encefalitis del cerdo”; o bien, en la parte peninsular de Malasia, “síndrome del cerdo con tos”. Los autores describen la nueva enfermedad, explican algunos de los hallazgos epidemiológicos realizados entre los cerdos infectados y presentan a grandes rasgos los programas de control implantados para contener el avance de la enfermedad en la cabaña porcina nacional.

Palabras clave

Reference