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THE OPERATION OF NIPAH VIRUS LABORATORY FOR THE NATIONAL SWINE SURVEILLANCE PROGRAMME

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The outbreak of Nipah virus disease in Malaysia, which has caused human fatalities, has been attributed to a viral infection in pigs. The outbreak in 1998/99 has been traced back to earlier incidences which were provisionally diagnosed as Japanese encephalitis (JE) (1). Since the discovery in March 1999 of the Nipah virus as the aetiological agent responsible for sickness and death of human beings associated with the pig industry (2), the Department of Veterinary Services (DVS) Malaysia, had introduced the National Swine Surveillance (NSS) programme in April 1999. The aim of this programme was to screen the pig farms in Malaysia so as to control the spread of the virus (3). The above epidemic was considered an emergency which was not experienced by the country previously and this article aims to entail the mode of operation in the Nipah virus laboratory at the Veterinary Research Institute (VRI), Ipoh.

The VRI in collaboration with the Australian Animal Health Laboratory (AAHL) Geelong, and Centers for Disease Control and Prevention (CDC) USA, developed a laboratory test to monitor antibodies in pigs due to Nipah virus infection in pig farms in the country. The DVS has established a 2- phase programme that focused on sampling pigs on 100% of the 889 pig farms in Malaysia and a surveillance program for abattoirs. One of the aims was to rapidly identify the positive farms in order to control the spread of the disease among pigs. The programme started on 21st April 1999 and was planned for completion on 31st July 1999.

The number of blood samples received for the period 20th April to 31st July 1999 is detailed in table 1. A total of 36,125 blood samples were received for the NSS programme from 889 pig farms where bleeding teams were established by the DVS field veterinary staff and authorized private veterinarians. Serum samples were also received from abattoirs conducting slaughter of pigs. Ante-mortem inspection and random blood sampling were performed by the staff of the abattoirs concerned.

The Regional Veterinary Laboratories (RVL) throughout the country namely Petaling Jaya, covering the states of Selangor, Melaka and Negeri Sembilan; Bukit Tengah, covering Penang, Kedah and Perlis; Johor Bharu, covering Johor; Kuantan, covering Pahang, and Kota Bharu, covering Kelantan undertook to process the blood samples collected from the farms and submit the serum samples to VRI for testing. Blood samples collected from farms in Perak were sent directly to VRI without processing. The Virology Section was involved in separating the serum from blood. The extraction of serum from blood in the RVLs and the VRI was performed in the biohazard class II cabinets.

An emergency Nipah virus testing laboratory in VRI was set up with the existing infrastructure. The laboratory was divided into 4 main work areas, namely, i) the registration room, ii) master plating room, iii) assay room and iv) storage room. Sample submitters from the various regional laboratories personally delivered the samples at the registration room.

Table 1: Number of blood samples received from pig farms and abattoirs from the period 20th April to 31st July 1999.

State	Type of Sample received	Total number of farms		Total number of samples		Total Number of samples from farms	Total number of samples from abattoir
		First bleed	Second bleed	First bleed	Second bleed		
Perils	Serum	1	1	18	18	36	0
Kedah	Serum	9	9	158	154	312	0
Penang	Serum	335	330	5051	5104	10155	110
Perak	Blood	197	191	4751	3148	7899	1594
S clangor	Serum	154	144	2663	2222	4885	1424
Melaka	Serum	98	93	1515	1751	3266	841
Negeri Sembila	Serum	1	1	15	18	33	10
Johor	Serum	77	73	1926	1641	3567	1130
Pahang	Serum	8 9	7 5	191	140	331	375
Kelantan	Serum			111	46	157	0
	Total	889	854	16,399	14,242	30,641	5484

The samples from the RVLs were received in sealed containers in sealed racks containing icepacks. Absolute care was taken to avoid any potential exposure of laboratory personnel to the infectious agent. Each blood specimen was regarded as a possible positive sample. Every step that involved exposure to the blood or serum samples was performed in the class 2 biohazard cabinet in a enclosed laboratory rooms. Opening the package sent from the submitter, sticking the label onto the serum vials and master plating of the sera into micro tubes (Biorad, USA) for further testing were performed in the biohazard cabinet. The details of the samples were registered on a Microsoft excel based spreadsheet recording the source of samples in the form of farm code, quantity of sample and details of the submitter. The identification label of each serum sample in a cryovial contained the NSS number, farm code and the date of blood collection for subsequent analysis. Duplicate labels for all samples was also maintained in a master record book. The cryovials were arranged in racks and stored temporarily at 4°C (cold room) before they were master plated.

The biohazard cabinet was also serviced and certified so as to assure that the air flow and filtering mechanism were functioning optimally. This was to guarantee the safety of the laboratory personnel involved in the work. The protective attire that had to be worn compulsorily by a laboratory personnel processing blood samples on a daily basis included, i) protective eye goggles or face shield, ii) HEPA filtered mask, iii) surgical rubber gloves and iv) laboratory gown. The laboratory gown worn and head cap were tailor-made specially based on the design recommended by the CDC and the AAHL.

To permit testing protocols to be carried out in an ordinary open bench laboratory, the serum samples were rendered non-infectious. This was done through a process called "masterplating". Aliquots of 100 µl of sera were pipetted from the serum cryovials into microtubes arranged in 8 x 12 arrays containing 400 µl phosphate buffered saline (PBS) containing the detergents, sodium dodecyl sulphate (SDS) and Triton X100 at 0.05%. The mixture was then submerged in a 56°C waterbath for 30 minutes for heat inactivation. The serological test employed was an indirect enzyme-linked immunosorbent assay (ELISA) to detect antibodies to the Nipah virus. The details of the ELISA are explained elsewhere (4).

The NSS programme required a good system for the storage and retrieval of the sera sent for testing and retesting or for further research work. Serum samples were stored in 2 ml. cryovials (Axygen USA). Each individual vial was assigned with a unique NSS number. The cryovials were arranged in 10 x 10 array cryoboxes (Nalgene USA, Simport Canada, and Corning USA), which contained 100 serum vials per box. The cryoboxes were placed in stainless steel tailor-made cryoracks that took 10 cryoboxes in each rack and placed in -20°C horizontal deep freezers. In addition to the normal electricity power supply, emergency electrical points were also installed to ensure continual power supply in the event of breakdowns. Thus a very effective and efficient system was established for receiving, processing and cryopreserving samples that permitted storing and retrieval of samples in a convenient way.

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