Laboratory Diagnosis of Viral Diseases of Domestic Animals at Universiti Pertanian Malaysia
By Electron Microscopy

A. LATIF IBRAHIM and LAI CHOOI MAY
Faculty of Veterinary Medicine and Animal Science, Universiti Pertanian Malaysia.

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SUMMARY
Clinical and postmortem specimens in the forms of warts, scabs, faeces, trachea and lungs from suspected cases of bovine papilloma, contagious ecthyma of goat, fowl pox, calf diarrhoea and avian infectious bronchitis were submitted for laboratory diagnosis. Examination by electron microscopy revealed coronavirus-like particles in the faeces of a calf with diarrhoea and in the allantoic fluid of embryonated eggs infected with materials from a fowl suspected of infectious bronchitis. Poxvirus-like particles were observed from the scab of a goat with clinical symptoms of contagious ecthyma and also from the scab of a chicken suspected of fowl pox. Parvovirus-like particles were detected from the faeces of a dog with diarrhoea. Examination of warts from two different cattle, both clinically diagnosed as bovine papilloma, revealed different results. In one case only papilloma virus was present while in the other, both papillomavirus and poxvirus-like particles were present.

INTRODUCTION
One of the constraints in the laboratory diagnosis of viral diseases of domestic animals in Malaysia is the difficulty in obtaining suitable tissue culture or specific pathogen free embryonated eggs for virus isolation and specific antisera for virus identification. Moreover, isolation and identification of viruses in tissue culture and embryonated eggs are time consuming and mixed infections are not readily diagnosed. Although the use of negative contrast electron microscopy (NCEM) in routine veterinary diagnostic work has been reported by several workers (England et al., 1976, England and Reed 1980, Flewett et al., 1974 Mc. Ferran et al., 1971, Mc. Ferran et al., 1978), there has been no information on the application of such a technique in Malaysia. In this paper, therefore attempts are made to demonstrate the usefulness of the NCEM as a rapid diagnostic tool and to discuss the advantages of this technique.

MATERIALS AND METHODS
Mounting and staining of materials
Carbon-formvar coated grids (200 mesh) were used. The grid was held carbon side down on to a drop of the virus suspension. The excess fluid was blotted off using the torn edge of a piece of filter paper and the grid was allowed to dry. Thereafter the carbon side was held down on to a drop of 3% phosphotungstate acid (pH 6.8 - 7.2). The excess fluid was removed as described earlier and the grid was then left to dry in a desiccator for half an hour before examination under the transmission electron microscope (Philips' HMG 400) at 60 - 80 kv.

Examination of clinical specimens
Faeces: A 10% suspension of faeces made in Hank's balanced salt solution was clarified by centrifugation at 5,000 r.p.m. for 30 minutes (Sorvall OD 50). The suspension was further
centrifuged at 100,000 × g for 3 hours. Following centrifugation, the supernatant was decanted and the pellet was resuspended in a drop of distilled water and then applied to a grid for examination.

Skin lesions: Scabs from animals suspected of fowl pox and caprine contagious ecthyma were received by the laboratory. In the laboratory the scabs were mashed on a glass slide, suspended in a few drops of distilled water, and applied to a grid.

Warts: Warts from cattle suspected of bovine papilloma were cut into two and the cut surface was smeared on to a microscope glass slide. The smear was then mixed with two drops of distilled water and then applied to a grid.

Examination of viruses grown in embryonated eggs

Allantoic fluid from embryonated eggs previously infected with lung and trachea homogenate from chicken suspected of infectious bronchitis was applied directly to a grid and dried before staining.

RESULTS

Direct examination of clinical materials

Faeces: Particles morphologically similar to coronavirus were observed in the faeces of calf with diarrhoea. The particles were easily recognised by its projections at the periphery (Figure 1). Parvovirus-like particles were detected in the faeces of a dog with diarrhoea. (Figure 2).

Skin lesions: Scabs from the mouth lesions of a goat with clinical symptoms of contagious ecthyma contained virus particles which belong to the poxvirus group (Figure 3). Poxvirus-like particles were also seen in the scabs of chickens suffering from fowl pox (Figure 4).
Warts: Examination of warts from a clinically diagnosed bovine papilloma case revealed the presence of papilloma virus (Figure 5). However, in another cow suspected of bovine papilloma, two distinct virus particles were observed, one resembling papilloma virus and the other poxvirus (Figure 6).

Examination of virus isolated from eggs

Allantoic fluid: Examination of allantoic fluid from a case of infectious bronchitis revealed the presence of coronavirus-like particles (Figure 7). This virus was later confirmed as infectious bronchitis virus by serum neutralization test.
DISCUSSION

Malaysia is now embarking on a massive programme to expand her livestock industry and the resulting intensification as well as increase in the number of livestock farms favour the outbreak and spread of viral diseases which can at times wipe out a large population of the livestocks. There is therefore a need to have a rapid technique for the diagnosis of viral diseases as no effective control measures can be initiated unless the viruses involved are quickly and properly identified.

The techniques commonly used for the laboratory diagnosis of viral diseases are isolation and identification and fluorescent antibody technique. Although virus isolation from clinical specimen is a very reliable method, it is time consuming and mixed infection as well as non cytopathic infection are not readily diagnosed. The fluorescent antibody technique is considered a rapid diagnostic tool for virus detection since it can demonstrate mixed infection and non cytopathic infection, but it is limited by the lack of specific reagents.

The present study has shown that negative contrast electron microscopy is a rapid and reliable laboratory diagnostic method for virus detection. This technique can be readily applied for the identification of viruses grown in embryonated eggs and from clinical specimens. Similarly, virus grown in tissue culture can also be identified by this technique. Morphologic identification of the virus by this technique, together with the clinical history, is sufficient to make a diagnosis of the disease and to suggest control and management measures. The technique is also useful in the diagnosis of infectious bronchitis which would normally take a longer time to yield results since the post mortem materials have to be passaged three times in embryonated eggs before evidence of curling and dwarfing of the embryo can be seen. According to McFerran et al., (1978) this technique is also valuable for detecting many strains of infectious bronchitis virus which fail to cause dwarfing after the third passage. This study also demonstrates the value of this technique for the detection of infectious agents such as papilloma virus, contagious ecthyma virus and bovine coronaviruses which normally do not grow in vitro. Furthermore, this technique can be used to detect mixtures of viruses when initially only one virus infection is suspected. This is shown in the case of bovine papilloma described earlier in which both bovine papillomavirus and poxvirus-like particles were detected.

It is of interest to mention here that this is the first time coronavirus, papillomavirus and poxvirus-like particles from a goat and cattle were detected in Malaysia. In the past the diagnosis of diseases caused by these viruses was based solely on clinical signs and pathological findings. It can be concluded that the electron microscopic identification offers several advantages. The technique, besides being rapid in that viruses can be detected as early as an hour after submission of the specimen, is also very useful in cases where the viruses cannot be cultivated in vitro, detected by in vivo technique or concluded from clinical or pathological findings. In cases where positive results are obtained from electron microscopic examination, no further virological technique is required.

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REFERENCES


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