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A SUSPECTED CASE OF EPIZOOTIC HAEMORRHAGIC DISEASE AND BLUETONGUE IN DEER (*CERVUS TIMORENSIS*)

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SUMMARY: Three carcasses of deer *Cervus timorensis* with generalised extensive haemorrhages were investigated. Non-inflammatory degenerative changes in the vascular walls with disseminated intravascular coagulation were the main histopathological findings. Serological evidence from convalescence deer is highly suggestive of Epizootic Haemorrhagic Disease Virus type 2 and Bluetongue Virus type 1.

Keywords: Bluetongue, epizootic haemorrhagic disease, deer.

INTRODUCTION

Epizootic haemorrhagic disease (EHD) is an acute, infectious, often fatal viral disease of wild ruminants. This disease, characterised by extensive haemorrhages, has been responsible for significant epizootics in Northern United States and Southern Canada (Trainer and Karstad, 1973). The occurrence and identification of EHD was first reported by Shope *et al.* (1955) in white-tailed deer (*Odocoileus virginianus*) in New Jersey where approximately 700 deer succumbed to the disease.

Bluetongue (BT) is primarily a disease of domestic sheep with all breeds susceptible (Howell, 1963). In wild ruminants, the blesbok (*Damaliscus albifrons*), bighorn sheep (*Ovis canadensis*) and white-tailed deer (*Odocoileus virginianus*) are susceptible to BT (Trainer, 1973). Stair *et al.* (1968) observed that white-tailed deer appeared to be affected more severely by bluetongue virus (BTV) than were domestic sheep or cattle which may manifest only a rise in body temperature. Deer must be considered important hosts in the epizootiology of BT since they are susceptible to the disease.

This paper reports a suspected case of EHD and BT in the deer (*Cervus timorensis*) in Malaysia and discusses its possible importance in the epidemiology of BT in this country.

HISTORY AND NECROPSY FINDINGS

Fifteen heads of deer (*Cervus timorensis*) were bought by MARDI in Bukit Ridan, Muadzam Shah in Pahang in February, 1991. These animals were imported from Mauritius, Africa in November, 1990.

The animals were housed in a paddock planted with *Brachiaria decumbens* and fenced up by a dark wire netting together with a dark house. The paddock was located near the fringe of the jungle and the farm also has sheep, cattle and buffaloes in it.

Three weeks after their arrival on the farm, seven of the deer (2 male and 5 female) died suddenly on the same day. Three of the deer were sent to the Regional Diagnostic Laboratory in Kuantan for autopsy. A wide variation in the morning temperature (17°C) and midday temperature (37°C) in the farm, was observed on that particular day.

The three carcasses presented had similar lesions consisting of extensive haemorrhages ranging from petechiae to ecchymoses in the epicardium, at the base of the pulmonary arteries, lungs, liver, omentum and generally the serosal surface of the gastrointestinal tracts. The fresh appearance of the blood in these haemorrhages indicated that they must have occurred in the later stages of the illness. In some instances, the blood was almost black due to the quantity of extravasated blood. Postmortem examination of the remaining four deer in the farm showed similar lesions. Two of the remaining eight deer were sick and convalescing.

LABORATORY FINDINGS

Tissues were fixed in 10 percent neutral buffered formalin, sectioned and stained with haematoxylin-eosin. Microscopical examination showed extensive haemorrhages ranging from petechiae to ecchymoses in the epicardium, liver, lungs, kidneys, spleen and brain. Non-inflammatory degenerative changes in the vascular walls with disseminated intravascular coagulation were the main features in these organs, especially the brain and the meninges. The most pronounced brain lesions were perivascular haemorrhages and oedema (Fig. 1), and extensive haemorrhages in the meninges (Fig. 2). The microscopic vascular changes, which were present throughout the body and which appeared to be associated with the haemorrhages, consisted mainly of dilation of vessels with degeneration and necrosis of the tunica media and adventitia.

Samples of the spleen and heart blood packed in ice were sent to Veterinary Research Institute, Ipoh for virus isolation. The samples were homogenised, diluted 1/10 in brain-heart-infusion broth and inoculated intravascularly into 10 to 11-day-old embryonated chicken eggs (ECE). All eggs inoculated were alive up to the 7th day of incubation, after which the liver and brain of the ECE were harvested. These were homogenised and passaged once in C6/36 (*Aedes albopictus*) for 5 days, the media harvested and further passaged twice in BHK-21 cells. There was no cytopathic effect (CPE) observed in the cell-lines. Immunoblot (immunoperoxidase) test conducted on the tissue-culture media harvested using polyclonal and monoclonal BTV and EHD serum did not show any detectable viral antigen.

Blind passages for viral isolation were also performed in calf testicle, bovine turbinate and BHK-21 for two passages. There was no CPE observed in all the cells.

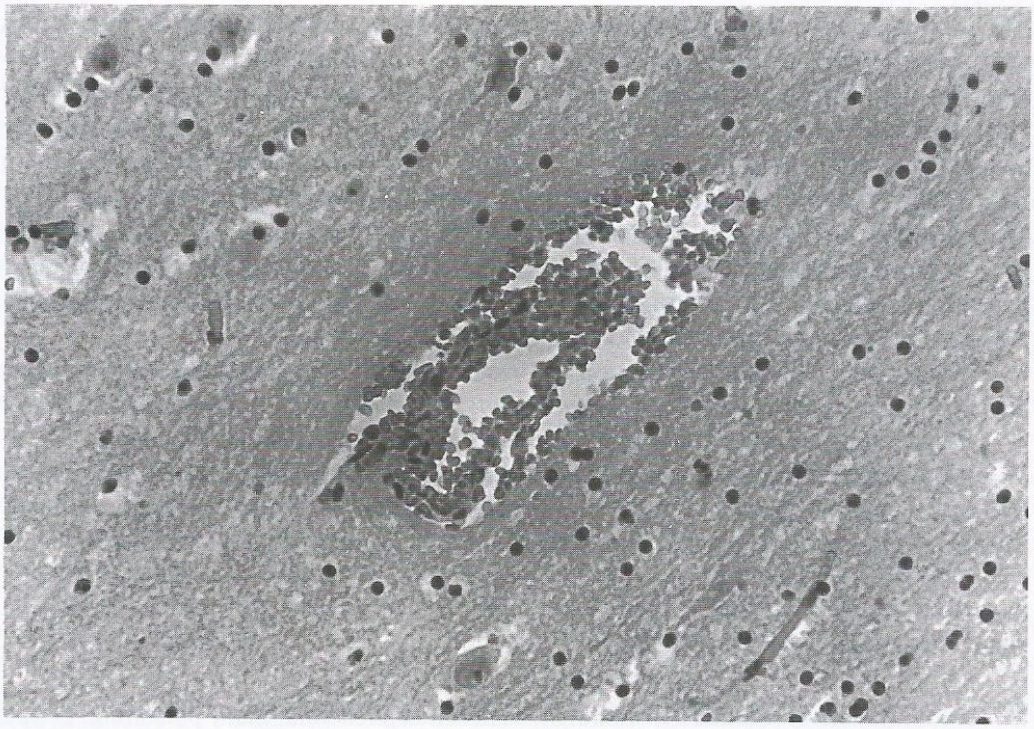

Figure 1. Pronounced perivascular haemorrhages and oedema in the cerebrum (HE X 100) of a deer (*Cervus timorensis*).

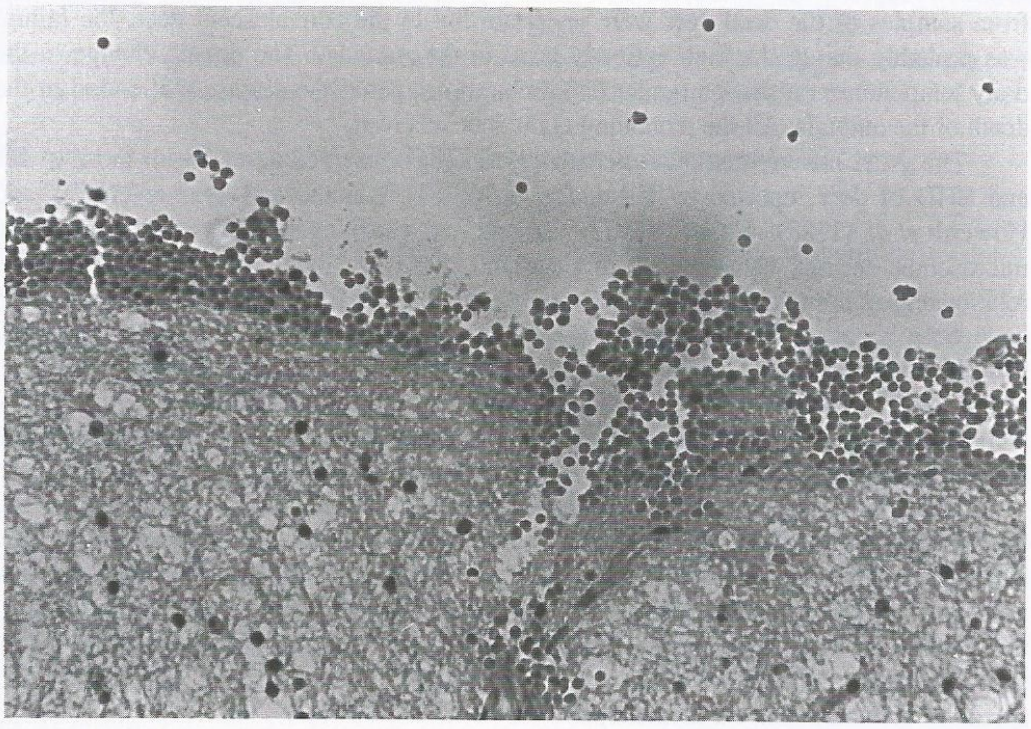

Figure 2. Extensive haemorrhages in the meninges (HE X 100) of a deer (*Cervus timorensis*) .

Serum samples from two of the deer in convalescence were taken and subjected to the agar-gel-immunodiffusion (AGID), plaque neutralisation (PN) and serum-neutralisation (SN) tests. The samples from both animals were positive in the AGID test. PN test revealed infection to EHD type 2 and BTV type 1. The PN and SN tests were conducted against only the 8 Australian BTV and 5 Australian EHDV serotypes*.

Virus isolation, also attempted from simultaneously bled heparinised samples from these two deer, failed to detect or recover any virus.

Profuse growth of haemolytic *Escherichia coli* was obtained from all the organs of the three carcasses on culture. *Salmonella sp., Pasteurella sp., Clostridia sp. and Pseudomonas pseudomallei* were not isolated from these organs.

Bacteriological culture of the soil and water samples taken from the paddock of the deer did not reveal the presence of *Pseudomonas pseudomallei.*

Organ impressions and intestinal examination did not revealed any parasite.

Analyses of the grass samples (*Brachiaria decumbens*) and the pelleted feed of deer did not reveal the presence of aflatoxin or a high copper content in these feeds. Mycology culture of the grass and feed also did not yield any pathological fungus.

DISCUSSION AND CONCLUSION

From the gross postmortem findings, bacterial septicaemia, plant poisoning, copper toxicity and virus infection were the differential diagnoses. However, the negative results of bacteriology and feed analysis suggested a virus infection. Although virus was not isolated from the dead deer, serological evidences from the convalescence deer is highly suggestive of EHD 2 and BTV 1 infections. Reasons for the failure to isolate the virus from samples of the dead deer were uncertain but in the convalescent deer, the failure was probably due to the high antibody level in the animals. The drastic change in the daily temperature on that particular day probably triggered the disease that ended in the death of the animals. All the remaining eight deer survived.

The gross postmortem and microscopic findings observed agreed with those of BT and EHD of deer reported by Karstad *et al.* (1961), Karstad and Trainer (1967), and Howerth *et al.* (1988). Karstad (1967) also reported a striking similarity of the gross and microscopic lesions between BT of deer and those of EHD and suggested a close relationship of the two diseases. Consecutive outbreaks of EHD of deer and BT of sheep have been reported by Dulac *et al.* (1988) in Okanagan Valley of British Columbia, Canada. In Malaysia, BT is endemic and serological testing showed that nearly all sheep tested have titres with no clinical signs except for the imported sheep (Chiang, 1989). Hence, there may be a possibility of BT outbreak in susceptible sheep followed by EHD and BT in deer or vice versa especially in our system of mixed farming.

With the large numbers of deer imported in Malaysia, strategies in the epidemiological study of BT and EHD should be formulated. Serological tests on the deer upon arrival in the country and at least 2-3 months later should be conducted. New serotypes of viruses brought in together with the deer and the susceptibility of the deer to the Malaysian BTV serotypes could then be identified. Sentinel herds should be set up and monitored regularly for EHD and BT in relation to the weather, distribution and spread of the competent *Culicoides sps.*

*BTV 1,3,9,15,16,20,21,23 and EHDV 2,5,6,7,8 - Provided by courtesy of Dr. G.P. Gard of the Department of Primary Industries and Energy, Canberra, Australia.

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RINGKASAN

KES PENYAKIT HEMORAJ EPIZOOTIK DAN LIDAH BIRU PADA RUSA (Cervus timorensis)

Tiga karkas rusa Cervus timorensis yang mengalami hemoraj teruk menyeluruh telah disiasat. Perubahan janarosot bukan keradangan dalam dinding vesel dengan penampilan penggumpulan intravesel tersebar merupakan penemuan histopatologi utama. Bukti serologi daripada rusa yang pulih kuat menyarankan Virus tip 2 Penyakit Hemoraj Epizootik dan Virus tip 1 Penyakit Lidah Biru.