ISOLATION OF BLUETONGUE VIRUS SEROTYPE 21 FROM MOSQUITOES IN WEST JAVA, INDONESIA

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ABSTRAK

Sendow, I., Sukarsih, Soleha, E. and Daniels, P.W. 1994. Isolasi virus bluetongue tipe 21 dari nyamuk di Jawa Barat, Indonesia. *Penyakit Hewan* 26(48): 21-25

Virus bluetongue tipe 21 telah berhasil diisolasi dari campuran kelompok *Aedes* spp. dan *Anopheles* spp., nyamuk, dari daerah dataran rendah di Jawa Barat dalam lampu perangkap nyamuk yang digunakan untuk menangkap insekta terutama *Culicoides* spp. Suspensi nyamuk diinokulasikan terlebih dahulu pada telur embryo tertunas sebelum diinokulasikan pada biakan jaringan *Aedes albopictus* dan BHK-21. Isolat virus diidentifikasi dengan uji immunodot blotting dengan menggunakan antibodi monoklonal yang spesifik terhadap bluetongue, dan yang bereaksi dikirim ke Laboratorium referen untuk konfirmasi dan serotipe.

Kata kunci: virus BT tipe 21, nyamuk, isolasi.

ABSTRACT

SENDOW, I., SUKARSIH, SOLEHA, E. and DANIELS, P.W. 1994. Isolation of bluetongue virus serotype 21 from mosquitoes in West Java, Indonesia. *Penyakit Hewan* 26(48): 21-25

Bluetongue virus serotype 21 was isolated from a pool of *Aedes* spp. and *Anopheles* spp. mosquitoes collected at a low altitude site in West Java in a light trap used for collecting *Culicoides* spp. A suspension prepared from the mixed pool was inoculated firstly into embryonated chicken eggs before passaging into *Aedes albopictus (C6/36) and baby hamster kidney (BHK-21) cells. Viral isolates were screened by immunodot* blotting using a monoclonal antibody specific for bluetongue group viruses. Those reacting were submitted to the international reference laboratory at Onderstepoort for confirmation and serotyping.

Key words: BTV type 21, mosquitoes, isolation.

INTRODUCTION

The bluetongue viruses (BTV) are arthropod borne viruses which infect ruminants (Erasmus, 1975). BTV can routinely be isolated from blood of infected ruminants (St. George, *et al.*, 1978; Gard *et al.*, 1988), or from insects (Standfast *et al.*, 1985; St. George and Muller, 1984). At present some *Culicoides* spp. are the known vectors (Muller, 1985; Standfast *et al.*, 1985). However, other arthropods have occasionally been reported to be infected with or carrying BTV. Brown *et al.* (1992) reported the isolation of a BTV from mosquitoes. Stott *et al.* (1985) demonstrated that ticks (*Ornithodoros coriceus*) may be infected with BTV. The sheep ked *Melophagus ovinus* has also been reported as a potential mechanical vector (Luedke *et al.*, 1965).

The identification of BTV in an arthropod may identify that species as a potential vector, or may merely indicate that the arthropod has recently fed on a viraemic animal. Care must be taken to ensure that specimens processed for virus isolation have not had a recent blood meal. Even if this criterion is satisfied, it is still necessary to demonstrate that the arthropod is capable of transmitting the infection to a vertebrate host before considering that the species is a proven vector (Standfast *et al.*, 1992).

In the present work a light trap was used to collect *Culicoides* spp. for BTV isolation, and in some collections *Aedes* spp. and *Anopheles* spp. were also present in small numbers. Isolation of viruses was attempted from these mosquitoes as part of a study of bovine ephemeral fever group viruses (Daniels, *et al.*, 1993; Soleha *et al.*, 1993). This paper records another isolation of a BTV from mosquitoes in Indonesia.

MATERIALS AND METHODS

1. Insect collection

Insects were collected weekly in 1991 in West Java, at a low altitude site, Depok; and a high altitude site, Cisarua; using a Pirbright light trap (Mohammed and Mellor, 1990). The collections were made near sentinel cattle (Sendow *et al.*, 1992; 1993a) from 4.30 to 8.00 pm. The method was used as described in Sukarsih *et al.* (1992). Insects were trapped directly into phosphate buffered saline (PBS) with 1000 ug/ml kanamycin, 500 ug/ml streptomycin and 500 IU/ml penicilin. Insects were sorted to sub-genus and species for *Culicoides* spp. and to genus for the mosquitoes. Only parous and unengorged female insects were used for viral isolation. Pools of insects processed for viral isolation contained not more than 250 insects. The *Culicoides* spp. pools contained members of the sub-genus *Avaritia,* sub-genus *Trithecoides* and sub-genus *Hoffmania,* with seperate pools for species *C. actoni* and *C. oxystoma* because of their abundance in the collections (Sukarsih, unpublished). Because of the low numbers in collections, all mosquitoes were processed as a single pool since the primary aim was to recover isolates of viruses.

2. Viral isolation

Each pool of insects was homogenized in 2 ml Minimum Eagle's Media (MEM, Flow Laboratories) with antibiotics and sonicated at 20 amplitudes for 10 seconds twice before clarification at 1000 G for 15 minutes (Sendow *et al.*, 1993b). The supernatant was filtered through a 450 nm Millipore filter (Sartorius). The sterile supernatant of 0.1 ml volumes was inoculated intravenously into four 10-11 days old embryonated chicken eggs (ECE). Inoculated ECE were observed for 5 days for the mortality of embryoes. Hearts were harvested from all dead embryoes, homogenized, sonicated, clarified and passaged into *Aedes albopictus* (C6/36) cells and BHK-21 cells. Inoculated BHK-21 cells were observed for 5 days for cytopathic effect (CPE), then passaged and observed twice more.

3. Preliminary identification of isolates

Inocula producing CPE in BHK-21 cells were considered to be viral isolates requiring further identification, to viral group in the first instance. The screening test was the immunodot blotting (IDB) test (Afshar, *et al.*, 1987; Sendow *et al.*, 1993b). Monoclonal antibody (Mab) specific to the BTV group was provided from the Australian Animal Health Laboratory (AAHL), Geelong, Australia (Lunt, *et al.*, 1988). Isolates reacting in the IDB test were submitted to the OIE Reference BTV Laboratory in Onderstepoort, South Africa, for confirmation and serotyping.

RESULTS

A total of 215 pools of *Culicoides* spp. and 14 pools of mosquitoes were processed during 10 months of collections in 1991 from low and high altitude areas (Table 1). There were differences in the insect collections with altitude. At high altitude site, the number of insects collected each night varied from 5 to 658 and the number of pools processed varied from 3 to 9. At low altitude site, the number of insects collected each night varied from 57 to 2692 and the pools processed for viral isolation varied from 6 to 12. From 215 pools of *Culicoides* spp. and 14 pools of mosquitoes processed for viral isolation, only 4 pools of *Culicoides* spp. and 1 pool of mosquitoes produced CPE in BHK cells (Table 2).

The isolate from mosquitoes was obtained from a pool containing 37 *Aedes* spp. and 7 *Anopheles* spp. The inoculum from this mosquito pool caused death of embryoes at days 3 to 5 after inoculation. The pathological changes observed included haemorrhages in the musculature and the heart was swollen and haemorrhagic. CPE was shown on the first passage in BHK-21 cells. This isolate from the pool of mosquitoes reacted in the IDB with the BTV Mab, and at the OIE reference Laboratory was confirmed as BTV serotype 21 (Erasmus, pers. comm). This isolate was collected on 12 May 1991 from the low altitude area.

Of the other isolates, one was from a pool of the sub-genus *Avaritia,* and 2 isolates were from pools of the sub-genus *Trithecoides.* The two isolates from pools of *Trithecoides* did not react with the BTV Mab in the IDB test, while the isolate from the *Avaritia* pool did react and has also been identified as BTV type 21 (Sendow *et al.*, 1993b).

DISCUSSION

The number of insects trapped was greater from the low altitude area than from the high altitude area. The difference may have been due to environmental factors which affect insect populations, such as temperature and breeding sites, particularly cattle faeces. The number of mosquitoes collected at each location was also lower

than for *Culicoides* spp. because the trap was designed for *Culicoides* spp.

All isolates that reacted in the IDB using the Mab specific to BTV were suspected of being BTV. Due to lack of a full panel of 24 BTV serotypes and the associated antisera in Indonesia, the suspected BTV isolates were sent for confirmation and serotyping to the Reference Laboratory for Bluetongue in Onderstepoort, South Africa.

In Indonesia, BTV serotype 21 has been isolated from *Culicoides* spp. (Sendow *et al.*, 1993b) and sentinel cattle blood (Sendow *et al.*, 1992; 1993a) in the study area in West Java, and now from mosquitoes. In Irian Jaya, some 4000 km from the study area, BTV serotype 21 was also isolated from blood of healthy sentinel cattle (Sendow *et al.*, 1993c). This indicates that BTV serotype 21 was widespread in Indonesia in 1991. The question arises whether the isolates are from the same population of viruses. Further studies must be conducted using molecular procedures as described by Gould, (1987); and Gould *et al.*, (1989) in which the sequence of nucleotides in normally conserved gene segments can be compared with those of isolates from known regions, to give an indication of the likely region of origin of an isolate, based on its similarities and differences with the BTV isolates from known regions.

At present only *Culicoides* spp. are considered as major BTV vectors. Some of the vector species have been defined, including *C. fulvus, C. brevitarsis, C. wadai,* and *C. actoni* in the Australian region (Standfast *et al.*, 1985; Muller, 1985; St. George and Muller, 1984). In Africa, *C. imicola* has been defined as the main BTV vector (Mellor, 1992; Boorman *et al.*, 1985) and in the Americas *C. variipennis* and *C. insignis* have been demonstrated to be BTV vectors (Greiner *et al.*, 1985; Loomis *et al.*, 1985; Mullen *et al.*, 1985). The possibly of other *Culicoides* spp. playing a role in BTV transmission has been investigated in Australia (Standfast *et al.*, 1985), but without any conclusive results other than those above. At present there is little information in Indonesia except the isolation of BTV serotype 21 from a mixed pool of *C. fulvus* and *C. orientalis* (Sendow *et al.*, 1993b).

Brown *et al.* (1992) reported isolation of a BTV from *Anopheles vagus* in Indonesia. *Anopheles vagus* is known to feed on large domestic animals, especially bovines (Horsfall, 1955). However, whether this mosquito may act as a BTV vector has not been established.

The current report offers some independent confirmation of this previously observed association, with the isolation in this case being from a mixed pool of *Anopheles* spp. and *Aedes* spp. Care was taken not to process mosquitoes showing clear evidence of a blood meal, but it is not yet proven that these mosquitoes will support replication of BTV.

Unfortunately the mosquitoes obtained in this study were not identified to species. However, the possibility arises that *Anopheles* spp. or *Aedes* spp. may play a minor role in transmitting BTV in livestock. Brown *et al.* (1992) reviewed the little information available on the association of other orbiviruses with mosquitoes. Epizootic haemorrhagic disease (EHD) viruses and Eubenangee virus have been isolated from mosquitoes, and African horse sickness (AHS) virus has been transmitted by mosquitoes. Further study on this aspect is needed for the findings of Brown *et al.* (1992) and the present report may offer a possible new dimension to BTV epidemiology, or may simply indicate that sensitive virus isolation procedures can detect viruses in the gut contents of insects having fed on viraemic animals.

It is noted that not only *Culicoides* spp. have been implicated as BTV vectors, but also a tick (*Ornithodoros coriaceus*) (Stott *et al.*, 1985). Their preliminary study identified BTV in the salivary glands of infected ticks and the ticks were capable of transmitting BTV to a susceptible cow on day 42 after the virus was ingested (Stott *et al.*, 1985).

The study of Gray and Bannister (1961) with sheep keds (*Melophagus ovinus*) was different, for this study simply showed isolation of BTV from keds after feeding on viraemic animals. Another small study demonstrated the transmission of BTV between sheep mechanical transmission (Luedke *et al.*, 1965), there having been inadequate time between feeding on the infected sheep and exposure to the recipient sheep to allow a cycle of virus replication in the keds. Such reports, including the present one, must be considered carefully for their significance in the overall epidemiology of BTV infections.

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Table 1. Pools of insect collected at high altitude and low altitude areas for viral isolation in 1991

Area	*Culicoides* spp.	Mosquitoes
Low altitude	120	10
High altitude	95	4
Total	215	14

Table 2. The number isolates obtained from insects in 1991

Area	*Culicoides* spp. Total	BTV group	Mosquitoes Total	BTV group
Low alt.	4	1	1	1
High alt.	0	0	0	0
Total	4	1	1	1

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